PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *CAPPARIS DECIDUA* AND *CITRULLUS COLOCYNTHIS*

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

by

PRASHANT KUMAR DHAKAD

Enrollment No. 1412302002

Department of Pharmacy

School of Medical and Allied Sciences

GALGOTIAS UNIVERSITY, GREATER NOIDA (U.P.)

JULY, 2018

DECLARATION

I hereby declare that the thesis entitled "PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *CAPPARIS DECIDUA* AND *CITRULLUS COLOCYNTHIS*" submitted by me, for the award of the degree of *Doctor of Philosophy* to Galgotias University is a record of bonafide work carried out by me under the supervision of **Prof. Pramod Kumar Sharma,** Dean, School of Medical and Allied Sciences, Galgotias University (Guide) and **Prof. Sokindra Kumar**, Principal, R.V. Northland Institute, Greater Noida (Co-Guide).

I further declare that the work reported in this thesis has not been submitted and will not be submitted, either in part or in full, for the award of any other degree or diploma in this institute or any other institute or university.

Place : Greater Noida
Date :

Signature of the Candidate

CERTIFICATE

This is to certify that the thesis entitled "PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *CAPPARIS DECIDUA* AND *CITRULLUS COLOCYNTHIS*" submitted by PRASHANT KUMAR DHAKAD (School of Medical and Allied Sciences) Galgotias University for the award of the degree of *Doctor of Philosophy*, is a record of bonafide work carried out by him under our supervision, as per the Galgotias University code of academic and research ethics.

The contents of this report have not been submitted and will not be submitted, either in part or in full, for the award of any other degree or diploma in this institute or any other institute or university. The thesis fulfills the requirements and regulations of the University and in our opinion meets the necessary standards for submission.

Prof. Pramod Kumar Sharma
Dean
School of Medical and Allied sciences
Galgotias University, Greater Noida (U.P.)

(Guide)

Prof. Sokindra Kumar
Principal
R.V. Northland Institute
Greater Noida (U.P.)

(Co-Guide)

ABSTRACT

The use of plants as source of medicine in treating disease is an ancient practice. In recent times, attention has been reverted back to plants as sources of therapeutic agents due to their higher properties. Two plants selected for the present investigations were *Capparis decidua* and *Citrullus colocynthis* those were used by the traditional people of Rajasthan. The plant parts selected for study of *Capparis decidua* was dried root, stem and leaves while for the *Citrullus colocynthis* was dried fruits. The selected plant parts were comminuted and dried powdered form was soxhlet extracted with water and alcohol in the ratio of 30:70 for the duration of 48 hrs. The extract was filtered and concentrated at 48˚C on a water bath and further the hydroalcoholic extract was stored in a sterile container. The dried crude powdered form of *Capparis decidua* and *Citrullus colocynthis* plants were undergone with physicochemical analysis. Phytochemical analysis of *Capparis decidua* and *Citrullus colocynthis* showed the presence of phenols, flavonoids, terpenoids, saponins, alkaloids, glycosides, steroids, carbohydrates and tannins. The dose of 100 mg/Kg and 200 mg/Kg of *Capparis decidua* while 50 mg/Kg and 100 mg/Kg of *Citrullus colocynthis* was administered to Wistar rats during the pharmacological studies. Anti-diarrheal and wound healing activities were studied in *Citrullus colocynthis* and anti-arthritic and aphrodisiac activities were performed in the *Capparis decidua* plant. Anti-diarrheal activity was evaluated using Castor oil induced diarrhea model and barium sulfate model. Wound healing activity was done using excision and incision wound models. Anti-arthritic activity was performed through FCA induced arthritis model. Aphrodisiac activity was evaluated using sexual behavior study. In these activities it was seen that dose of 200 mg/Kg of *Capparis decidua* and 100 mg/Kg of *Citrullus colocynthis* showed better results in different parameters evaluated. The present results validate the use of a hydroalcoholic extract of *Capparis decidua* and *Citrullus colocynthis* and presence of phytoconstituent are hold responsible for their pharmacological actions in treatment of diseases.

Keywords: *Capparis decidua*, *Citrullus colocynthis*, phytochemical analysis, hydroalcoholic extract, pharmacological studies.

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Place : Greater Noida **Prashant Kumar Dhakad**

Date :

Certificate

This is certify that the project title **"Phytochemical and Pharmacological Investigation of *Capparis decidua* & *Citrullus colocynthis*"** has been approved by the IAEC.

Protocol No. : RVNI/IAEC/2016/04
No. of Animal approved: 84 M/F Rats (Wistar)
Duration of project: 06 Months.

Name of Chairman/ Member Secretary IAEC:
Dr. Sokindra Kumar/Ramji Gupta

Name of CPCSEA nominee:
Dr. Anil Kumar Sharma

S Kumar 18/08/2016

Signature with date

Signature with date

Ramji Gupta 18/08/16

Chairman/ Member Secretary of IAEC:

Anil 18.8.16

Main Nominee CPCSEA:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

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UNIVERSITY OF RAJASTHAN, JAIPUR

No. Bot/16/3780 Dated: 19th March,2016

AUTHENTICATION CERTIFICATE

This is certified that Mr. Prashant Kumar Dhakad Ph. D. Research Scholar of GALGOTIAS UNIVERSITY, GREATER NOIDA has deposited plant specimen on 19.03.2016 and plant is identified as:

Capparis Decidua L. (R.NO:RUBL211604) belongs to Family. Capparidaceae

Convener 19.3.16
Herbarium Committee
(Dr R.D. Agrawal)

Head 19.3.16
Department of Botany
Department of Botany
University of Rajasthan
JAIPUR

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No. Bot./2016/ 5205 Date: 23/12/16

AUTHENTICATION CERTIFICATE

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- *Citrulllus colocynthis* (R.No: RUBL 211645) belong to Family: Cucurbitaceae

Manju Sharma 23/12/2016
Convener
Herbarium Committee

23.12.16
Head
Department of Botany

HEAD
Department of Botany
University of Rajasthan
JAIPUR

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LIST OF ABBREVIATIONS/SYMBOLS

S.No.	Abbreviation/Symbol	Full Form
1.	WHO	World Health Organisation
2.	NO	Nitric Oxide
3.	ED	Erectile Dysfunction
4.	CT	Computerised Tomography
5.	Ig	Immunoglobulins
6.	T cells	Thymus Cells
7.	TNF α	Tumor Necrosis Factor α
8.	RA	Rheumatoid Arthritis
9.	IL	Interleukins
10.	GCMS	Gas Chromatography–Mass Spectrometry
11.	HPLC	High-Performance Liquid Chromatography
12.	DNA	Deoxyribose Nucleic Acid
13.	RNA	Ribose Nucleic Acid
14.	ELISA	Enzyme Linked Immunosorbent Assay
15.	MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
16.	FCA	Freund's Complete Adjuvant
17.	CPCSEA	Committee For The Purpose Of Control And Supervision Of Experiments On Animals.
18.	COID	Castor Oil Induced Diarrhea Model
19.	BSM	Barium Sulfate Milk Model
20.	WBC	White Blood Cells
21.	RBC	Red Blood Cells
22.	PLT	Platelets
23.	ESR	Erythrocyte Sedimentation Rate
24.	ALT	Alanine Aminotransferase
25.	AST	Serum Aspartate Transaminase
26.	ALP	Alkaline Phosphatase
27.	SGPT	Serum Glutamic Pyruvic Transaminase
28.	LDH	Lactate Dehydrogenase
29.	NADH	Nicotinamide Adenine Dinucleotide
30.	SGOT	Serum Glutamate Oxaloacetate Transaminase
31.	MDH	Malate Dehydrogenase
32.	EDTA	Ethylenediaminetetraacetic Acid
33.	ML	Mount Latency

34.	IL	Intromission Latency
35.	EL	Ejaculation Latency
36.	MF	Mount Frequency
37.	IF	Intromission Frequency
38.	SD	Standard Deviation
39.	CCE	Citrullus Colocynthis Extract
40.	ANOVA	Analysis Of Variance
41.	*C.decidua*	*Capparis decidua*
42.	*C.colocynthis*	*Citrullus colocynthis*

LIST OF CHEMICALS/EQUIPMENTS

S. No.	Ingredients	Manufacturer
CHEMICALS		
1.	Lead acetate	Central Drug House, New Delhi
2.	Sodium hydroxide	Central Drug House, New Delhi
3.	Ethanol	Fischer Scientific
4.	Conc. Sulfuric acid	Central Drug House, New Delhi
5.	Chloroform	Fischer Scientific
6.	Hydrochloric acid	Central Drug House, New Delhi
7.	Wagner's reagent	Central Drug House, New Delhi
8.	Dragendorff's reagent	Central Drug House, New Delhi
9.	Ferric chloride	Central Drug House, New Delhi
10.	Glacial acetic acid	Central Drug House, New Delhi
11.	α-napthol solution	Central Drug House, New Delhi
12.	Barfoed's reagent	Central Drug House, New Delhi
13.	Molisch reagent	Central Drug House, New Delhi
14.	Benedicts reagent	Central Drug House, New Delhi
15.	Fehling's reagent	Central Drug House, New Delhi
16.	Castor oil	Jayant Agro Organics, Mumbai
17.	Loperamide	Arene Life Sciences, Andhra Pradesh
18.	Barium sulfate	Oasis Fine Chem, Vadodara
19.	Freund's complete adjuvant	Sigma-Aldrich Ltd, USA
20.	Povidone iodine ointment	Cipla India Ltd
21.	Ethyl ether	Central Drug House, New Delhi
22.	Diclofenac sodium	Afton Pharma, Gujarat
23.	Sildenafil citrate	Cadila Pharmaceutical Ltd, Gujarat
24.	Ethinyl oestradiol	Sigma-Aldrich Ltd, USA
25.	Progesterone	Sigma-Aldrich Ltd, USA

EQUIPMENTS		
1.	Plethysmograph	MEDICAID SYSTEMS (PM041609)
2.	Vernier Calipers	Swastika scientific
3.	Hot air oven	HICON
4.	Digital balance	SHIMADZU Corporation Japan
5.	Heating mantle	HICON
6.	Desiccators	MGI
7.	Hand grinder	Lucky Scientific House
8.	Mixer	Gold line (MG301)
9.	Copper sieve	Unique manufacturers
10.	Rota rod apparatus	HICON
11.	Eddy's Hot plate apparatus	HICON
12.	Centrifuge	HICON

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Chapter 1

Introduction

A large number of individuals today rely on the natural prescriptions since they trust in them and see them as their medication as compared to the "allopathic" (modern) system of medicine. These restorative herbs are accessible locally and are recommended by the experts of conventional medicine. Indeed, even in Western nations, there is currently an expanded utilization of herbal medicines, as a result of more undesirable reactions due to western drug. Many people carry impression that medicines derived from natural plants are harmless. Although natural medicines induce fewer side effects than conventional drugs, there are plants that cause severe side effects. Research on medicinal plants should be carried out to determine whether western therapeutics could add to its armamentarium a few new drugs obtained from medicinal plants used in the traditional systems. One such area could be hepatitis, since the traditional system of medicine has plants for centuries for protecting the liver and for treatment of liver dysfunction. *Picrrorhiza kurroa, Phyllanthus amorous* and *Andrographis paniculata* are some of the widely used hepatoprotective herbs. In view of their mention in ancient literature laboratory experiment need be carried out with the plants so that they could be used widely in chemical practice. Similarly, plants should be investigated in the field of bronchial asthma. It is difficult to accept that there are no more drugs waiting to be discovered from plants, in spite of unrewarding experiences in this field during the last 30 years. There may still some such discoveries ahead of us (Chaudhury 1995).

In Ayurvedic texts, there is an exhaustive description of herbs and their clinical uses which can be further explored using modern scientific methods to establish their therapeutic usefulness and detect the wonder molecules. Ayurveda, thus, has an important role in bioprospecting of further medicine. Possibly because of different therapeutic principles, many difficulties are faced during this process of drug development, which can be adequately overcome by an appropriate correlation of principles for diagnosis and treatment of both the systems of medicine. This in turn depends on an appropriate

interpretation of Ayurvedic texts in contrast to mere translation in different languages (Arora and Kumar 2002).

Rigveda is the earliest celebrated treatise mentioning the use of some medicinal plants. The work of Agnivesha resulted in the compilation of Charaka-Samhita by Charaka Sushruta, a brilliant discipline of Dhanvantri, was known for his knowledge of surgery and medicine. Some Portuguese and Dutch scientist in sixteenth century came to India for the study of medicinal plants. Van Rheed's Horetus Malabericus is the monumental work in 12 volumes on the study of Indian plants published between 1678 and 1703. William Roxburgh, the father on Indian Botany, worked a lot on Indian plants and his work was published by Carey in 1820 to 1824. Heber Drury published his monumental work 'Useful plants of India, Dymock's Vegetable Material Medica of India appeared in 1883 and his comprehensive work Pharmacographic Indices was prepared in collaboration with Warden and Hooper, Sir George Watt produced a voluminous dictionary of economic plants of India in six volumes. These are outstanding work wealth of information on economic plants (Kaul 1997).

1.1 IMPORTANCE AND SCOPE OF HERBAL MEDICINE

We can unquestionably expect that the mending properties of a few plants were found by primitive people genuinely early and they figured out how to utilize them. Individuals increase important encounters by gathering and utilizing restorative plants and passed on their insight to their ages. One of the important concerning therapeutic pharmaceuticals and sedative substances was found on a mud tablet in Assyrian cuneiform substance backpedaling to 2,700 BC. The tablet indicates a darker medicine, girl of poppy, which implies opium. In old Egypt, restorative science and the utilization of therapeutic substances have a well-established custom. The Egyptian Pharmacopeia dependably had a supply of prescriptions of plant and creature, and also mineral sources. There were 25 sorts of therapeutic plants, as fundamental nourishing and restorative plants, onion, garlic, lettuce; lentils, olives, and caraway were utilized (Gupta and Chitme 2002).

The expression "traditional medicine" has a few definitions and elucidations. The most thorough is where the WHO has characterized it as a sum of all the information and

practices which are help in finding and aversion of ailments and in addition the disposal of mental, social, physical unevenness, which trust just on reasonable inclusion and notes passed on starting with one age then onto the next through composing or verbally (Thaibinh 2002).

The conventional drug is by and large grabbing pervasiveness over allopathic pharmaceutical because of the going with reasons:

1. Climbing costs of therapeutic planning.
2. As these are from characteristic root, they are free from reactions.
3. It gets to the root drive and disposes of it with the goal that the malady does not come again.
4. Treatment for some hardheaded diseases.
5. Simple attainable quality of pharmaceuticals from regular establishments.

Herbal remedies popularized because of their efficacy, easy obtain ability, low price and somewhat being lacking of serious toxic effects. A significant number of conventional recuperating herbs were compound examination conspicuous to the isolation of reparable mixes. Beginning from 1800 A.D., the segregation and portrayal of herbal extracts turned into a noteworthy piece of pharmacopeias (Dandgi 2008).

The wild plants have been used by local group restoratively to cure cuts, wounds, consumes and other illness sicknesses having dietary or pathogenic beginning. Rajasthan state is having to a great degree dry climatic conditions and neighborhood group is combined with destitution and cataclysmic event like dry spell which is trying for them to adapt up to sustenance and drug. Be that as it may, the vegetation of this state involves dry spell safe plants having photochemical and mineral fixings to complete almost all organic responses of body. According to the reports, it is assessed that 70– 80% of total populace, particularly in creating nations, relies upon plant drug to avoid and cure sicknesses. What's more, it has been accounted for that around 25% of the blended medications are being gotten from therapeutic plants (Sharma et al, 2016).

1.2 *CAPPARIS DECIDUA*

Capparis decidua (Forssk.) Edgew. (Kair) is a multipurpose perennial woody plant, of caper family (Capparaceae), found chiefly in hot arid region of different parts of world.

The caper family includes 650 species of plants found in 30 genera located principally in tropical and warm temperate regions. Nearly 26 of these species are reported to occur in India. Because of its xerophytic adaptive nature this plants grows well under the harsh climatic conditions of arid regions. *Capparis decidua* is salt-tolerant and grows along saline hard planes in Thar Desert. Mature plants develop extensive root systems that penetrate deeply into the soil. Leaf stipules form into spines to reduce transpiration. It also protects birds and animals from scorching heat during summers (Sharma et al, 2016).

1.2.1 TAXONOMICAL CLASSIFICATION

Kingdom: Plantae
Division: Phanerogamae
Subdivision: Angiospermae
Class: Dicotyledonae
Subclass: Polypetalae
Order: Thalamiflorae
Suborder: Parietales
Family: Capparaceae
Genus: *Capparis*
Species: *C.decidua*

1.2.2 BOTANICAL DESCRIPTION

Capparis decidua (Kair) is a spiny, much dense and slender branched, green twiggy looking shrub or small tree growing gregariously bearing dense spherical crowns. The stem bark is smooth, green when young and turns yellow or whitish grey as it matures. Leaves are deciduous, glabrous, small caducous, succulent that appear for maximum of one month, on new shoots. New leaves sprout from January to November, being sessile with very short petioles, pointed and small (2–12 mm in length and 1–3 mm in width). Fruits are globose, borne on a long stalk, green when immature and red or pink when ripened. Ripe fruits contain a sweet yellow pulp with many seeds. Flowering occurs on young shoots of the current year. Narrow leaves and stipular spines on shoots help reduction in loss of water due to transpiration in extremely drought conditions. It has well developed tap root systems which uptake water within ground at a depth of up to 4m. The

natural habitat of *Capparis decidua* is on the lower side of plains all over the hot and dry regions, semi- stabilized dune peripheries. *Capparis decidua* flourishes well on shallow hard soils and rocky outcrops but not on shifting sand dunes or water logged areas. It grows abundantly in sandy, saline and gravy soils of pH 6.5-8.5. It can tolerate temperatures as high as 50˚ C and as low as 0˚ C. It has also been found to thrive well on saline soils. This species shows wide genetic variability in plant size, morphology, fruit production and dimorphism in seeds (Sharma et al, 2016) **(Figure 1).**

1.2.3 TRADITIONAL & THERAPEUTIC USES

Capparis species have been utilized in medicine since ancient times. These plants were used first time about 2000 years BC by Sumerians. The roots, flowers, and fruits of these plants with potential medicinal benefits, have been in use since that time against infectious diseases without any side effects. Sharma and Kumar (2008) suggested that the biological effects of *Capparis decidua* may be ascertain to presence of antimicrobial bioactive compounds, like phenolics, flavonoids, polyamine alkaloids, glucosinolates, and vitamins that decrease the growth of microorganism, and are negligibly harmful for their hosts. The medicinal use of *Capparis decidua* is also mentioned in ancient books. By Kavirajas, the plant is regarded as acrid, laxative, counterirritant and stimulant. They often prescribe it in heart diseases, colic pains, scurvy and phthisis. The plant act therapeutically in flatulence, anorexia, respiratory disorders, skin diseases, in general weakness and also act as anthelmintic and diuretic. Infusion of *Capparis decidua* is used externally for eruptions, boils, joint diseases and internally in cough and as an antidote in case of poisoning. Juice of fresh plant is used to kill worms in ear. It acts as a good substitute of senega. Crushed bark is applied as poultice for treatment of wounds. Roots are acts as sudorific, thermogenic, expectorant, carminative, digestive, stimulant, antibacterial, aphrodisiac, anodyne, anthelmintic and useful in arthritis, dyspepsia, constipation, lumbago, odontalgia, amenorrhoea and dysmenorrhoea. Root bark is known to be astringent, alterative, acrid, diaphoretic, alexeteric. Powder or infusion of root bark is used in gout, rheumatism, cough, dropsy, palsy, asthma, intestinal worms and intermittent fever. The root powder is applied externally on malignant ulcer. Coal paste obtained after burning the wood is applied to muscular injuries. Fresh leaves and young

shoots, when chewed, relieve toothache immediately. The local people of India and Pakistan consider caper fruits having anti-diabetic, eye smoothing, and laxative properties so, they use caper fruits in pickles and curry. Hakeem's in India, suggest using Caper fruit powder mixed with sugar ameliorate rheumatism and diarrhea in livestock animals. Plants of genus Capparis contain spermidine, glucosinolates, alkaloids, phenols, glycosides, and flavonoids, which have various pharmacological properties and anti-inflammatory activities. Polyamine alkaloid called as spermidine, reported in caper species, delays aging in yeast, flies, worms, and human immune cells through the induction of autophagy. Pichiah et al, (2011) suggested that spermidine is used for treating type 2 diabetes. Isocodonocarpine, isolated firstly from *Capparis decidua* found useful against inflammation and asthma. β-Sitosterol showed a significant anti-inflammatory activity, similar to indomethacin, in carageenan-induced rat paw edema. β-Sitosterol showed to inhibit ear inflammation induced by multiple applications of tetradecanoylphorbol-13-acetate in mice. It also inhibited adjuvant-induced rat paw edema by inhibition of cyclooxygenase and 5-lipoxygenase pathways. Phenols, flavonoids, and indoles are reported as bioactive constituents with anti-inflammatory effects in many other plants. Compounds extracted from Capparis species have also shown to be useful for controlling the metabolism of lipids. Alcoholic extracts of bark, flowers, and roots of *Capparis decidua* (Forssk.) Edgew reduced cholesterol, triglycerides, LDL (low-density lipoproteins), and VLDL (very low-density lipoproteins) levels, whereas, *Capparis decidua* (Forssk.) Edgew fruit extract showed beneficial effect on blood sugar levels, glycated hemoglobin levels, and lipid profiles in diabetic and normal male rats. Rahmani et al,, (2013) study concluded that consumption of *Capparis decidua* (Forssk.) Edgew fruits might decrease levels of sugar in blood and improve lipid profile (Sharma et al, 2016).

1.2.4 PHYTOCHEMISTRY

Isocodonocarpine, Spermidine alkaloid, Capparisinine, Capparidisine, Capparine, and capparinine have been isolated from Caper roots. Codonocarpine, capparisine, cadabacine-26-O-d-glucoside, andcapparipine-26-O-d-glucoside have also been isolated from dry root bark of *Capparis decidua*. N-acetylated spermidine alkaloids- 15-N-acetyl

capparisine and 14-N-acetyl isocodonocarpine obtained from the root bark of *Capparis decidua*. It can be considered that roots of Capparis species are rich in spermidine alkaloid compounds and can be used as a natural source for isolation of these polyamine alkaloids for formation of phytomedicines. Spermidine and spermine polyamines exhibit antioxidant and anti-allergenic activities, and suppression on glycation process. Spermidine is a class of multifunctional polyamines, found in some animals and microorganisms. Spermidine and spermine polyamines are essential in the proliferation, growth, and development of mammalian cells. These polyamines exhibit antioxidant and anti-allergenic activities, and suppression on glycation process. Polyamines prevent arteriosclerosis and promote healthy hair growth attributed to their anti-inflammatory properties and cell proliferative properties. β-Sitosterol is a principal phytosterol present in several plant including *Capparis decidua (*Forssk.) Edgew has partial antimicrobial effect through inhibition of cyclooxygenase and 5-lipoxygenasepathways. Flavonoids are known to be the most abundant plant compounds in human diet. Flavonoids are commonly found in cell vacuoles of the outer coloring parts of the flowers, fruits, and leaves and show anti-stress effects in plants. Seemingly, the concentrations of phenolics and flavonoids vary depending on the extraction methods, genetic factors, and climatic/growing conditions of different sites. Baghiani et al, (2012) reported that ethyl acetate extracts of *Capparis decidua* leaves showed higher amounts of phenolic compounds and flavonoids, followed by the chloroform extracts of roots. Mann et al, (2013) also investigated that the content of different compounds in extracts of *Capparis decidua* alters depending on the solvent used. *Capparis decidua* fruits also contain carotene, ascorbic acid, phytic acid and oxalic acid. According to previous studies, water extract from roots of Capparis species exhibited better purgative effect as compared to alcoholic extracts indicating that different extracts can exhibit different pharmacological potential. Oil extract from leaves of *Capparis decidua* contains phenyl propanoid, terpenoids, isothiocyanates, and n-alkalenes. Recently, isothiocyanates have shown as anti-cancer agents. In another investigation, the oil extract of *Capparis decidua* showed presence of thymol, isopropyl isothiocyanates, butyl isothiocyanates, and 2-hexenol. Quaternary ammonium compounds and alkaloids were isolated from *Capparis decidua* leaves (Sharma et al, 2016).

1.2.5 PHARMACOLOGICAL ACTIVITIES: Pharmacological activities of various parts of *Capparis decidua* have been shown in Table 1.

Table 1: Representation of pharmacological activities shown by various plant parts of *Capparis decidua* and phytoconstituents responsible for pharmacological activities.

Plant parts	Pharmacological activity	Phytoconstituents	References
Capparis decidua stems and flowers	Antibacterial, antifungal, antiparasital activity	Quarternary ammonium compounds, Glucosinolate	Upadhyay et al, (2006), Martınez-Carballo et al, (2007)
Capparis decidua root, stem, fruit	Antimicrobial activity, antifungal activity	Phenolic and flavanoid compounds	Sharma and Kumar, (2008), Ravi et al, (2010)
Capparis decidua seeds	Antimicrobial activity	Isothiocyanat-e aglycon	Muthana (1993), Gaind et al, (1972), Juneja et al, (1971)
Capparis decidua leaves	Antioxidant activity	Phenolic compounds, Polyphenols, Tocopherols, Carotenoids	Tepe et al, (2006), Sommer and Davidson, (2002)
Capparis decidua leaves	Antiplaque activity	Volatile oil-Thymol	Rathee et al, (2011), Upadhyay (2013)
Capparis decidua stem	Hepatoprotective activity	Flavonoids, Cyanogenic glycosides, Triterpenes, Vit. C	Ali et al, (2009)
Capparis decidua root bark	Anthelmintic activity	Spermidine alkaloids, Tannins	Ahmad et al, (1992), Dash et al, (2002), Shivkar et al, (2003)
Capparis decidua powdered fruit	Antidiabetic activity	Alkaloids	Yadav et al, (1997), Sharma et al, (2010)
Capparis decidua Fruit and shoot	Antisclerotic activity	Vitamins, Alkaloids, Phenolic compounds	Goyal and Grewal (2003), Purohit and Vyas (2005), Koshy et al, (2001), Anila et al, (2002)

Capparis decidua fruit, flower, bark	Antihyperlipidemic activity	Saponins, Tannins	Goyal and Grewal (2003)
Capparis decidua plant	Antisebum activity	β-sitosterol, Essential fatty acids,Thioglu-cosides	Zaman et al, (2012)
Capparis decidua flowers, stem	Sedative and anticonvulsant activity	Alkaloids	Goyal and Nagori (2009)
Capparis decidua stem	Analgesic and antinociceptive activity	Tannins, Diterpenes, Triterpenes, steroids	Dev et al, (2015)
Capparis decidua stem, root, root bark	Antiinflammatory activity	Isocodonocarpine, β-sitosterol	Perianayagam et al, (2008)
Capparis decidua stem, flower	Anti-termite activity	Heneicosylhexadecanoate, triacontanol, 2-carboxy-1, 1-dimethylpyrrolidine, 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one	Upadhyay et al, (2012)

(a)

(b)

Figure 1: Stem, leaves of *Capparis decidua* plant are shown in (a) and flowers, fruits of *Capparis decidua* are shown in (b).

1.3 *CITRULLUS COLOCYNTHIS*

Citrullus colocynthis (L) Schrad (Indrayan) belongs to the Cucurbitaceae family. The plant is widely available in the Sahara and Arabian deserts, Sudan and Southern part of Asia including Pakistan, India and Southern Islands. The fruit was introduced by the Arabs in the middle ages to Spain and Cyprus (Sharma et al, 2017)

1.3.1 TAXONOMICAL CLASSIFICATION

Classification

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Citrullus

Species: *Citrullus colocynthis*

1.3.2 BOTANICAL DESCRIPTION

Citrullus colocynthis is a perennial herbaceous vine that produces small flowers. The stems are angular, rough and having rough hairs; leaves are alternately arranged on the petioles and rough to touch, 5–10 cm in length, 1.5–2 cm in width, deeply 3–7 lobed; solitary pale yellow blooms. Flowers are yellow and seen on the axils of the leaves. It is monecious, single and pedunculated. Each plant produces 15–30 round fruits, about 7–10 cm in diameter, green with undulate yellow stripes, becoming yellow all over when dry. The fruit of *Citrullus colocynthis* is bitter and globular with smooth texture. It is hard and has a rind around it and contains 200–300 seeds/gourd. Seeds are small (6 mm in length), ovoid, compressed, smooth and brownish when ripe. Seeds contained about 75% of the weight of fruit (Sharma et al, 2017) **(Figure 2)**.

Cucurbitaceae family is one of the best genetically assorted accumulations of restorative plants in the plant kingdom. Most plants of this family are dry season tolerant, intolerant to wet, frost-sensitive and ineffectively drained soils. In the Vedic literature, it is

mentioned that "There is no man on this planet that is inept and there is no plant which has no medical use. Where everything is available, actually, a man to oversee them legitimately is rarely accessible. Practically speaking, a plant is called restorative plant, when it is very used in the system of medicine. Nowadays, therapeutic plants get attention to researchers because of their special significance in safety of humanity. The curative properties of therapeutic plants are predominantly because of the presence of different chemical constituents of various compositions which exists as secondary metabolites. A few dynamic synthetic constituents of *Citrullus colocynthis* plant were surveyed. They are grouped as saponins, carbohydrates, tannins, glycosides, alkaloids, flavonoids and essential oils. Plant-based characteristic constituents can be obtained from any part of the plant like leaves, roots, flowers, stems, fruits, and seeds. Various plant secondary metabolites including flavonoids and cucurbitacins have already been accounted for from *Citrullus colocynthis*. *Citrullus colocynthis* has a wide range of therapeutic and nutritional uses. Traditionally this plant is used in the treatment of diseases like cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases (Sharma et al, 2017)

1.3.3 TRADITIONAL AND THERAPEUTIC USES

Citrullus colocynthis has the conventional use in treatment for cancer, carcinoma, endothelioma, and leukemia, tumors of the liver, spleen, and eye. A decoction of the entire plant, made with the juice of fennel is said to help indurations of the liver. Roots may likewise be utilized as a laxative and for treatment of rheumatism, urinary diseases, jaundice and in snake poison. *Citrullus colocynthis* is broadly utilized as a part of society prescription for quite a long time and as a vitality source too such as oilseed and biofuel. The leaves are diuretic and utilized as a part of the treatment of jaundice and asthma. The root is used as a treatment measure during inflammation of the breasts, rheumatism, joint pains, and amenorrhea and is also used externally in uterine torments and ophthalmia. The fruit is pungent, cooling laxative, antipyretic, anthelmintic and carminative. They are used in cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia's and throat

diseases while fruit pulp acts as antiepileptic, purgative, diuretic and used against gonorrhea (Sharma et al, 2017).

1.3.4 PHYTOCHEMISTRY

A few bioactive compounds of *Citrullus colocynthis* fruit have been elucidated in the studies so far. They are included as carbohydrates, alkaloids, fatty acids, glycosides, flavonoids and essential oils. The cucurbitacins are prevalently found in the Cucurbitaceae family. As per chemical structures, cucurbitacins can be divided into 12 categories, yet all are not found in *Citrullus colocynthis* Because of the cytotoxic conduct, cucurbitacins seem to assume an important part in medicate disclosure, especially in anticancer medication advancement. Among different cucurbitacins, cucurbitacin E was discovered richly in *Citrullus colocynthis* fruit pulp. Colocynthoside A and colocynthoside B, were isolated from the methanolic concentrate of the fruits. Different cucurbitacins secluded from the butanol part were, cucurbitacin L 2-O-β-D-glucopyranoside, hexanocucurbitacin I 2-O-β-D glucopyranoside, cucurbitacin K 2-O-β-D glucopyranoside and khekadaengoside E, cucurbitacin J 2-O-β-D glucopyranoside, cucurbitacin I 2-O-β-D glucopyranoside. Some flavonoid glycosides e.g., isoorientin 30-O-methyl ether, isovitexin & isosaponarin and two cucurbitacin glycosides e.g. 2-O-β-D-glucopyranosyl cucurbitacin L & 2-O-β-D-glucopyranosyl cucurbitacin I were also identified in butanol fraction of the methanolic extract of *Citrullus colocynthis* fruits. The major fatty acids found in *Citrullus colocynthis* seed oil includes palmitic (8.1–17.3%) and stearic acids (6.1–10.5%) that form principal saturated fatty acids of this oil. Linoleic and oleic acids are the primary monounsaturated fats, and this high content of Linoleic acid (50.6–60.1%) in seed oil, which is an essential unsaturated fat, makes this oil restoratively profitable. The unsaturated fat profile of the seed oil uncovers that it falls in the class of Linoleic-oleic acid oils and closely resembles a few other vegetable oils. In this way, the *Citrullus colocynthis* oil, similar to some other cucurbit seed oils, is probably going to have potential uses as cooking oil. Previous studies reported that the seed oil composition of this plant was like that of safflower oil, with an aggregate of 80–85% unsaturated fats. An investigation detailing the physical-compound portrayal and the fatty acid composition of the fixed oil of the seeds revealed that it is a decent wellspring

of characteristic cancer prevention agents like uncovered that it is a decent wellspring of characteristic cancer prevention agents like natural antioxidants e.g., α-tocopherol, γ-tocopherol and β-carotene with respective composition of 45.1, 435 and 0.18 mg/kg. Many examinations revealed the presence of alkaloids in the *Citrullus colocynthis* fruits however just a couple of reports are accessible on the isolation and identification of individual alkaloids. A study was done in 1973 in which choline and two unidentified alkaloids from fruit pulp of *Citrullus colocynthis* were isolated. *Citrullus colocynthis* is a superb wellspring of various amino acids as methionine, arginine, and tryptophan. The biological files its protein quality has been depicted as: "lower than soybean however similar to or higher than generally oilseeds." *Citrullus colocynthis* fruits and seeds possess many vitamins and minerals that play an important role in the diet. The potential of *Citrullus colocynthis* seed as a source of calcium and niacin is encouraging to the low milk-consuming zones of the world. *Citrullus colocynthis* seeds contain ash-2.00g/100g, protein- 13.19 g/100 g, moisture- 4.91 g/100 g, fat-18.59 g/100 g and mineral such as Calcium-569 mg/100 g, Potassium- 465 mg/100 g, Magnesium- 210 mg/100 g, Phosphorous 30.0 mg/100 g, Sodium- 11.9 mg/100 g, Iron- 11.6 mg/100 g, Copper- 5.1 mg/100 g & Zinc- 1.1 mg/100 g (Sharma et al, 2017).

1.3.5 PHARMACOLOGICAL ACTIVITIES

Pharmacological activities have been explicated in **Table 2**.

Table 2: Representation of pharmacological activities shown by various plant parts of *Citrullus colocynthis* and phytoconstituents responsible for biological activity.

S. No	Plant Part	Extract	Pharmacological activity	Possible constituents responsible for activity	References
1.	Fruit	Methanolic extract	Antioxidant activity	Phenolic compounds	Kumar et al, (2008)
2.	Pulp and seeds	Hydro-methanolic extract	Anti-hyperlipidemic effect	Saponins	Zamani et al, (2007)
3.	Fruit	50% Ethyl alcoholic extract	Anti-fertility effects	Decrease in cholesterol levels	Chaturvedi et al, (2003)
4.	Whole Plant	Ethanolic & aqueous extracts	Anti-ulcer activity	Flavonoids, saponins, alkaloids, and tannins	Reddy (2012)
5.	Whole Plant	Hydroalcoholic extract	Anticonvulsant Activity	Flavonoids	Mehrzadi et al, (2016)
6.	Leaf	Aqueous & Methanolic extracts	Antimicrobial effect	Alkaloids, tannins, flavonoids	Gurudeeban et al, (2016)
7.	Fruit	Hydroalcoholic extract	Antifungal activity	Glycosides and resins, colocynthin and colocynthin alkaloids	Eidi et al, (2015)
8.	Fruits & leaves	Ethanolic extract	Antibacterial activity	Alkaloids, flavonoids, and glycosides	Najafi et al, (2010)
9.	Whole Plant	Butanolic extract	Insecticidal activity	Cucurbitacin E Glycoside	Torkey et al, (2009)
10.	Stems, roots, leaves & maturation stages of	Aqueous and diluted acetone	Antibacterial and anticandidal activity	Tannins, steroids, pigments and flavonoids, alkaloids,	Marzouk et al, (2009)

	its seeds & fruit			iridoids	
11.	Roots	Aqueous extract	Hypoglycaemic activity	Glycosides (saponins glycosides), triterpenoids, alkaloids, flavonoids, and resins	Agarwal et al, (2012)
12.	Rind of fruits	Aqueous extract	Anti-hyperglycemic effect	Saponins, glycosides	Abdel-Hasan (2000)
13.	Fruits	Petroleum ether fruits extract	Antidiabetic effect	Saponins, flavonoids, and glycosides	Jayaram et al, (2009)
14.	Fruits	Aqueous extract	Analgesic effect	Alkaloids, iridoids, flavonoids, steroids	Marzouk et al, (2013)

(a)

(b)

Figure 2: Complete Fruits and leaves of *Citrullus colocynthis* shown in (a) and transverse section of fruits showing seeds inside in (b).

1.4 PATHOPHYSIOLOGY OF DISEASES

Diarrhoea

Wound Healing

Erectile Dysfunction

Arthritis

1.4.1 PATHOPHYSIOLOGY OF DIARRHEA

The looseness of the bowels refers to expansion in the amount of stool or number of times of defecation. It is a standout amongst the most well-known clinical indications of gastrointestinal infection, yet additionally shows essential issue apart from stomach related framework. Absolutely, scatters influencing either the little or substantial gut can prompt looseness of the bowels. For some individuals, looseness of the bowels speaks to an infrequent burden or inconvenience, yet no less than 2 million individuals on the planet, for the most part, incredible the results of the runs every year. There are various reasons for looseness of the bowels, yet in all cases, this issue is an appearance of one of the four essential instruments portrayed beneath. It is likewise basic for more than one of the four components to be associated with the pathogenesis of a given case (Whyte and Jenkins 2012). Summarized information is available on **Figure 3** and **Table 3** regarding the pathophysiology and causes of diarrhea respectively.

Osmotic diarrhea:

Ingestion of water in the gastrointestinal tract is dependent on attractive maintenance of solutes. On the off chance that exorbitant measures of solutes are held in the intestinal lumen, water won't be consumed and looseness of the bowels will come about. Osmotic loose bowels ordinarily come about because of following circumstances:
Intake of an inadequately assimilated substrate: The culpable particle is normally a sugar or divalent particle. Regular illustrations incorporate mannitol or sorbitol, epson salt and a few stomach settling agents like magnesium hydroxide.

Inability to ingest certain starches is the most broadly perceived deficiency in this order of detachment of the entrails; in any case it can occur essentially any kind of malabsorption. An average instance of malabsorption, tormenting various adults' individuals and pets is lactose fanaticism coming to fruition due to a need in the brush periphery compound lactase. In such cases, an immediate measure of lactose is eaten up (as a general rule as deplete), however the intestinal epithelium is deficient in lactase, and lactose can't be effectively hydrolyzed into glucose and galactose for maintenance. The osmotically-dynamic lactose is held in the intestinal lumen, where it "holds" water. To make an officially troublesome circumstance far and away more terrible, the unabsorbed lactose goes into the interior organ where it is developed by colonic microorganisms, achieving making of unreasonable gas. A recognizing highlight of osmotic looseness of the bowels is that it stops after the patient is fasted or quits expending the ineffectively retained solute.

Secretory diarrhea:

Huge amount of water are ordinarily emitted into the little intestinal space, yet a huge lion's share of this water is efficiently retained before achieving the digestive organ. The diarrhea happens when emission of water into the intestinal lumen surpasses ingestion. A large number of individuals have passed on of the secretary looseness of the bowels related with cholera. The dependable life form, Vibrio cholerae, induces cholera poison that unequivocally actuates adenylyl cyclase, causing a drawn out increment in intracellular centralization of cyclic AMP inside grave enterocytes. It brings about deferred opening of the chloride coordinates that are instrumental in release of water from the catacombs, allowing uncontrolled outflow of water. Additionally, cholera harm impacts the enteric tangible framework, achieving a free shock of release. Introduction to poisons from a few different sorts of microscopic organisms (e.g. E. coli warm labile poison) incites a similar arrangement of steps and gigantic Secretory looseness of the bowels that is frequently deadly unless the individual or creature is forcefully treated to look after hydration. Notwithstanding bacterial poisons, an extensive number of different operators can actuate Secretory looseness of the bowels by turning on the intestinal Secretory pathways:

•Few purgatives

•Hormones discharged by specific sorts of aggressive cells (e.g. vasoactive intestinal peptide)

•Some medications (e.g. asthmatic drugs, anti-depressants, cardiovascular medications)

•Metals, natural poisons, and plant parts (Whyte and Jenkins 2012)

Inflammatory and infectious diarrhea:

The stomach epithelium is protected from attack by different parts constituting the gastrointestinal check, however like numerous hindrances, it can be ruptured. Interruption of the epithelium of the digestive system because of microbial or viral pathogens is an extremely basic reason for the runs in all organisms. Destruction of the epithelium occurs not simply in exudation of serum and blood into the lumen yet frequently is connected with in all cases pummeling of absorptive epithelium. In such cases, maintenance of water happens inefficiently and detachment of the guts comes to fruition. Instances of pathogens once in a while associated with compelling free entrails incorporate microorganism like salmonella, campylobacter, rotaviruses, parvovirus's, coccidia species, giardia etc.

The safe response to provocative conditions in the entrails contributes substantively to change of the runs. Start of white platelets drives them to release blazing center individuals and cytokines which can empower outflow, thus constraining a secretory portion over a flammable detachment of the entrails. Open oxygen species from leukocytes can hurt or execute intestinal epithelial cells, which are supplanted with young cells that generally are lacking in the brush periphery enzymes and transporters basic for maintenance of supplements and water. Thusly, parts of an osmotic (malabsorption) the runs are added to the issue (Whyte and Jenkins 2012)

Abnormal mucosal permeability:

In health tight junctions between enterocytes closely regulates movement of solutes across the mucosa. Inflammatory, infiltrative or ulcerative diseases or portal hypertension

can disrupt mucosal integrity resulting in leakage of fluid into the intestinal lumen. In severe cases protein will be lost resulting in protein losing enteropathy (Whyte and Jenkins 2012).

Diarrhea associated with deranged motility:

All together if supplements and water need to get productively consumed, the bowel substance should be sufficiently presented to the epithelium and held sufficiently for longer duration to permit assimilation. Clutters in gastrointestinal movement than quicken travel time might diminish assimilation, bringing about looseness of the bowels regardless of whether the absorptive procedure in essence was continuing appropriately. Adjustments in intestinal motility (normally expanded impetus) are seen in numerous kinds of looseness of the bowels. What isn't usually clear, and exceptionally hard to illustrate, is whether essential adjustments in motility are really the reason for the runs or just an impact (Whyte and Jenkins 2012).

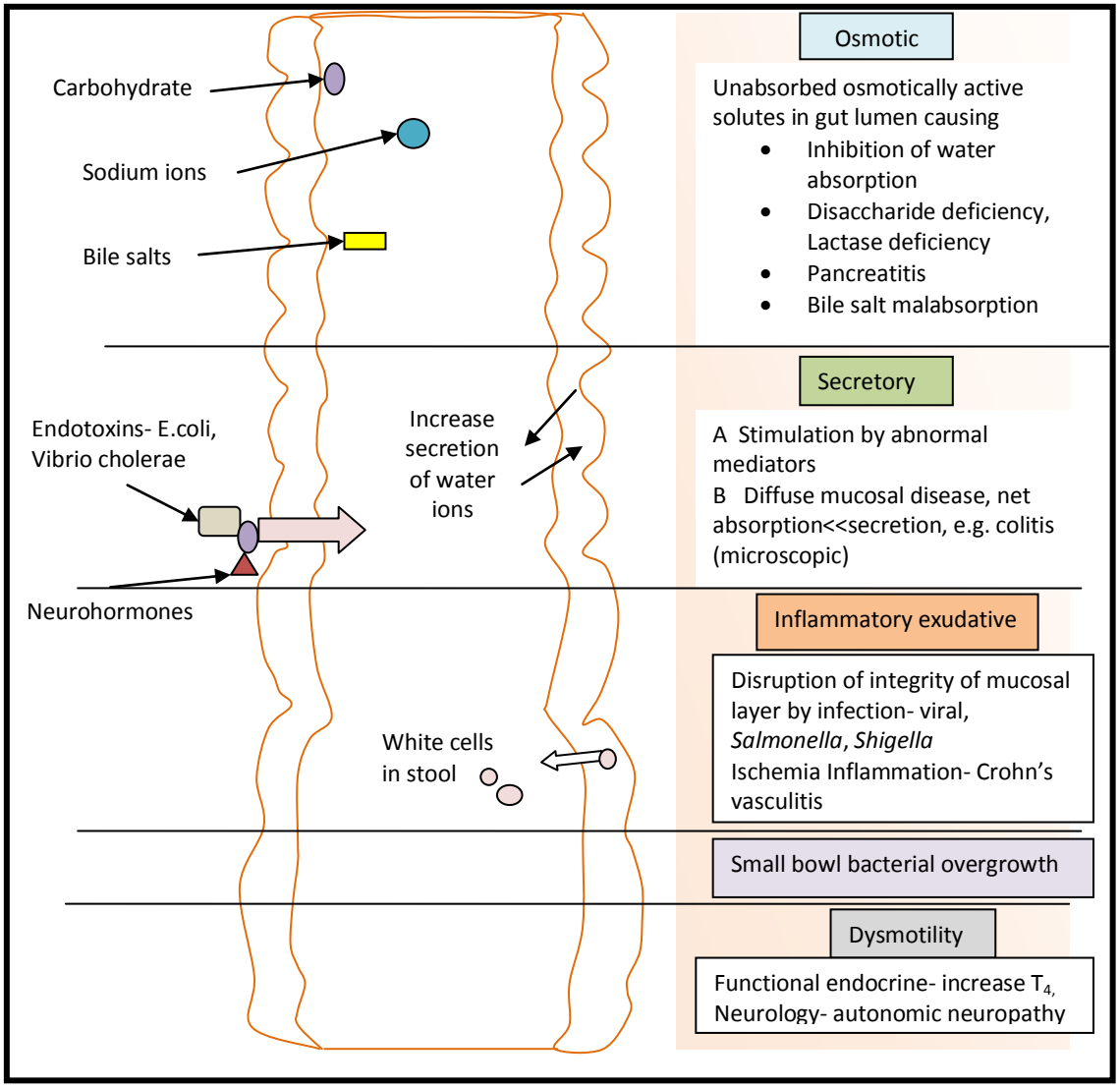


Figure 3: Representation of different factors relating to pathophysiology of diarrhea.

Table 3: Representation of various causes of diarrhea

Different causes of diarrhea	
Osmotic	Sudden change in diet
	Maldigestion
	Overeating
	Malabsorption
	Scavenging
Secretary	Unconjugated bile acids from bacterial fermentation
	Pathogenic bacteria toxins e.g. toxins produced by Clostridium perfringens, Salmonella typhimurium
	Giardiasis
	Hydroxylated fatty acids form bacterial fermentation
Abnormal mucus permeability	Inflammation
	Gastrointestinal ulceration
	Portal hypertension
	Lymphangiectasia
	Infiltration (neoplasia)
Abnormal motility	Scavenging
	Sudden change in diet
	Irritable bowel syndrome
	Feline hyperthyroidism
	Secondary motility disorders - inflammation, ischemia, infection, anticholinergic drugs

1.4.2 PATHOPHYSIOLOGY OF WOUND HEALING

An injury is a disruption in the outer membrane of body and joined by disturbance in the organization and capacity in basic ordinary tissue. An injury occurs because of exact disturbance of tissue by the specialist's blade (cut) to broad harm of tissue (e.g. significant injury, consumes). An injury may likewise come about because of a wound, hematoma, gash or a scraped spot. The coherence of the skin must be reestablished speedily on the grounds that it assumes a urgent part in looking after homeostasis. The recuperating of intense injuries includes an unpredictable, dynamic, all around coordinated arrangement of occasions.

Different periods of recuperating are condensed in **Figure 4** and part of different cells in wound mending is compressed in **Figure 5** (Stuart and David 2005).

Healing of acute wounds:

Mending of intense injuries (as found in essential recuperating) happens as a painstakingly controlled, foundational course of covering forms that require the organized finishing of an assortment of cell exercises, including mitogenesis, chemotaxis, phagocytosis and amalgamation of parts of the extracellular network. This action happen in a timeframe that connects with presence of various cells composes in the injury amid different phases of the mending procedure. These procedures (activated by tissue damage) include four covering (however all around characterized) periods of haemostasis, irritation, expansion, and redesigning and scar development (Stuart and David 2005).

Haemostasis:

Tissue harm is portrayed by micro vascular harm and extravasations of blood into the injury. Loss of auxiliary uprightness starts the coagulation course and choking of vessel dividers; the subsequent coagulation development and platelet conglomeration constrains additionally blood misfortune. The platelets caught in the coagulation are basic for haemostasis and a typical incendiary reaction. The platelets degranulate and discharge their alpha granules, which discharge a couple of improvement factors, including: platelet-determined development factor, insulin-like development factor-1, epidermal

development factor, changing development factor-β, platelet factor-IV. These proteins start the injury mending course by pulling in and initiating fibroblasts, endothelial cells and macrophages. These occasions additionally enact four noteworthy intensification frameworks (supplement course, coagulating component, kinin course, plasmin age), which add to haemostasis and the ensuing phases of the recuperating procedure (Leaper and Harding 1998)

Early and late phage inflammatory phases:

Early provocative stage (days 1–2): inflammation starts with the actuation of the traditional and option pathways of the supplement course. This prompts invasion of the injury with neutrophil granulocytes that are pulled in to the injury site inside 24– 48 hours of damage by various agents like parts of extracellular structure protein, changing development factors, supplement segments peptide items from microorganisms.

Inside a brief span apportioning, the leukocytes start to hold fast to the endothelial cells in the nearby veins (margination) and begin to reasonably experience the vessel divider (diapedesis). Once in the harm condition, they phagocytose little animals and other outside particles, and butcher them by discharging tainting proteins and free radicals got from oxygen. Amidst this period, basal cells at the cut edge of the epidermis start to show broadened mitotic advancement. Inside 24– 48 hours, epithelial cells from the two edges start to move and copy along the dermis, keeping pieces of storm cellar layer as they advance. The movement of polymorphonuclear leukocytes as a rule stops inside a few long stretches of hurting (after the dirtying minute living things have been cleared). Repetitive cells are cleared from the harm by launch to the harm surface as swamp or by phagocytosis by macrophages. The fundamental furthest reaches of polymorphonuclear leukocytes is to limit bacterial debasing of the harm, along these lines avoiding contamination, and they add little to the average procedure of twisted recouping past this stage.

Late provocative stage - on meeting up at the damage site, blood monocytes encounter a phenotypic switch to twist up tissue macrophages. Monocytes are pulled in to the damage by an arrangement of chemo attractants, like clotting factors, Ig G, subparts of elastin, complementary system, and cytokines.

Macrophages are the most basic cells show in the later (48– 72 hours) periods of the searing method and appear to go about as the key authoritative cells for repair. They fill in as phagocytic cells and furthermore being the fundamental creator of advancement factors responsible for the rise in smooth muscle cells, increase generation of the extracellular matrix and increased endothelial cells causing healing.
In like manner, macrophages release proteolytic proteins (e.g. collagenase) that help to debride the damage. In the occasion that depleted, surrounding monocytes and tissue macrophages cause genuine changes in wound repairing, provoking poor debridement of the damage, conceded development of fibroblasts, lacking angiogenesis, and poor fibrosis (Leaper and Harding 1998).

The proliferative stage begins on 3rd day and goes on for 2 month subsequent to injuring and is portrayed by fibroblast movement, testimony of the outer lattice and formation of granulation cells. During proliferative stage, the temporary fibrin grid is supplanted by the recently framed granulation cells (Leaper and Harding 1998).

Fibroblasts show up in the injury 2– 4 days subsequent to injuring and endothelial cells take after around multi day later. Following damage, fibroblasts are pulled in to the injury by various components, including platelet-determined development factor and changing development factor-β. Once inside the injury, fibroblasts multiply and deliver the framework proteins fibronectin, hyaluronan and, later, collagen and proteoglycan (Stuart and David 2005).

Formation of the extracellular matrix:

Other than giving turgid to delicate tissues and inflexibility to bone, extracellular lattice supplies a substratum for cell bond and basically directs the development, development and separation of the phones inside it. The extracellular network comprises of sinewy basic proteins and an interstitial framework made out of cement glycoprotein implanted in glycosaminoglycan gel and proteoglycan (Leaper and Harding 1998).

Collagens:

These are incorporated by fibroblasts acting as bottomless group of proteins found in our body. They give quality and trustworthiness to all cells thus assumes an indispensable part in wound healing. Platelet cell-determined development factor, fundamental fibroblast development factor, changing development factor-β, interleukin-1, and tumor rot factor actuate collagen combination amid the differentiating and redesigning stages. These are composed of 3 protein α-chains interlaced into a rope-like triple helix; the individual chains can entwine firmly in light of the fact that each α polypeptide has one glycine particle at each third position. Progressively that 30 particular α-chains frame around 18 distinctive collagen writes (some of which might be one of a kind to particular cells and tissues). Some collagen writes (e.g. I, III, V) frame fibrils because of sidelong cross linking of the triple helices, and shape the vast majority of the connective tissue in mending wounds; different collagens (e.g. type IV) are non-fibrillar and progressed toward becoming segments of the cellar layer (Leaper and Harding 1998).

Glue glycoprotein's are fundamentally different proteins that connection the segments of the extracellular grid to each other and to cells, and incorporate fibronectin, laminin, and thrombospodin (Leaper and Harding 1998).

Proteoglycan comprise of glycosaminoglycan connected to a protein spine; they help to control the structure and porousness of the extracellular network. Proteoglycan can balance the development and separation of cells. Glycosaminoglycan without a protein center is imperative constituents of the extracellular grid (Stuart and David 2005).

Formation of granulation tissue within three to five days, granulation tissue (demonstrative of ideal recuperating) is settled. Granulation cells show pink, delicate, granular structure, (for example, that seen underneath the scab of a skin wound). It is signified by multiplying fibroblasts and circles of vessels in a free extracellular framework. This stage is portrayed by angiogenesis or arrangement of fresh recruit's vessels from previous vessels at the site of damage.

The new vessels are swelled due to inadequately confined inter-endothelial crossing points and extended cytosis. A couple of factors (checking endothelial improvement factors, platelet induced advancement factors, basic fibroblast advancement factors and

changing advancement factor-β) activate healing. Vascular hair like sprouts assaults the fibrin-rich damage bunch and inside two or three days orchestrate into a micro vascular sort out all through the granulating cells. The thickness of veins diminishes since collagen totals in the granulating cells to make scar; disrupting impact of this active system may affect the change of wearisome wounds (Leaper and Harding 1998).

Epithelialization: inside just after damage, a lone layer of epidermal tissue migrates from the damage edges to shape a delicate covering over the revealed unrefined zone, a strategy known as 'epiboly'. From around 12 hours, there is a checked augmentation in mitotic development inside the basal epithelial cells of the damage edges. These cells move as a sheet, widening lamellipodia along the movement edge. A short time later, they discharge their normally firm associations with the central dermis, empowering them to move in a 'hop' frame over the transitory system. While advancing epithelial cells meet, propel improvement is finished by 'contact limitation' and another tempest basement layer recuperates; help advancement and partition of epithelial cell re-develops the stratified epithelium. Epithelialization requires a soaked space, acceptable sustenance and bacteriological control, and is changed by a couple of advancement factors, including keratinocyte improvement factor, epidermal improvement factor, and fundamental fibroblast improvement factor (Leaper and Harding 1998).

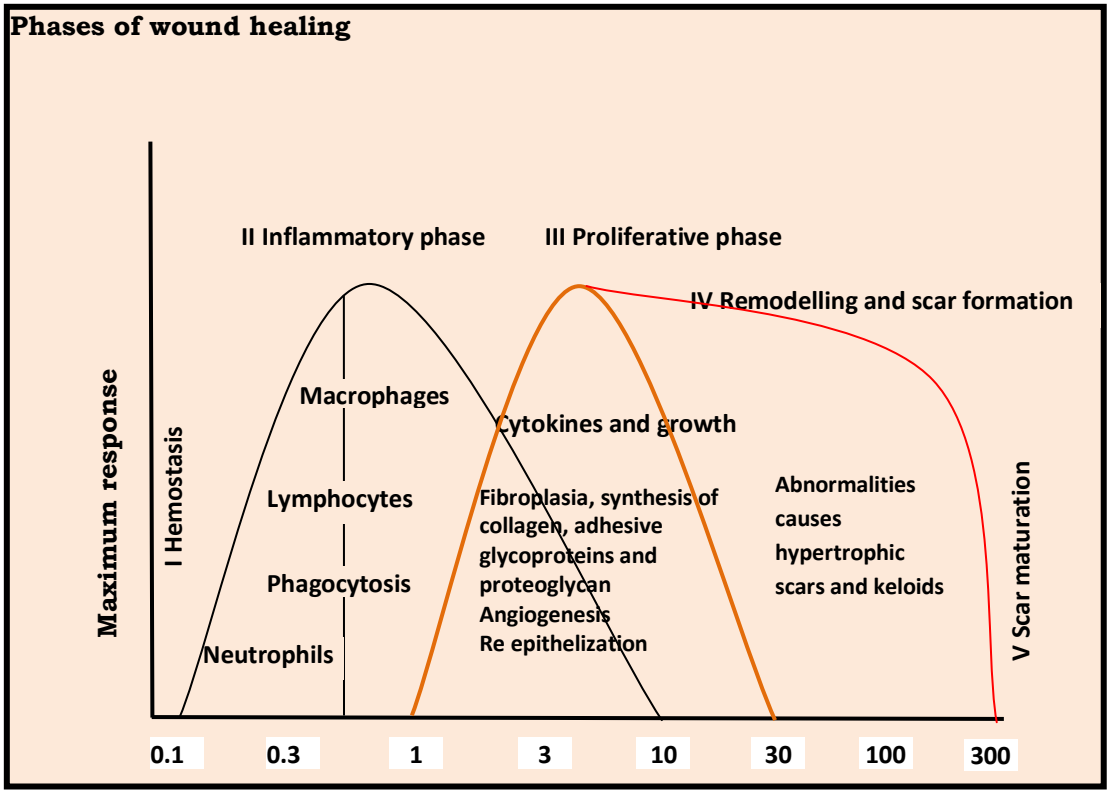


Figure 4: Various phases of wound healing

Cells involved in wound healing	
Cell type	Function related to wound healing
Platelets	Involved in thrombus formation
	α granules are rich source of inflammatory mediators including cytokines (e.g. TGF-β, PDGF, β-thromboglobulin, platelet factor-4)
	Major initial stimulus for inflammation
Neutrophils	First cells to infiltrate site of injury
	Phagocytosis and intracellular killing of invading bacteria
Monocytes (macrophages)	Phagocytose and destroy invading bacteria
	Clear debris and necrotic tissue
	Rich source of inflammatory mediators including cytokines
	Stimulate fibroblast division, collagen synthesis and angiogenesis.
Lymphocytes	May produce cytokines in certain types of wound
Fibroblast	Produce various components of ECM, including collagen, fibronectin. hyaluronic acid, proteoglycan
	Synthesize granulation tissue
	Help to reorganize the provisional ECM
Key words- TGF: Transforming growth factor; PGDF: Platelet derived growth factor; ECM: Extracellular matrix	

Figure 5: Representation of role of various cell types in wound healing.

1.4.3 PATHOPHYSIOLOGY OF ERECTILE DYSFUNCTION

The way toward accomplishing penile erection includes the reconciliation of mental, neurological, and vascular procedures, which join to start a physiologic reaction inside the penile vasculature. Endothelial intervened expansion of arteriolar smooth muscle brings about expanded blood stream into the sinusoids of the corpora cavernosum and resulting filling while all the while unwinding to build consistence. This filling blocks venous outpouring from the penis by pressure of the veins against the tunica albuginea, bringing about penile erection. Erectile brokenness is characterized as a trouble in starting or keeping up penile erection satisfactory for sexual relations. Diagrammatic portrayal of erectile brokenness in various illnesses is depicted in **Figure 6** (Seidman et al, 2002).

Factors of erectile dysfunction include psychogenic, iatrogenic, vascular, endocrine, cellular and neural.

Physiology of penis erection:

Boosts that prompt erection incorporate material jolts to penis which make a reflex erection, and paying little mind to whether visual, sound-related, olfactory or imaginative moreover convey erection by a framework which incorporates the paraventricular center and normal preoptic zone of the hypothalamus. A 3rd framework is locked in with the age of evening erections that occur in all men in the midst of REM rest. While the right central system related with such strategies incorporates undefined, dropping pathways from the hypothalamus finally incite extended parasympathetic and reduced attentive neural activity inside the penis. The parasympathetic nerves discharge various neurotransmitters inside the penis, and most vital is nitric oxide (NO). In any case, the neural landing of NO is bolstered by release from the endothelium and causes loosening up of the smooth muscle in the penile veins and in the versatile tissue of the corpora cavernosal. In this manner, there is vein dilatation, cavernosum loosening up and extended drainage of blood inside the trabeculum of cavernosum. As a result, the veins are pressed against the tunica albuginea, provoking diminished venous surge, and this gathered 'veno-occlusive' part realizes the unyielding penile erection (Seidman et al, 2002).

Psychogenic erectile dysfunction:

Psychogenic problems are associated with men suffering with erectile dysfunction, regardless of whether we now realize that in the larger part of men the prevailing pathophysiological forms are natural. Such is the central importance of penile erection to the male personality, that even the most minor regular breakdown can realize mental results, which can progress to implied 'execution related uneasiness'. All things considered, an arrangement of components can add to psychogenic ED, and these can be beneficially isolated into three get-togethers, slanting, empowering and taking care of factors. One of the clinical features that prescribe a basic psychogenic portion in a man's ED is the proximity of normal evening time erections. If they are accessible and common, by then it is likely that the neural, vascular and endocrine instruments that are trademark

for run of the mill penile erection are generally set up, and there is a staggering psychogenic portion to the pathophysiology of the ED (Feldman et al, 1994).

Neurogenic erectile dysfunction:

A few mental conditions, for example, misery, can bring about ED, and are considered as natural causes rather than psychological. Actually connection amongst sadness and ED is a mind boggling one. Melancholy can unquestionably bring about ED, yet mellow gloom can likewise come about because of ED, to such an extent that it has turned out to be certain that ED treatment in men with gentle despondency enhances the depressive indications and additionally the ED. Spinal line damage influences erectile capacity, however the photo relies on the level and degree of the damage. Extensively, men with high sores can in any case get reflex erections (by means of in place reflex pathways) while men with low string injuries can in some cases keep on getting psychogenic erections (Johannes et al, 2000).

Endocrine mechanisms in erectile dysfunction:

Testosterone is vital to ordinary male sexual limit having a basic part in both penile erection and sexual drives. Regardless, a reduced level of testosterone has variable effects upon sexual limit. Men with hypogonads don't generally lose reflex and psychogenic erections. They act, be that as it may, have lessened nighttime erectile movement, with both diminished span and unbending nature of the erection. Once more, there are an expansive number of reasons for lessened blood testosterone level, yet these are extensively arranged into essential hypogonadism, where the essentially it influences the hypothalamus or pituitary gland. A remarkable endocrine explanation behind ED is a prolactin producing tumor of the pituitary. The showing reactions are normally ED, galactorrhea and gynaecomastia. The serum testosterone is by and large diminished and the finding is made by CT checking of the pituitary (Braun et al, 2000).

Vasculogenic erectile dysfunction:

Vascular ailment is probably the most broadly perceived explanation behind ED, and of all the vascular causes, the commonest is atherosclerosis. Regardless, is atherosclerosis related with ED, and its danger factors, to be particular smoking, hypertension,

hyperlipidemic and diabetes, are also associated with the change of ED. At a cell level, it has been prescribed that a reduced vein inflow prompts relative hypoxia inside the penis with resulting cell impacts. The critical cell middle person gives off an impression of being Transforming Growth Factor Beta 1, which rises in hypoxia and incites transition in the cavernosum smooth muscle. Exactly when there is frustration of the veno-occlusive instrument, the ponder of venous spillage happens. This is an essentially radiological ponder seen in the midst of particular radiological imaging of the penis (cavernosography). Right when at first portrayed, it was envisioned that the irregular veins addressed the fundamental over the top variety from the standard, and for a couple of years surgical ligation of these veins was held onto as a strategies for treating ED (Parazzini et al, 2000).

Cellular factors of ED:

Two sorts of cavernosal cells are fundamental to penile erection; to be particular smooth muscle cells and endothelial cells. The vascular endothelial cells line the trabecular spaces of the cavernosal sinusoids and release a grouping of vasoactive compounds which manages smooth muscle tone within penis. The most incredulous of these is Nitric oxide. Ailments which hurt the endothelium ruin the vascular response of the penis to neural shocks. Different diseases hurt the endothelium, including hyper-cholesterolaemia, however the most basic is diabetes type II. The helper changes in the endothelium that are conveyed by diabetes are joined by utilitarian changes that result in upset smooth muscle loosening up. The ailments of smooth muscle that can achieve erectile brokenness have quite recently been suggested already. Developing realizes decreased penile smooth muscle, as doe's atherosclerosis, while renal dissatisfaction achieves smooth muscle brokenness. Right when the smooth muscle breakdowns, vein dilatation is lacking, cavernosal loosening up fails to happen and the veno-occlusive framework misses the mark (deTejada et al, 1989; Cartledge et al, 2001).

Iatrogenic erectile dysfunction:

Countless drugs can weaken sexual capacity, either by an impact upon erectile capacity, ejaculatory capacity or sex drive. Utilization of these medications seldom delivers ED all alone, their activity as a rule being a subordinate to another pathophysiological system.

Surgery and radiotherapy can likewise disable erectile function. The commonest surgical reason for ED is radical pelvic surgery for rectal tumor, bladder growth or prostate disease. The parasympathetic nerves that sub serve penile erection run nearby the prostate and are frequently harmed amid such radical surgery (Walsh and Donker 1982).

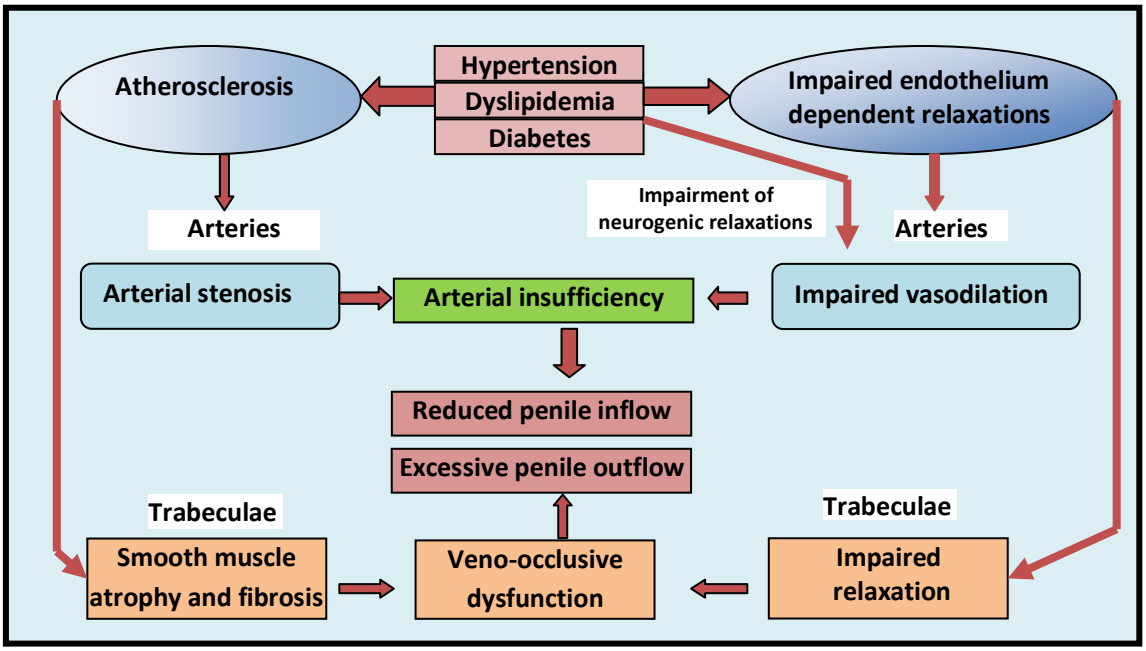


Figure 6: Representation of pathophysiology of erectile dysfunction.

1.4.4 PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

According to Scheel et al, (2011) in RA, naive and memory B cells infiltrate and accumulate in synovial tissue, in which there appears to be continuous activation of selected B cell clones with a high migratory capacity. Flow cytometry analysis has shown that peripheral B cells from RA patients have an altered expression of key molecules, with high CD86 (co stimulatory molecule) and low FcgRIIb (inhibitory receptor for IgG immune complexes – required for feedback inhibition) levels. Articulation was diminished and expanded individually by TNF hindrance, proposing that CD86 and FcgRIIb might be deregulated by aggravation, conceivably adding to resilience breakdown and advancement of humoral auto-insusceptibility (Catalan et al, 2010).

Catalan et al, (2010) described that FOXO3a is a transcription factor implicated in cell cycle regulation and survival. In microarray experiments, FOXO3a mRNA was increased in RA patient blood, primarily in polymorphonuclear cells. FOXO3a over expression lead to the prolonged survival of these cell types in RA, thereby contributing to chronic inflammation. According to Azuma et al, (2009) and Hsu et al, (2009) high levels of sH4 (soluble B7–H4) in majorly found in RA patient sera. B7–H4 is a cell surface inhibitory molecule of the B7–CD28 signaling pathway, and mouse models hint that sH4 exacerbates arthritis by competing with the cell-surface molecule. ZAP-70 is an important molecule in the T cell receptor (TCR) signaling pathway, whose functions include recruitment of downstream effector molecules. In a progression of rich transgenic tests, transformation of two key platform deposits brought about decreased T cell advancement and impeded reaction to TCR incitement, disturbed positive and negative thymic determination and lessened quantities of administrative T cells. Administrative T cells assume a vital part in the upkeep of invulnerable resilience. Following TCR flagging administrative T cells (Tregs) enroll less protein kinase C theta (PKCu) to the invulnerable neural connection than effector T cells, with less downstream enactment of NF-kB. In fact, PKCu acts as a negative regulator of Treg function, and TNFα increases its localization to the immune synapse, consistent with the inhibitory effect of TNF α on Treg function in RA. Furthermore, inhibition of PKCu enhances Treg function and

prevents inhibition by TNF α. The PKCu pathway could therefore provide an important target in diseases characterized by immune deregulation (Zanin-Zhorov et al, 2010).

As per Schmutz et al, (2010) monocytes and macrophages are enter players in RA pathogenesis, discharging master incendiary cytokines such as TNF α, IL-1 and IL-6. In RA, articulation of chemokine receptor CCR9, fundamental for the movement to, and maintenance of leukocytes in Chen et al, (2010), RA joints was expanded in the CD14þ populace of fringe monocytes and synovial macrophages (Church et al, 2010). CCL25 (CCR9 ligand) was discovered colocalised with macrophages in the synovium of both RA patients and controls. In RA synovial liquid osteopontin (an extracellular grid protein) levels corresponded essentially with IL-17 generation and the recurrence of Th17 cells and, in vitro, osteopontin impacts Th17 T cell separation. Th17 T cells and their 'mark' cytokine item, IL-17, are progressively implicated in RA irritation and joint pulverization. Synovial liquid mononuclear cells had diminished IL-22 levels (a Th17 and Th1 determined effector cytokine) and don't express IL-23R, which is essential for Th17 extension and survival. At the point when contrasted and fringe blood and synovium, there was an unobtrusive improvement of IL-17-creating CD4 cells in synovial liquid however the general extent of these phones was low, recommending there might be an elective wellspring of intra-articular IL-17 (Huebe et al, 2010).

Nistala et al, (2010) reported that in kids with joint inflammation the dominant part of synovial liquid IL-17-emitting cells have a phenotype amongst Th17 and Th1, for instance communicating both T-wager and RORC2 (Th17/Th1). Moreover, synovial liquid Th17/Th1 and Th1 cells communicated CD161, for the most part a marker of Th17 cells, and imparted TCR clonality to Th17 cells. In vitro, an ace fiery milieu containing low TGFb and high IL-12 advanced separation of Th17 cells into Th17/Th1 cells. In this way the Th17 phenotype might be unsteady, with the possibility to change over to both Th1 and Th17/Th1 cells in light of aggravation. IL-17 isn't the main professional incendiary cytokine that might be created from capricious sources. Platelet microparticles (MP) have been distinguished in incendiary joint pain synovial liquid (counting RA), in which they may stick to leukocyte surfaces. In the K/BxN serum exchange model of fiery joint inflammation, platelet exhaustion altogether repressed improvement of joint pain. A progression of exquisite tests thusly proposed that glycoprotein VI, a collagen receptor

communicated by platelets, is the system whereby fibroblast-like synoviocytes (FLSs) and extracellular lattice trigger microparticle age from platelets. Microparticles communicated layer bound IL-1a and IL-1b, which animated FLSs to discharge ace fiery IL-6 and IL-8 (Boilard et al, 2010).

VanEijk et al, (2010) reported that microparticles are likewise activators of the supplement course, a capacity that was unaffected by calming treatment. Hashimoto et al, (2010) described that brokenness of supplement pathways is related with incendiary issue and, in auto-safe inclined SKG mice, supplement actuation by b-glucan evoked a Th17-interceded incessant auto-insusceptible joint inflammation. Conversely, coordinate restraint of the elective supplement pathway utilizing properdin hindered murine joint pain advancement and tissue harm (Kimura et al, 2010).

Joyce et al, (2010) and Charpin et al, (2010) reported that other pathways and mediators remain to be fully explored, such as myeloid DAP12-associating lectin (MDL)-1. By recruiting substantial numbers of bone marrow-derived inflammatory macrophages and neutrophil and promoting osteoclast activation, MDL-1 can promote synovial inflammation and bone erosion in vitro. Pathways involved in inflammation and destruction in the rheumatoid joint has been shown in **Figure 7.**

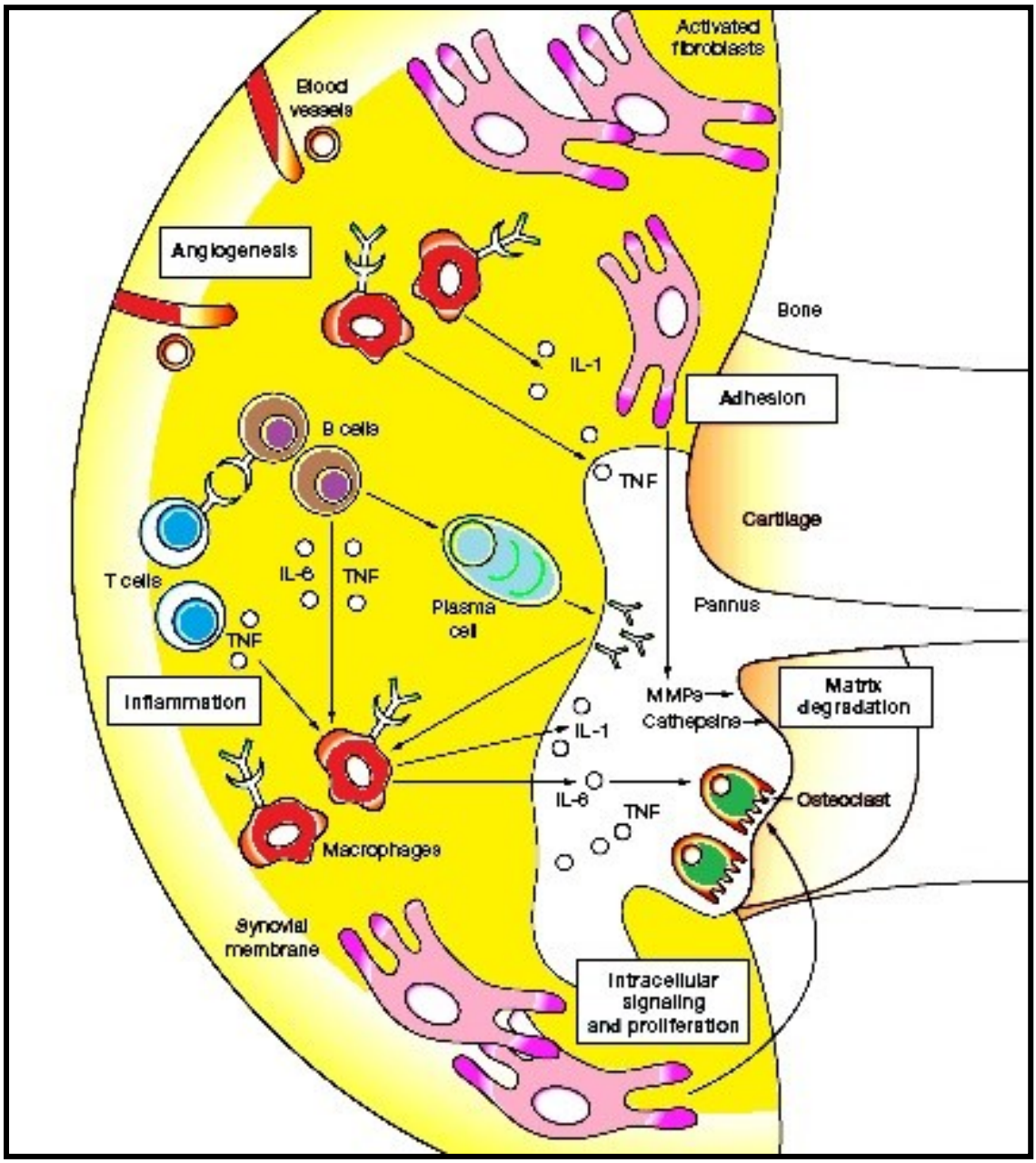


Figure 7: Pathways associated with irritation and obliteration in the rheumatoid joint.

Chapter 2

Literature Review

Rai et al, (2018) evaluated the aphrodisiac potential and reproductive safety profile of water extract of *Tamarindus indica* in male Wistar rats. The fluid concentrate was set up by maceration of mash took after by decrease of volume in rotavapor under warmth took after by solidify drying. The readied remove was portrayed for substance of aggregate phenol, flavonoid, and saponins. It was additionally subjected to phytoconstituent examination utilizing GCMS. Further, the concentrate was assessed for intense harmfulness consider. The sexual enhancer and conceptive poisonous quality potential were assessed in creatures in the wake of collection them in four with six creatures each to be specific, ordinary control, standard (Sildenafil citrate, 4 mg/kg p.o.) and concentrate of *Tamarindus indica* treated groups in 2 measurements doses, 125 and 250 mg/ kg orally. The examination was directed for 54 days with day by day once dosing of concentrate and standard. Level with number of females was gathered without treatment for assessment of parameters of sexual want (mount recurrence and intromission recurrence) and parameters of sexual excitement (mount inertness and intromission idleness). These parameters were assessed on day 14, 28, 42 and 54. Creatures were relinquished on day 54, testicles were evacuated and contemplated for histopathological changes. The concentrate indicated 6.6 mg Gallic corrosive identical/g of aggregate phenol, 2.3 mg catechin proportionate/g of flavonoid and 11.6% saponins. Forty synthetic constituents were recognized by GCMS examination.

Biswas et al, (2017) aim of the study was scientific validation of the plant for wound healing activity in detail. Watery concentrate of the plant was readied and phytochemical constituents were distinguished by HPLC examination. Intense and dermatological poisonous quality investigation of the concentrate was performed. Pharmacological testing of 15% treatment (w/w) of the concentrate regarding fake treatment control and standard comparator framycetin were done on full thickness punch twisted in Wistar rats and impacts were assessed in view of parameters like injury compression estimate (mm^2), elasticity (g) tissue DNA, RNA, protein, hydroxyproline and histological examination.

The salve was connected on chosen clinical instances of interminable and entangled injuries and adequacy was assessed on premise of scoring on granulation, epithelialization, vascularity and additionally routine hematological examinations.

Chokpaisarn et al, (2017) the study *w*as focused on utilization of *Quercus infectoria* (Qi) as a topical agent for chronic wound treatment. Twenty Qi plans (QiFs) were pharmaceutically planned and antibacterial action of all details was performed. The best definition in view of an antibacterial movement was chosen for assessment of wound recuperating property. Add up to phenolics, add up to flavonoids, and a hostile to oxidant movement of the chose definition were likewise explored. Wound recuperating action was surveyed in streptozotocin-incited diabetic rats and control rats. Streptozotocin infusion (50 mg/kg) was found to actuate stamped hyperglycaemia, contrasted and citrate-infused controls. Two injuries were made on the upper back of every creature. QiF was topically connected three days in the wake of injuring to one of the copy wounds on every creature and physiological saline (control) was connected to the next. All injuries were cleaned once per day until the point when wound conclusion. QiF10, which showed antibacterial and hostile to oxidant exercises, had the capacity to upgrade the injury recuperating process in diabetic rats with copious cell penetration, collagen statement, and re-epithelialization.

Hirapara et al, (2017) evaluated wound healing activity of alcoholic extract of *Jasminum grandiflorum Linn*. (*J. grandiflorum*) flowers in diabetic rats. Streptozotocin-incited diabetic Wistar pale skinned animals were partitioned into six groups. Three groups – diabetic control, positive control (that got Glibenclamide) and treatment (that got *J. grandiflorum* Linn. bloom extricate) were worked for extraction wounds (EW). These groups were assessed for wound compression and re-epithelization. The other three groups were worked for entry point wounds (IW) and dead space wounds (DW). Entry point and dead space wounds were delivered in similar rats. IWs were broke down for wound breaking quality and the granulation tissues from DWs were examined for dry weight, hydroxyproline substance, and histology. IWs and DWs demonstrated huge change in wound breaking quality (265.8±10.4 versus 332.5±8.2 $p<0.05$), granulation tissue dry weight (26.1±0.6vs 40.4±0.3 $p<0.01$) and hydroxyproline content (19.3±0.5

versus 32.6±0.8 $p<0.01$) in treatment aggregate when contrasted with control gathering. Neo-angiogenesis was additionally high in treatment gathering. Wound compression was before (day 14) in treatment amass contrasted with diabetic group (day 20). No noteworthy transition was seen in re-epithelization in treatment gathering.

Birri et al, (2017) investigated and determined that the decoction of this plant elicits pro-ejaculatory activity and increases the ejaculatory potency in the Fictive Ejaculation Model. The extraction strategy (decoction) was approved through Selectivity, Accuracy and Precision, by distinguishing proof of the dominant part alkaloids, communicated as sauroxine. Male (sexually experienced and noncopulating) and female (open) Wistar rats were utilized to decide sexual conduct. Sildenafil was utilized as positive control. The accompanying factors were assessed: Ejaculation Latency, Post Ejaculatory Interval, Intromission Latency, Mount Latency and in addition the Mounts and Intromissions Number. In sexually experienced male rats, *P. saururus* decoction animates sexual excitement and encourages sexual execution. In noncopulating male rats, this decoction actuates lovemaking with behavioral attributes like sexually experienced creatures.

Babu et al, (2017) the study was aimed at investigating the effect of methanolic extract of *Buchanania axillaris linn*. (Anacardiaceae) on general mating conduct, charisma, and antagonistic impacts on sexually ordinary male rats. Methanolic separate was regulated p.o at the measurement of 100, 200, and 400 mg/kg, to various groups of male animals (n = 8) once a day for 14 days. Every one of the measurements brought about noteworthy increment in mount recurrence, intromission recurrence and anogenital sniffing when contrasted with ordinary. The methanolic concentrate of *Buchanania axillaris* leaves at higher fixation (400 mg/kg body weight) demonstrated huge love potion action on male Wister pale skinned person rats as confirm by an expansion in number of mounts and mating execution. Along these lines, in exploratory rats, the aftereffects of the present examination recommend that the methanolic concentrates of *Buchanania axillaris* apply huge sexual enhancer action.

Carro-Juárez et al, (2017) in this study, the aphrodisiac properties of the purple corn (*Zea mays*) in male rats were analyzed. The fluid unrefined concentrate of purple corn (at

25, 50, and 75 mg/kg) was regulated to (a) making love male rats and (b) anesthetized and spinal rope transected male rats. Behavioral parameters of copulatory conduct and parameters of the genital engine example of discharge past to its restraint, affected by the purple corn extricate, are depicted. Organization of the fluid unrefined concentrate of purple corn altogether encourages the excitement and execution of male rodent sexual conduct without noteworthy effects on the walking conduct. What's more, purple corn remove inspire a huge increment in the quantity of releases of the ejaculatory engine designs and in the aggregate number of genital engine designs evoked in spinal rats. The discoveries demonstrated that the fluid rough concentrate of purple corn has love potion movement.

Dwivedi et al, (2017) investigated antimicrobial, wound healing, and antioxidant activity of extract of *Pongamia Pinnata leaves*. Methanolic concentrates of *P. pinnata* leaf were considered for wound recuperating effectiveness, and was evaluated by the rate of wound withdrawal, rigidity, breaking quality, hydroxyproline and hexosamine content, alongside its impact on expert fiery and calming cytokines was surveyed utilizing extraction and entry point model of twisted repair in Wistar rats. Antimicrobial action against ten microorganisms was likewise surveyed. In vivo cell reinforcement movement was performed to comprehend the instrument of wound mending strength. The outcomes showed that *P. pinnata* remove has intense injury recuperating limit as apparent from the injury withdrawal and expanded elasticity. Expanded injury withdrawal and rigidity, increased hydroxyproline and hexosamine content, ant oxidative action and direct antimicrobial movement bolster the early twisted mending showed by *P. pinnata*.

Mbiantcha et al, (2017) this study aimed at evaluating immunomodulatory and anti-arthritis capacity of aqueous and methanol extracts of stem bark of *Piptadeniastrum africanum* (Mimosaceae). ROS creation from phagocytes, expansion of T-cells, TNF-α and IL-1β generation and cytotoxicity were performed by utilizing chemiluminescence system, fluid glimmer counter, ELISA and MTT measure, individually. Hostile to ligament action was assessed utilizing a model of adjuvant actuated joint inflammation. Methanol and fluid concentrates of *Piptadeniastrum africanum* altogether ($P<0.001$) repressed extracellular and intracellular ROS creation. These concentrates additionally

have noteworthy (P<0.001) inhibitory movement on T-cell multiplication other than decreased TNF-α and IL-1β generation. *Piptadeniastrum africanum* likewise essentially showed anti-arthritic action in entire Freund's adjuvant initiated joint pain in rodent related with a huge calming and hostile to hyperalgesia action .

Chen et al, (2017) investigated the anti-arthritic activity of the ethanol extract of *Claoxylon indicum* (CIE) on mice with adjuvant induced joint arthritis. Adjuvant joint inflammation was prompted in mice by subcutaneous infusion of finish FCA into the plantar surface of right rear paw. Joint pain seriousness was assessed by ligament score, rear paws oedema and spleen file, and histological examinations. Serum tests were gathered for assurance of malondialdehyde (MDA) and soluble phosphatase (ALP) levels. The statement of interleukin-1 beta and tumor putrefaction factor alpha in the examples of knee joints was controlled by standard safe histochemical procedures

Roy et al, (2017) The present study was conducted to evaluate if MTX-pioglitazone combination therapy has an add-on benefit over monotherapy with MTX or pioglitazone on disease activity in male Wistar rats in adjuvant-induced arthritis model. Joint inflammation was initiated by single subcutaneous infusion of finish Freund's adjuvant (CFA) in thirty male Wistar pale skinned person rats. They were then separated into five equivalent groups, which included two control groups (ligament and nonarthritic), pioglitazone-treated (1.35 mg/kg every day), MTX-treated (0.225 mg/kg day by day), and MTX + pioglitazone-treated. There was a critical lessening of sickness movement in the MTX monotherapy aggregate when contrasted and malady control. Be that as it may, pioglitazone monotherapy aggregate neglected to exhibit any huge impact on infection action. The MTX-pioglitazone blend bunch showed more noteworthy concealment of ailment action when contrasted with MTX and pioglitazone monotherapy and ailment control gathering (P< 0.05).

Yuniarti (2017) evaluated the effects of leaf extracts of *Madeira vine (A. cordifolia (Ten.) Steenis)* on skin burn healing process in rats as an animal model. In this examination, there were four treatment groups: G0, G1, G2, and G3, each comprising of five rats. Every one of these rats were given skin consumes, utilizing hot metal plates. At

that point, sulfadiazine was given to G0, 2.5% leaf concentrate of Madeira vine was given to G1, 5% remove was given to G2, and 10% concentrate was given to G3, for straight 14 days topically, 3 times each day. Toward the finish of the treatment time frame, skin extractions were directed, and histopathological examination was completed. Infinitesimal perception on the injury recuperating process on the collagen affidavit, polymorphonuclear penetration, angiogenesis, and fibrosis demonstrated that G2 had a huge distinction with G0, G1, and G3.

Zhang et al, (2017) *Phlomis younghusbandii* (Labiatae) is reported to be effective in the treatment of rheumatoid arthritis (RA). In the present examination, the mitigating and against ligament impacts of phlomisoside F (PF), disconnected from P. *younghusbandii* (Labiatae), were researched in male Wistar rats subjected to carrageen-incited paw edema and finish Freund's adjuvant (CFA)- initiated joint pain. Joint pain scores were assessed by a 5-point ordinal scale (scores 0– 4). Articulation levels of IL-1β, TNF-α, IL-10, IL-6, Cox-II and 5-Lox were resolved by means of ELISA and western smear measures. Consequent to setting up the edema and joint inflammation models, oral organization of PF (5, 10 and 20 mg/kg) fundamentally hindered mean edema rate, contrasted and the control assemble in carrageenan-incited paw edema test. Also, organization of PF (5, 10 and 20 mg/kg/day) for 28 days particularly showed a hostile to ligament action by counterbalancing the body weight reduction, restraining the paw edema, decreasing the joint pain scores and the files of thymus and spleen, hindering the articulation levels of IL-1β, TNF-α, COX-2, IL-6, and 5-Lox, and expanding the outflow of IL-10, when contrasted and the individual control aggregate in CFA-prompted joint inflammation examine.

Li et al, (2017) performed in vivo real-time monitoring of the male aphrodisiac pheromones released by the small white cabbage male butterfly *(Pieris rapae* Linnaeus) using confined direct analysis in real time (cDART) mass spectrometry. cDART is another technique effectively adjusted to the examination progressively of chemicals discharged into the earth by for all intents and purposes any creepy crawly. The real compound discharged by the male Pieris rapae was distinguished as ferrulactone. The test comes about revealed here show that the arrival of ferrulactone happens under 1 s after

the male envisions its accomplice, and achieves a greatest after around one half moment. This investigation is the main revealed in vivo location and checking of butterfly male love potion pheromones progressively.

Estrada-Reyes et al, (2017) determined whether an aqueous extract of *C. mexicana* stimulates rat male sexual behavior in the sexual exhaustion paradigm. Sexually depleted male rats were treated with Cm (80, 160, and 320mg/kg), a watery concentrate of *T. diffusa* or yohimbine. The sexual depletion state in the control assemble was described by a low level of guys displaying mounts, intromissions, and discharges and no guys exhibiting mating conduct after discharge. *C. mexicana* (320mg/kg), *T. diffusa*, or yohimbine essentially expanded the extent of sexually depleted rats that discharged and continued sex after discharge. In guys that showed inversion of sexual fatigue, *C. mexicana* (320mg/kg) enhanced sexual execution by diminishing the quantity of intromissions and contracting discharge latency. The prosexual impacts of *C. mexicana*, and also those of *T. diffusa*, are built up at a focal level, which underpins the customary utilization of *C. mexicana* for fortifying sexual movement.

Barka et al, (2017) evaluated the anti-diarrheal activity of *Rhus tripartita* root methanolic extract (RRE). The anti-diarrheal action of *Rhus tripartita* extract oral measurements (50, 100, 200 and 300 mg/kg) was assessed utilizing the castor oil-prompted the runs, the intestinal liquid exhausting technique and the typical intestinal travel test. Looseness of the bowels tests demonstrated a defensive impact of the *Rhus tripartita* extract which created a noteworthy and measurements subordinate diminishment of all the runs parameters. It deferred the beginning of the runs, created a huge reduction in the recurrence of crap and the loose bowels score seriousness and diminished the volume of intestinal liquid prompted by castor oil and in addition the impetus intestinal travel.

Patel et al, (2016) assessed the counter ligament action of *Pathyadya Churna* ethanol extricate (PCE) in rats. Formaldehyde (2% v/v) or (CFA 0.1 mL) was infused in the left rear paw of male Wistar rats to create joint pain. These rats were treated with three doses (135, 270, and 540 mg/kg) of PCE and one estimation (10 mg/kg) of indomethacin.

Against joint activity of the think was assessed by observing paw volumes, rheumatoid factor (RF), blood parameters, and histological changes.PCE treatment reduced paw swelling in joint irritation caused by both formaldehyde and CFA. In CFA-treated rats, a vital lessening ($P < 0.001$) was found in hemoglobin (13.92 g/dL to 9.97 g/dL), red platelet count (7.32 million/mm3 to 6.58 million/mm3), and stuffed cell volume (44.04% to 30.56%). There were furthermore important ($P < 0.001$) ascents in platelets (2.46– 4.15 lakhs/mL), white platelet check (8220/ – 11,420/mm3), low-thickness lipoprotein (14.34–19.32 mg/dL), erythrocyte sedimentation rate (3.76– 8.03/60 min), triglycerides (71.69–96.60 mg/dL), mean cholesterol (96.85– 145.05 mg/dL), RF (7.17– 26.77 IU/mL), low-thickness lipoprotein (53.11– 109.60 mg/dL.

Kim et al, (2016) *Ganghwaljetongyeum* (GHJTY) has been used as a standard treatment for arthritis for approximately 15 years at the Korean Medicine Hospital of Dongshin University. GHJTY is made out of 18 restorative herbs, of which five essential herbs were chosen and named new *Ganghwaljetongyeum* (N-GHJTY). The motivation behind the present examination was to watch the impact of N-GHJTY on joint pain and to decide its component of activity. In the wake of affirming joint pain enlistment utilizing complete Freund's adjuvant (CFA) in rats, N-GHJTY (62.5, 125, and 250 mg/kg/day) was regulated once per day for 10 days. Edema in the paw and knee joint of N-GHJTY-treated rats was essentially diminished at 6, 8, and 10 days after organization, contrasted with that in the CFA-control gathering, while weight reliably expanded. Rats in N-GHJTY-treated groups likewise recouped from the CFA-initiated obsessive changes and demonstrated a noteworthy decrease in cytokine levels. Results demonstrated that N-GHJTY organization was viable in restraining CFA-incited joint pain through mitigating impacts while advancing ligament recuperation by controlling cytokine levels.

Zhao et al, (2016) evaluated the therapeutic effect of total flavonoids from *J. sabina* (JSTF) on RA-induced by CFA in rats. Wistar rats (200 ± 20 g) were inoculated by intradermal infusion of 0.1 mL of CFA into the correct rear metatarsal footpad. JSTF was directed p.o at the measurement of 125 mg/kg, 250 mg/kg and 500 mg/kg on 14 days after the acceptance of adjuvant joint inflammation. Tripterygium glycoside (20 mg/kg) was utilized as a positive control. Paw swelling, joint score, body weight reduction,

serum cytokines, incendiary go betweens, and histological change were estimated. JSTF enhanced paw swelling of CFA rats, and essentially restrain ligament score (P< 0.05). The overproduction of tumor corruption factor alpha and interleukin 1beta were astoundingly smothered in the serum of JSTF (125,500 mg/kg) treated rats (P < 0.05). Histopathological contemplates additionally demonstrated a checked diminishing of synovial incendiary penetration and synovial covering hyperplasia in the joints of JSTF-treated creatures.

Hirapara et al, (2016) evaluated wound healing activity of cow urine ark in diabetic rats. Streptozotocin-induced diabetic Wistar albino rats were randomly divided into six groups (n = 6). Three groups - diabetic control, dynamic control (glibenclamide), and treatment (bovine pee ark) were worked for extraction wounds (EWs). Rats in these groups got refined water 1 ml/day, glibenclamide 0.5 mg/kg body weight/day, and cow pee ark 5.5 ml/kg body weight/day orally till finish recuperating of the EWs. EWs were assessed for twisted constriction on third, seventh, and eleventh day and for re-epithelization on eleventh day. The other three groups were worked for entry point wounds (IW) and also dead space wounds (DW) in a similar creature which got the above operators orally for 11 days. IWs were examined for wound breaking quality and DWs were broke down for dry weight, hydroxyproline substance, and histology of granulation tissue. EWs indicated essentially expanded injury conclusion in the treatment bunch when contrasted with the diabetic and in addition dynamic control bunches at third (P < 0.001) and eleventh (P < 0.05) present injuring day and on the main diabetic control gather at seventh (P < 0.01) post-injuring day. The bovine pee ark was possibly powerful in advancing recuperating of diabetic injuries by expanding granulation tissue arrangement and collagen content, be that as it may, additionally ponders are required for its clinical application.

Du et al, (2016) study aimed at investigating whether low dose oral prednisone combined with escin could inhibit the progression of adjuvant-induced arthritis (AIA) in rats. Adjuvant joint pain was prompted in SD rats started day 1 for 28 days. Paw swelling, ligament list, histological and radiographic changes were surveyed to assess the counter joint impact. Histopathological and radiographic examination demonstrated a stamped reduction of synovial incendiary penetration, synovial hyperplasia and bone

disintegration by blend treatment, which additionally particularly stifled the declaration of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin6 (IL-6). Taking everything into account, the blend treatment had synergistic hostile to ligament adequacy and decreased unfavorable impact, which may assume a part in the administration of human RA.

Kumar et al, (2016) investigates the anti-arthritic activity of *Picrorhiza kurroa* (PK), on formaldehyde and adjuvant induced arthritis (AIA) in rat. Organization of *Picrorhiza kurroa* rhizome extricate (PKRE) fundamentally restrained joint aggravation in both creature models. In AIA-instigated joint rodent, treatment with PKRE extensively diminished synovial articulation of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor rot factor receptor-1 (TNF-R1) and vascular endothelial development factor as contrasted and control. The counter ligament action was observed to be very much substantiated with noteworthy concealment of oxidative and incendiary markers as there was diminished malonaldehyde, Nitric oxide, tumor rot factor alpha levels went with expanded glutathione and superoxide dismutase, catalase exercises.

Mu et al, (2016) studied a novel nano-porous L-Bandage, and provide results from a mouse full-thickness wound model investigating its mechanism of action on wound healing. Distinctive attributes, for example, porosity, mechanical properties and water vapor transmission rate (WVTR) were resolved. The L-Bandage shaped film had a permeable system structure with mean distance across of 18 nm that could adequately keep the bacterial intrusion, and good properties of elasticity, extension, and WVTR. The L-Bandage treated injury showed quickened mending, with decreased aggravations, improved injury re-epithelialization, constriction, granulation tissue development, and fast angiogenesis.

Jain et al, (2015) investigate the effect of diacerein on the histopathology of articular cartilage and subchondral bone of the femorotibial joint in rats. Osteoarthritis was actuated in rats after single intra-articular infusion of sodium iodoacetate. In view of histopathological and biochemical discoveries, diacerein treatment indicated chondroprotective impact. Moreover, the chondroprotective impact of diacerein was

observed to be more articulated following 12 weeks when contrasted with two months in the two cases (i.e., post 1 and 2 weeks of iodoacetate infusion). Comparable outcomes were seen by examination of chondroitin sulfate amid biochemical investigation, demonstrating the chondroprotective impact. Taking everything into account, diacerein shows chondroprotective impact in rats with late beginning of activity.

George et al, (2015) study the antioxidant, antitumor, and wound healing properties of *Rubus ellipticus*. *R. Ellipticus* leaf methanol (RELM) extract, which showed higher in vitro antioxidant activity, was taken for the evaluation of in vivo antioxidant, antitumor, and wound healing properties. A significant wound healing property was observed in incision, excision, and Staphylococcus aureus-induced infected wound models in the treatment groups compared to the control group.

Rahman et al, (2015) explored the anti-diabetic and the anti-diarrheal effects of alcoholic extracts of *Cynodon dactylon Pers*. shoot parts in Wistar rats. Castor oil-prompted diarrheal and GIT motility test with barium sulfate drain display were performed for assessing the anti-diarrheal impacts at measurements of 1 g/kg, 750 mg/kg individually. After organization of EECA at the dosage of 1 g/kg, the concentrate demonstrated huge ($P < 0.05$) anti-diarrheal action in castor oil-initiated diarrheal model. The outcomes were likewise critical ($P < 0.05$) in barium sulfate drain show for similar measurements by utilizing previously mentioned creatures.

Kiessoun Konaté et al, (2015) evaluated anti-diarrheal and antimicrobial activities of the bioactive fraction of Trichilia emetic. It was discovered that ethyl acetic acid derivation part powerful weakening factor (EAF) evokes the higher aggregate phenolics and aggregate flavonoids substance contrasted with the concentrates of leaves of *Trichilia emetica*. EAF of *Trichilia emetica Vahl*., has constructive outcomes in a measurements subordinate way against the runs initiated by castor oil in exploratory mice.

Moghadamtous et al, (2015) evaluate the wound healing potential of ethyl acetate extract of A. *muricata* leaves (EEAM) towards excisional wound models in rats. The movement of cell reinforcements, to be specific catalase, glutathione peroxidase and superoxide dismutase, and malondialdehyde (MDA) was estimated in wound tissue

homogenate. Plainly visible and tiny examination of wounds exhibited a noteworthy injury mending movement appeared by EEAM at two dosages. Treatment of wounds with salve containing EEAM caused critical surge in cancer prevention agents exercises and abatement in the MDA level of wound tissues contrasted and vehicle control. EEAM displayed a promising injury recuperating potential towards excisional twisted models in rats.

Akindele et al, (2014) evaluated the anti-diarrheal activity of the hydroethanolic leaf extract of *Pupalia lappacea* (PL). The anti-diarrheal action of PL was assessed utilizing the typical and castor oil-prompted intestinal transit, castor oil-initiated looseness of the bowels, gastric purging and intestinal liquid collection tests in rodents. Results demonstrated that the hydroethanolic leaf concentrate of *Pupalia lappacea* has anti-diarrheal movement potentially interceded by antimuscarinic receptor action.

Asuntha et al, (2014) studied the aphrodisiac potentials of ethanol extract of *Argemone mexicana L.* (*A. mexicana*) of Papaveraceae family in sexually sluggish male Wistar rats. The latencies of mount, intromission and discharge were essentially diminished. The EEAM has created checked variety in sexual conduct attributes and could raise the serum testosterone levels ($P<0.01$) on par to that of Sildenafil citrate. The EEAM has hoisted sexual dysfunctions in male rats.

Patil et al, (2014) investigated the in vivo aphrodisiac activity of various extracts obtained from aerial parts *Cocculus hirsutus (C. hirsutus*) and evaluated spermatogenic activity by oral administration of *C. hirsutus* in male rats. The concentrates fortified the spermatogenic movement and extra regenerative organs in pale skinned person rats. Phytochemical screening indicated positive tests comes about for steroids, saponins, oils and fats, phenolic mixes and tannins. *C. hirsutus* displayed surprising increment in spermatogenic activities.

Choudhary et al, (2014) evaluate anti-arthritic potential of ethyl acetate fractions of chloroform extract from leaves of *Barleria prionitis*. Joint pain evaluation, body weight, paw volume, motor in-coordination and pain limit were estimated. Hematological evaluations of red and white platelets, erythrocyte sedimentation rate, and in addition

histopathological ponders were additionally done on day 21, after creatures were relinquished. Dosage reliant and huge hindrance of oedema was seen in both intense and endless models. The concentrate at dosage 250 mg/kg indicated most intense and noteworthy paw oedema hindrance which is bolstered by the aftereffects of biochemical parameters, body weight, motor in-coordination and nociceptive edge in Freund's Complete Adjuvant-instigated joint pain display.

Ara et al, (2014) evaluated the Application of response surface methodology for the optimization of supercritical carbon dioxide extraction and ultrasound-assisted extraction of *Capparis spinosa* seed oil. The trial parameters of SFE and UAE were optimized utilizing a rotatable focal composite plan. The most astounding yield for SFE was acquired at a weight of 355 bar, temperature of 65◦C, modifier volume of 140L, extraction time of 10 and 35 min, separately and for UAE was picked up at dissolvable volume of 23 mL, sonication time of 45 min and temperature of40◦C. This brought about a most extreme oil recuperation of 25.1% and 27.9% for SFE and UAE, separately. The concentrates with higher yield from the two strategies were subjected to transesterification and GC– MS investigation. SFE and UAE forms specifically extricated the greasy oils with high level of omega-6 and omega-9-unsaturated fats. The significant parts of the separated oils from the two techniques were Linoleic, oleic, its positional isomer cis-vaccenic and Palmitic corrosive.

Boga et al, (2014) evaluated the antibacterial activity of roots of *Capparis spinosa L*. A decoction of *Capparis spinosa L*. roots, generally utilized as a part of the conventional medicine of Italy was arranged and submitted to antibacterial action tests, which demonstrated an intriguing bacteriostatic movement on the development of Deinococcus radiophilus. Heterocyclic mixes were too recuperated from the chloroform concentrate of the roots.

Kataria et al, (2013) studied the methanol extract of *Corchorus depressus* for *in-vitro* aphrodisiac activity on rabbit corpus cavernosum smooth muscle. The chloroform portion (CDC) was observed to be the most dynamic and thusly examined facilitate on general

mating conduct, drive and strength of typical male Wistar pale skinned person rats in comparison with the standard medication, Sildenafil citrate.

Xiao et al, (2013) determined anti-diarrheal activities of *Polygonum chinense L*. and to identify its active components through bioactivity-guided isolation technique. Creatures were orally regulated with the concentrate of *Polygonum chinense L*. The counter diarrheal impacts of 75% ethanol remove, four divisions with various polarities from 75% ethanol separate, diverse eluates gathered from Diaion HP-20 macroporous tar chromatography, ellagic corrosive and corilagin, were analyzed in view of mouse models of castor oil-and magnesium sulfate-initiated looseness of the bowels. The outcomes demonstrated that the 75% ethanol concentrate of *Polygonum chinense L.* displayed huge against diarrheal exercises in a dosage subordinate way in two mouse models.

Ladhari et al, (2013) evaluated the phytotoxic activity of *Capparis spinosa L*. and its evaluated active components. Authors segregated and recognized the bioactive mixes in benefactor plant. Leaf isolates were most phytotoxic to lettuce growth and development, finish hindrance happened with watery concentrate at 20 g/L and 65.9 % restraint by alcoholic extricate at 6 g/L. The alcoholic buildup was consecutively extricated with ether, ethyl acetic acid derivation and alcohol-water. The ethyl acetic acid derivation separate caused 75.5 % restraint in lettuce germination and development at 6 g/L, while other two concentrates caused hindrance of 60.1%. Twelve sub fractions were acquired from the ethyl acetic acid derivation separate, among which two were most poisonous (31.7 and 64.4 % hindrance). Chromatographic fractionation showed three bioactive chemicals as: quercetin, quercetin-3-O-β-D–glucopyranoside and kaempferol 3-O-β - D – glucopyranoside.

Yang wei-jun et al, (2013) evaluated the Protection of *Citrullus colocynthis* Fruit Extracts on Carbon Tetrachloride-induced and Bacillus Calmette-Guerin plus Lipopolysaccharide-induced Hepatotoxicity in Mice. To examine the hepatoprotective exercises of the concentrates from *Citrullus colocynthis* (ECC), a local plant utilized as conventional Uigur Medicine on intense liver damage in mice. Techniques The exercises of ECC of oil ether (ECCPE), chloroform (ECCC), ethyl acetic acid derivation (ECCEA),

n-butyl liquor (ECCBA), and water (ECCW) were assessed in vivo utilizing two exploratory models, carbon tetrachloride (CCl4)- and bacillus calmette-guerin (BCG) in addition to Lipopolysaccharide (LPS)- initiated intense Hepatotoxicity in mice. The substance of alanine aminotransferase and aspartate aminotransferase in serum were resolved and the liver histological examination was completed, separately. Results The pretreatment with ECC for 7 d clearly decreased the effect of CCl4 poisonous quality on the serum markers of liver harm, ECCEA and ECCC with a noteworthy contrast of AST ($P < 0.01$, 0.05, individually) and ALT ($P < 0.05$, 0.01, separately). The defensive action was reconfirmed against BCG + LPS-actuated damage and the serum enzymatic levels were clearly lifted, for ECCEA and ECCC with a critical contrast of AST ($P < 0.05$, 0.01, separately) and ALT ($P < 0.01$, 0.05, individually).

Dhabale et al, (2012) investigated the physicochemical and phytochemical evaluation of *Capparis zeylanica* linn. The present examination endeavor to assess the physicochemical and phytochemical parameters of *Capparis zeylanica* leaves have a place with family Caparadaceae is a climbing bush found in all through India. The plant is utilized as a part of society pharmaceutical to treat, ailment, stomach ulcers and hernia, swelling, tingling, hepatitis, liver tonic, bug harming and mitigating. Be that as it may, there is no institutionalization work gave an account of *Capparis zeylanica* clears out. Physicochemical parameters, preparatory portrayal and phytochemical investigation were completed.

Argentieri et al, (2012) investigated the bioactive compounds from *Capparis spinosa* subsp. Rupestris. Point of this examination was to decide the substance of bioactive phytochemical in *Capparis spinosa* subsp. rupestris (syn. *C. orientalis*), a less researched types of escapade and look at the substance profile of this species with that of other examined Capparis sp. furthermore, particularly with the related cultigens *C. spinosa* subsp. spinosa . Concoction organization of seed oil and glucosinolates and in addition of glucosinolates and flavonoids from the elevated parts of the plant has been resolved and information revealed here. Oil from the plant seeds is rich in unsaturated and uncommon lipids, for example, cis -vaccenic corrosive the primary glucosinolates is glucocapperin. The aeronautical parts are described by rutin as the prevailing flavonoid. The general

phytochemical information got from the investigation of *C. spinosa* subsp. rupestris demonstrate that this species speaks to an extremely rich wellspring of bioactive mixes of nutraceutical importance despite the fact that the compositional profile does not separate this subspecies from *C. spinosa* subsp. Spinosa.

Chaudhari et al, (2012) evaluated anti-inflammatory, analgesic and anti-arthritic activity of leaves of *Cassia uniflora Mill*. Treatment with separate indicated noteworthy and measurement subordinate increment in paw licking duration. In squirming test extricates fundamentally diminished no. of squirms. A measurement needy and huge restraint of edema was seen via carrageenan actuated paw oedema. The concentrate showed pain relieving, calming and against ligament action which can be credited to phytochemical constituents.

Mazumder et al, (2012) investigated the anti-arthritic potential of *B.lupulina* leaves and its immunomodulation. Methanolic concentrates of *B. lupulina* was tried for its anti-arthritic action for its different models. The accompanying exploration demonstrated critical outcomes and contributes that *B. lupulina* demonstrated anti-arthritic movement and immunomodulatory action.

Soni et al, (2012) evaluated ethanolic extract of *P. foetida* on sexual behavior and testosterone level in male rats. Ethanolic concentrate of the leaves (50 mg/kg, 100 mg/kg and 200 mg/kg bw) was contemplated for their impact on body and auxiliary sexual organ weight, sexual conduct, spermatogenesis and serum testosterone level in male pale skinned person rats. Oral organization of the concentrate in pale skinned person rats demonstrated articulated anabolic and spermatogenic impacts in creatures in the treated groups. The concentrate altogether expanded both mount and intromission recurrence. Furthermore, it additionally fundamentally diminished both mount and intromission inactivity.

Pinal et al, (2012) evaluated the immunomodulatory, analgesic and anti-inflammatory activities of methanolic and aqueous extracts of mycelium of *P. sajorcaju* and finding suggested that extracts of *P. sajorcaju* can be used against autoimmune disease and inflammation. In this way, *P. sajorcaju* inspected for its anti-arthritic movement. For

anti-arthritic movement 500 and 1 000 mg/kg of the two concentrates were arranged and directed by oral course. Paw edema (irritation), body weight, spleen weight, hematological parameter, histological and radiological examination evaluated in rats with Freund's adjuvant prompted paw aggravation. The two concentrates demonstrated noteworthy and measurement subordinate mitigating and against joint impacts contrasted with control gathering.

Sharma et al, (2012) assessed hostile to diarrheal action of fluid and alcoholic concentrate of the leaves of *Murraya koenigii* (*M. koenigii*)by utilizing models of castor oil incited looseness of the bowels, charcoal dinner test and PGE2 initiated the runs. The outcome recommended that *Murraya koenigii* acts midway and hinder the PGE2 to give against diarrheal impacts. After-effect of charcoal feast test additionally recommended its against muscarnic action. These discoveries demonstrated that watery concentrate of the leaves of *M. koenigii* shows great against diarrheal action, certifying the society utilization of *M. koenigii* arrangements and contributing for its pharmacological approval.

Mali et al, (2011) evaluated the *Phyllanthus amarus Schum*. for its anti-arthritic activity. The institutionalized fluid concentrate of *P. amarus* separate (PAE) (Phyllanthin and hypophyllanthin) was tried against Freund's total adjuvant instigated joint rats. Joint inflammation evaluation, joint distance across, paw volume, nociceptive edge and mechanical hyperalgesia estimated. PAE altogether diminished the joint inflammation which was obvious with joint inflammation file, paw volume and joint distance across. It likewise altogether expanded the nociceptive limit and mechanical hyperalgesia.

Singh et al, (2011) studied the lyophilized powder of the dried fruits of *Tribulus terrestris* for sexual behavior effects of acute and sub chronic administration in male albino rats, and comparison been made with standard sexual stimulant drug, Sildenafil citrate. The creatures were assessed on different parameters of sexual conduct, anabolic impacts, and testosterone level and in-vitro sperm checks. Oral organization of 100mg/kg of test medicate has demonstrated anabolic impact as prove by bodyweight pick up in the body and conceptive organs. Change in sexual conduct of male rats was portrayed by expanded sum and intromission recurrence. Penile erection record (PEI) was likewise

extensively improved with no recognizable lethality, and the testosterone level and sperm count additionally essentially expanded, and the outcomes were practically identical to that of standard medication, Sildenafil citrate.

Al-Safadi et al, (2011) investigated the Improvement of caper (*Capparis spinosa L.)* propagation using in vitro culture and gamma irradiation. The quantity of unusual shoots rising up out of trick stems refined in vitro expanded from 2.2 shoots for each explants when the development medium contained 2 mg/L of gibberellic corrosive (GA3) to 5.5 when the development medium contained 2 mg/L zeatin riboside (ZR) and 1 mg/L naphthalene acidic corrosive (NAA). The best medium for callus arrangement from leaf and stem parts contained the development controllers 1 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L NAA and the best medium for plant recovery contained 1 mg/L kinetin and 0.1 mg/L indole-3-acidic corrosive (IAA). The impact of gamma light on the development of escapade shoots in vitro was additionally examined. 10 Gy measurements of gamma light fortified development of shoots up to 200% and expanded shoot attaching rate from 75 to 100%. Techniques for scratching the seed coat with press particles and treating the entire seeds with concentrated H2SO4, ultrasonic waves and gamma beams were utilized for breaking the seed lethargy. Treating the seed with H2SO4 for 20 min alongside scratching was extremely viable in invigorating germination (46% as contrasted and 0% for the control). Lighting escapade seeds with 100 Gray (Gy) dosage of gamma beams prompted half seed germination in vitro and 70% on peatmoss one month in the wake of refined.

Suntar et al, (2010) investigated the *Hypericum perforatum L*. (Hypericaceae) for the treatment of wounds through *in vivo* incision and excision wound models. Aerial parts of plant was removed with ethanol, significant injury recuperating movement profile was seen with the injury models in the vicinity of 18.3% and 95.6% in extraction demonstrate and from 13.9% to 100.0% hindrances in entry point show were resolved. The outcomes demonstrated that flying parts of *Hypericum perforatum* have exceptional injury mending and calming effect.

Ekambaram et al, (2010) study states the impact of the watery concentrate and the entire seed powder of *Strychnos potatorum Linn* seeds on the FCA instigated ligament rodent body weight changes, paw edema and adjustments in biochemical and hematological parameters in both creating and created periods of joint inflammation. Histopathology of proximal interphalangeal joints and radiology of back legs were analyzed. In FCA started tendon rats, there was vital addition in rat paw volume and diminishing in body weight increment, while SPP and SPE treated gatherings, exhibited basic lessening in paw volume and run of the mill place on in body weight. The balanced biochemical parameters and hematological parameters in the tendon rats were basically reclaimed to close normal by the SPP and SPE treatment at the dose of 200 mg/kg/p.o in both making and made times of joint irritation.

Amabeoku (2009) investigate the anti-diarrheal activity of the leaf aqueous extract of *Geranium incanum* in mice. The leaf watery concentrate of Geranium incanum essentially lessened faecal yield in castor oil - instigated the runs and furthermore altogether diminished the quantity of diarrheal scenes. *Geranium incanum* fundamentally deferred the beginning of loose bowels prompted by castor oil and altogether lessened the number of creatures showing looseness of the bowels. Loperamide, a standard anti-diarrheal tranquilize, delivered comparative impacts to the leaf fluid concentrate of *Geranium incanum* on castor oil-instigated looseness of the bowels. Both *Geranium incanum* and Loperamide altogether decreased the intestinal drive of charcoal supper in mice. The phytochemical examination of the leaves uncovered the nearness of tannins, saponins especially steroidal saponins, and flavonoids.

Deshmukh et al, (2009) investigate the effects of *Calotropis gigantea* root bark on wound healing role in rats by incision, dead space wound healing and excision models in animals. Topical utilization of *Calotropis gigantea* in extraction wound model expanded the level of wound withdrawal. Scar region and epithelization time were diminished. In cut injury and dead space wound breaking quality of wounds and hydroxyproline was expanded. *Calotropis gigantea* quickened twisted mending in rats and in this way underpins its conventional utilize.

Guohua et al, (2009) examined the effect of *Allium tuberosum* seeds extract upon the expression of male rat sexual behavior, in order to know whether *Allium tuberosum* seeds extract possess aphrodisiac property. The concentrate (500 mg/kg body weight/day) and L-dopa (100 mg/kg body weight/day) were directed orally by gavages for 40 days. Mount inertness (ML), intromission inactivity (IL), discharge dormancy (EL), mounting recurrence (MF), intromission recurrence (IF), discharge recurrence (EF) and post ejaculatory interim (PEI) were the parameters saw previously and amid the sexual conduct learn at day 0, 10, 20, 30 and 40. The n-BuOH separate diminished altogether ML, IL, EL and PEI ($p < 0.05$). The concentrate likewise expanded fundamentally MF, IF and EF ($p < 0.05$). These impacts were seen in sexually dynamic and latent male rats.

Zamble et al, (2008) studied action of *Caesalpinia benthamiana* on sexual conduct and some examines on potential methods of activity were additionally performed. The Spanish fly properties of AECB regulated orally by gavage (50 mg/kg body weight) to male rats were assessed by watching the sexual conduct of creatures. *C. benthamiana* establishes were rich in phenolic mixes (Gallic corrosive, resveratrol and tannins). The outcomes demonstrated that AECB had huge vasorelaxing properties. With respect to the Spanish fly movement of AECB in male rats, comes about demonstrated that sexual parameters are invigorated.

Zamble et al, (2008) studied the aphrodisiac properties of *Microdesmis keayana J. Le´onard* root extract and major isolated alkaloids were investigated by the sexual behavior of male rats. Watery concentrate (150 mg/kg bw) and unadulterated alkaloids (3 mg/kg bw) were regulated orally by gavage to male rats. Dormant circumstances of perception, intromission and discharge, mounting conduct, number of mating and intromissions exhibitions were assessed and contrasted with those got with untreated rats within the sight of open and non-responsive females. The outcomes demonstrated that watery concentrate and alkaloids of *M. keayana* fortify sexual parameters in rats' sexual conduct.

Mbagwu et al, *(*2008) studied effect of the aqueous extract of *Mezoneuron benthamianum* (MB) on experimentally induced diarrhoea, intestinal propulsive

movement (IPM) and intestinal fluid accumulation (enteropooling) in rats and mice. The concentrate (400, 800 and 1600 mg/kg, orally) delivered a huge ($p < 0.05$) and measurement subordinate decrease in drive in the castor oil-instigated intestinal travel in mice. The mean peristaltic file (%) for these measurements of concentrate, control, morphine, (10 mg/kg, s.c.) and (distilled water, 10 ml/kg, p.o.) were 73.48, 69.34, 57.27, 89.93 and 31.56, separately. The impact of the concentrate at the most noteworthy dose was fundamentally ($p < 0.05$) lower than that of the standard medication. The outcomes acquired demonstrate that MB has hostile to diarrheal action because of its inhibitory impacts on gastrointestinal drive and intestinal liquid collection. The hostile activities of yohimbine in the examinations propose a part for the a2-adrenergic receptor framework.

Ratnasooriya et al, (2008) studied the male sexual stimulant activity of black tea brew (BTB) of *Camellia sinensis* (L.) *O. Kuntze* (Theaceae). Results demonstrated that BTB has checked Spanish fly movement (as far as prolongation of inactivity of discharge shortening of mount-and intromission latencies and rise of serum testosterone level). The love potion activity had a quick beginning and gives off an impression of being intervened by means of hindrance of uneasiness and rise of serum testosterone level.

Pokharkar et al, (2008) investigated the Synthesis and Characterization of Fatty Acid Methyl Ester by In-Situ Transesterification in *Capparis decidua* Seed. Unsaturated fat methyl ester is made virgin or utilized vegetable oils (both palatable and non-consumable) and creature fats. Unsaturated fat methyl ester works in pressure start motors like petro-diesel. Unsaturated fat methyl ester can be mixed in any proportion with oil diesel fills. It can be put away simply like the oil diesel fuel. Petro diesel can be supplanted by biodiesel because of its prevalence. It has different focal points. The seeds of *Capparis decidua* possess non-eatable oil in the scope of around 63.75 %. The level of biodiesel yield increments with convergence of KOH as an impetus. The present study showed the financially savvy new wellspring of vitality by single step response i.e. creation of oil by joining extraction and response of concentrate with the blend of alcohols.

Xiao Pu Fu et al, (2008) evaluated the new spermidine alkaloids from *Capparis spinosa* roots. Three new spermidine alkaloids capparisine, capparisine 26-o-β-D-glucoside and cadabicine 26-o-β-D-glucoside hydrochloride were confined from the foundations of Capparis spinosa. Their structures were set up based on spectroscopic investigation, including 1D and 2D NMR tests (1 H– 1H Cozy, HSQC, HMBC).

Rouf et al, (2007) studied methanol extract of *Xylocarpus granatum* bark for its anti-diarrheal properties in experimental diarrhoea, induced by castor oil and magnesium sulphate in mice. At the measurements of 250 and 500 mg/kg per oral, the methanol separate demonstrated critical and dosage subordinate anti-diarrheal action in the two models. The concentrates additionally altogether decreased the intestinal travel in charcoal supper test when contrasted with atropine sulphate (5 mg/kg i.m.). The outcomes demonstrated that the concentrates of *Xylocarpus granatum* bark have a critical anti-diarrheal movement and backings its customary uses in home grown prescription.

Ahmed et al, (2007) *Capparis cartilaginea* and *C. deserti* developing in Egypt were researched for their glucosinolates and rutin content. From Capparis cartilaginea four isothiocyanates were secluded and identified using GC and EI/MS methods. These mixes were 6-methylsulphonylhexyl isothiocyanates, butyl isothiocyanates, 5-benzylsulphonyl-4-pentenyl isothiocyanates and 7-methylsulphonylheptyl isothiocyanates. Notwithstanding mixes and two other mixes were segregated and distinguished from *Capparis deserti*. These mixes are [11-(2-butenylthio) 6-undecenyl isothiocyanate] and 3-methylthiopropyl isothiocyanates. The principle flavonoid segment in the considered species was segregated and distinguished asrutin by contrasting the information and those announced. The butanol division from *C. cartilaginea* and *C. deserti* demonstrated the most noteworthy cancer prevention agent properties.

Arora et al, (2007) evaluated the Corrosion Inhibition of Aluminium by *Capparis decidua* in Acidic Media. The restraint effectiveness of ethanolic concentrate of various parts of *Capparis decidua* (Ker) in acidic medium has been assessed by mass misfortune and thermometric strategies. Estimations of restraint effectiveness got from the two

strategies are in great understanding and are reliant upon the convergence of inhibitor and corrosive.

Ghule et al, (2006) investigated the Immunostimulant effects of *Capparis zeylanica* Linn. leaves. The present examination was attempted to investigate the immunomodulatory movement of ethanolic and water concentrates of *Capparis zeylanica* Linn. (Capparidaceae) leaves on neutrophil attachment test, humoral reaction to sheep red platelets, deferred type extreme touchiness, phagocytic action and cyclophosphamide-actuated myelosuppression. Pre-treatment of water extricate (300 mg/kg, oral) of *Capparis zeylanica* evoked a huge increment in neutrophil attachment to nylon filaments. The expansion of humoral safe reaction to sheep red platelets by ethanolic and water extricates (150– 300 mg/kg) is prove by increment in counter acting agent titers in mice. A measurement related increment in both essential and auxiliary counter acting agent titre was watched. Oral organization of ethanolic and water concentrates of *Capparis zeylanica* leaves, at measurements of 150 and 300 mg/kg in mice, dosage conditionally potentiated the deferred compose extreme touchiness response actuated by sheep red platelets. Immunomodulatory movement was likewise evaluated by serological and hematological tests. *Capparis zeylanica* separates averted myelosuppression in mice treated with cyclophosphamide medicate. The examination involved the intense danger and preparatory phytochemical screening of the ethanol and water removes.

Panico et al, (2005) investigated the protective effect of *C. spinosa* on chondrocytes. This plant, regular to the Mediterranean bowl, has been utilized by the customary drug for its diuretic and antihypertensive impacts and furthermore in certain obsessive conditions identified with uncontrolled lipid peroxidation. The concentrate contains numerous constituents, specifically a few flavonoids (kaempferol and quercetin subordinates) and hydrocinammic acids with a few referred to natural impacts, for example, the mitigating and the cancer prevention agent ones. In this investigation, we examined the impact of LECS on human chondrocytes societies invigorated by proinflammatory cytokine interleukin-1h (IL-1h) and we decided the creation of key atoms discharged amid unending provocative occasions (nitric oxide, glycosaminoglycan, prostaglandins and responsive oxygen species). We watched that LECS could neutralize

the unsafe impacts initiated by IL-1h. This insurance gave off an impression of being more prominent than that inspired by indomethacin, which is typically utilized in joint illnesses. Since LECS have a chondroprotective impact, it may be utilized as a part of the administration of ligament harm amid the fiery procedures.

Dursun et al, (2005) investigated the Some Physical Properties of Caper Seed. Some physical properties of escapade seed were assessed as a component of dampness content. In the dampness go from 6 03 to 16 35%d.b., the measurements of the real, medium and minor tomahawks fluctuated from 3 09 to 3 67 mm, 2 40 to 2 90 mm and 1 52 to 1 87 mm, individually. In the above dampness go, the number juggling and geometric mean distances across expanded from 2 34 to 2 81 mm and from 2 24 to 2 71 mm, separately. The sphericity, surface territory, anticipated zone and thousand seed mass expanded from 0 725 to 0 738, from 15 81 to 23 07 mm 2, from 7 37 to 9 25 mm 2, and from 6 60 to 7 75 g, separately, while the mass thickness, genuine thickness and porosity diminished from 438 to 399 kg m 3, 806 to 678 kg m 3 and 45 7 to 41 1%, individually. The max speed and edge of rest expanded from 6 1 to7 7ms 1 and 21 to 321, separately, and the static coefficient of grating on four auxiliary surfaces expanded from 0 547 to 0 698 for elastic, 0 523 to 0 656 for plywood (with wood grains parallel to the heading of development), 0 402 to 0 469 for aroused metal sheet and 0 356 to 0 456 for aluminum sheet.

Eddouks et al, (2004) evaluated the anti-hyperglycemic effects in diabetic rats. The hypoglycaemic impact of fluid concentrates of *Carum carvi (CC)* and *Capparis spinosa L.* (CS) organic product were researched in ordinary and streptozotocin (STZ) diabetic rats. After a solitary measurement or 14 day by day dosages, oral organization of the fluid CC and CS extricates (20 mg/kg) created a noteworthy lessening on blood glucose levels in STZ diabetic rats ($P<0.001$) the blood glucose levels were almost standardized 2 weeks after day by day rehashed oral organization of both watery CC and CS separates (20 mg/kg) ($P<0.001$). No exceedingly critical changes on blood glucose levels were seen in typical rats after both intense and ceaseless medications with CS and CC. Investigators reason that watery concentrates of CC and CS display a powerful hostile to hyperglycemic action in STZ rats without influencing basal plasma insulin fixations.

Ekramul Haque et al, (2004) investigated the E -octadec-7-en-5-ynoic acid from the roots of *Capparis zeylanica*. Once more unsaturated fat, E-octadec-7-en-5-ynoic has been separated from chloroform concentrate of the underlying foundations of *Capparis zeylanica*. The structure of this compound was built up fundamentally by 1D and 2D-NMR spectroscopy.

Ramachandran et al, (2004) studied the aphrodisiac activity of *Butea frondonsa* Koen. Ex Roxb (Papillionaceae) bark extract. The concentrate (400 mg/kg body wt./day) was directed orally by gavage for 28 days. Mount idleness (ML), mounting frequency (MF), discharge dormancy (EL), intromission inactivity (IL), intromission frequency, discharge recurrence and post-ejaculatory interim (PEI) parameters were seen previously and amid the sexual conduct learn at day 0, 7, 10, 14, 21, and 28. The concentrate lessened altogether ML, IL, EL and PEI ($p < 0.05$). The concentrate likewise expanded altogether MF, IF and EF ($p < 0.05$). These impacts were seen in sexually dynamic and latent male rats.

Murthy et al, (2004) the methanolic concentrate of dried pomegranate (*Punica granatum*) peels demonstrated the nearness of a high substance of phenolic mixes (44.0%) alongside different constituents. This concentrate was figured as a 10% (wt/wt) water-solvent gel and was contemplated for its injury mending property against an extraction twisted on the skin of Wistar rats. The action was contrasted and that of a business topical antibacterial candidate. The injury recuperating action was surveyed by estimating the percent withdrawal in skin and estimation of collagen content as far as hydroxyproline content. Mended skin was additionally subjected to histopathological concentrates to look at the minuscule changes. The creatures treated with 2.5% gel indicated direct recuperating (55.8% and 40.8% mending contrasted and negative and positive controls, separately), though the gathering treated with 5.0% gel demonstrated great recuperating (59.5% and 44.5% recuperating contrasted and negative and positive controls, individually).

Rouf et al, (2003) evaluated partitioned ethyl acetate, n-hexane and residual methanol extracts of the root of *Rumex maritimus* for anti-diarrheal activity in mice using serotonin

and castor oil induced diarrhoea and also charcoal motility test at 50, 100 and 20 mg/kg body weight dose. Among these, the methanol remove most fundamentally drawn out the ideal opportunity for enlistment of loose bowels diminished the recurrence of diarrheal scenes and furthermore diminished the impetus of charcoal dinner through the GIT tract.

Gokhale et al, (2002) studied the *Saussurea lappa*, *Argyreia speciosa* and *Achyranthes aspera for* inflammatory conditions. S. lappa and A. speciosa were found to essentially hinder paw edema actuated via carrageenan and Freund's total adjuvant and to forestall gathering of fiery cells in carrageenan-instigated peritonitis at dosages of 50– 200 mg/kg. A. aspera hindered these fiery reactions at measurements of 100– 200 mg/kg. The examinations uncover that the ethanolic concentrates of A. speciosa, A. aspera and S. lappa have mitigating and against ligament movement and bolster the method of reasoning behind the customary utilization of these plants in inflammatory conditions.

Calis et al, (2002) investigated the (6S)-Hydroxy-3-oxo- a -iodole glycosides from *Capparis spinosa* fruits. Two new (6S)- hydroxy-3-oxo-an iodole glycosides, together with corchoionoside C ((6S,9S)- roseoside) and a prenylglucoside, were disengaged from develop products of *Capparis spinosa.* The structures were built up based on spectroscopic, chiroptic and compound proof. Furthermore, the 13 C-reverberation of C-9 was observed to be of specific symptomatic incentive in relegating the outright setup at that inside in iodole glycosides. The an ionol subsidiaries are metabolites of (+)-(S)-abscise corrosive.

Shoba et al, (2001) evaluate the effect of methanolic and aqueous plant extracts of *Pongamia glabra* leaves*, Acorus calamus* rhizome*, Strychnos nux-omica* root bark and *Aegle marmelos* unripe fruit for their anti-diarrheal potential against COID in mice. The methanolic plant extricates were more compelling than fluid plant removes against castor-oil incited looseness of the bowels. The methanolic plant separates fundamentally diminished enlistment time of the runs and aggregate weight of the defecation. The outcome acquired set up the viability of these plant removes as anti-diarrheal specialists.

Nagappa et al, (2001) studied the aqueous extract of *T. populnea* fruit which showed noteworthy injury recuperating action in the extraction wound and entry point twisted models in rats following topical and oral organization, individually.

Park et al, (2001) studied methanolic extract of *Opuntia ficus-indica* stems and its hexane, ethyl acetate, n-butanol and aqueous fractions for their wound healing activity in rats. The concentrate and less polar portions indicated huge impacts.

Mukherjee et al, (2000) evaluated methanol extract of *Hypericum patulum Thumb*. leaves for wound healing potential on different experimental models of wounds in rats. The impact created by the concentrate treatment, as far as wound contracting capacity, wound conclusion time, recovery of tissues at wound site, rigidity of the injury and histopathological qualities were practically identical to those of a standard medication nitrofurazone balm.

Saha et al, (1997) evaluated methanol extract of *L. lavandulaefolia* for its wound healing activity both in the form of an ointment as well as an injection in two types of wound model in rats: the excision wound model and the incision wound model. Both the infusion and the balm of the methanol concentrate of the plant material delivered a critical reaction in both of the injury compose tried. The outcomes were likewise tantamount to those of a standard medication, nitrofurazone, regarding wound contracting capacity, wound conclusion time, elasticity and recovery of tissues at the injury site.

Chapter 3

Scope, Objective, Need of Study and Plan of Work

3.1 SCOPE

Plants have been utilized as helpful regimen for a great many years, in view of involvement and society cures and keep on drawing wide consideration for their part if there should be an occurrence of mellow and unending sicknesses. As of late, the enthusiasm for the utilization of natural items and the attention on plant inquire about has developed significantly in the western world and also created nations. Restorative herbs as potential wellspring of helpful guide have achieved a noteworthy part today in wellbeing framework everywhere throughout the world, in the infected condition as well as potential material for keeping up legitimate wellbeing. Amid the most recent couple of decades, there is a resulting development in the worldwide enthusiasm for restorative capability of plants because of rising and re-rising contaminations, wild medication safe maladies and tremendous ascent in way of life illnesses.

Plant-based medications could go about as remedial specialists to supplement present day drug in administration of these irrepressible sicknesses and help to achieve positive wellbeing. Unfortunately, one of the hindrances in the wider acceptance of herbal drugs is inadequacy or lack of quality control standards. Because of tremendous variety of compound constituents in the crude plant material gathered from various topographical sources and the act of corruption or substitution, appropriate quality control of the home grown crude material and completed items has turned out to be basic for institutionalization, viability, security and consistency of the medications. Accordingly, it is basic to create sufficient models to abuse the viability and guarantee wellbeing of the long-standing customary herbals in the light of current tools and techniques. In addition, despite the fact that dominant part of the restorative plants have colossal helpful potential for misuse in perspective of the estimation of their financial items for use as medication, they are either under-used or are yet to be investigated experimentally. In this manner, pharmacological screening of these therapeutic plants is a more compelling methodology

for finding new synthetic substances as sheltered and powerful medications and to meet the developing needs of the human.

Capparis decidua has been used as an ethnic medication in different parts of the world. A couple of examiners unquestionably took a premium and explored this plant for recognizing, detaching, and isolating potential remedial constituents which exhibited diverse pharmacological activities. A significant number of pharmacological exercises have just been specified yet at the same time a great deal of concentrate should be tended to with respect to this plant. In spite of the large number of pharmacological exercises appeared by this plant a few exercises like antirheumatic, sexual enhancer which is generally outstanding yet has not been confirmed by any specialist yet. Additionally clinical examinations have not been led so far to fit in with the consequences of preclinical investigations.

A few plants parts like leaf, stem, roots, natural products, and seeds of *Citrullus colocynthis* has been considered widely by famous researchers and scientists. In addition, natural product holds much organic and concoction criticalness to the extent this investigation is concerned. Biological activities appeared by various parts of this plant show a multidisciplinary utilization of this plant in treating a few infections. Although a number of compounds and many pharmacological activities have been elucidated in this plant species still more research is needed to be done as many traditional uses been reported so far are required to be authenticated by research.

3.2 OBJECTIVE

To carry out phytochemical screening and evaluation of pharmacological activities as anti-arthritic activity, aphrodisiac activity in *Capparis decidua*, and anti-diarrheal activity, wound-healing activity in *Citrullus colocynthis* plant species.

3.3 NEED OF STUDY

Emergence of new diseases, re-emergence of old, side effects of some currently available drugs including toxicity and other undesirable effects in patients are a few major problems which require immediate attention to combat diseases with effective drugs of high therapeutic index. Therefore, there is an immense need for the development; and discovery of new and safer bioactive compounds. Natural products are usually derived from microorganisms, plants or animals.

The research project entitled as "Phytochemical & Pharmacological investigation of *Capparis decidua* & *Citrullus colocynthis*" deals with study of those pharmacological activities which are yet to be explored & have not been studied prior. As still a number of patients suffer from diseases like sexual dysfunction, arthritis, diarrhea, and wounds resulted from various diseases. This research evaluated the pharmacological activities of plant extracts. Pharmacological activities studied involve aphrodisiac activity, anti-arthritic activity, anti-diarrheal activity & wound-healing activity.

Plants continued to remain a rich source of therapeutic substances since extinct civilization. Moreover, even today, a major portion of new drugs are obtained from natural products or their derivatives. So this research is innovative and provides a platform for future studies & clinical trials of these plants.

3.4 PLAN OF WORK

The research work was carried out according to a well planned and systematic scheme. The steps in systematic work plan are as under.

1. Literature review and Selection of plants- A comprehensive review was undertaken on all contemporary literature available related to the phytochemical and pharmacological profiles of selected plants, *Capparis decidua* & *Citrullus colocynthis* and that of pharmacological models in rats, attempted in the study. A high caliber and solid restorative data from the web was recovered just from the certified websites like CAM-PubMed, Entrez PubMed [Medline], Allied and Complementary Medicine Database, Embase and Cochrane library, Natural Medicine Comprehensive Database, apart from the hand-picked research articles from library. The data bases were searched to 2018.

2. Collection and authentication of Plants- Plants parts were collected from Jaipur and Jodhpur cities of Rajasthan for the conduction of study and the plants were authenticated.

3. CPCSEA approval for experimentation on animals- The present study was approved by the Institutional Animal Ethics Committee.

4. Solvent extraction by soxhlet apparatus

5. Phytochemical screening

6. Pharmacological investigations of plant extracts:

a. Anti-diarrheal activity

b. Anti-arthritic activity

c. Aphrodisiac activity

d. Wound healing activity

7. Compilation of data from experimental results

8. Thesis writing

Chapter 4

Experimental Investigations

4.1 IDENTIFICATION, COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

Capparis decidua and *Citrullus colocynthis* plants wildly found in districts of Jaipur and Jodhpur in Rajasthan, India. The fresh young and mature root, stem and leaves of *Capparis decidua* were collected from Jagatpura area and Rajasthan Ayurveda University campus Jodhpur in the month of March, 2016 during vegetative season. While *Citrullus colocynthis* fresh fruits were also collected from same regions in the month of August, 2016 during vegetative season. These species were systematically distinguished and confirmed by Dr. Manju Sharma, and Dr. Kailash Agarwal in University of Rajasthan. Specimen (R.No: RUBL 211604) for *Capparis decidua* and another voucher specimen (R.No: RUBL 211645) for *Citrullus colocynthis* has been stored at the Herbarium, University of Rajasthan, Jaipur, India.

4.2 PREPARATION OF PLANT EXTRACT SAMPLES

Capparis decidua root, stem and leaves were washed with tap water followed by distilled water and then cut and dried under the shade. The dried plant parts were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. Similarly *Citrullus colocynthis* fruits were undergone the washing followed by drying under shade and further comminuting and passage across sieve no. 40 and finally storage in closed container.

4.3 PHYSICOCHEMICAL ANALYSIS

4.3.1 DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING)

Precisely measured 10 g of medication (without preparatory drying and cut in parts of around 3 mm thickness) was put in a tare dissipating dish. The medication was then dried at 105°C for 3 h and weighed. Drying and weighing was proceeding at 30 minutes interim until the point when contrast between two progressive measuring related to not over 0.25%. Consistent weight was considered to achieve when two progressive weighing subsequent to drying cooling in desiccators, indicate not in excess of 0.01 g distinction (Anonymous 2001).

4.3.2 DETERMINATION OF ASH VALUES

(a) Determination of total ash

3 g of precisely measured root, stem and leaf powder was burned in a pot (tare silica dish) at a temperature not surpassing 450°C of every a suppress heater until the point that it was free from carbon, and afterward cooled and weighed. In the event that carbon free fiery remains couldn't be gotten in this way, the roasted mass was depleted with around 2 ml of heated water or an immersed arrangement of ammonium nitrate, the deposit was gathered on an ash less channel paper, dried and afterward touched off to a consistent weight. The slag in this way acquired was then cooled, weighed and level of fiery debris was computed with reference to the air-dried powdered medication (Anonymous 2002).

(b) Determination of acid insoluble ash

The fiery debris (ash) acquired from above system was included drop wise 25 ml of weaken hydrochloric corrosive and sifted utilizing an ash less channel paper (Whatman 41) to gather insoluble issue and washed with boiling water until the point when the filtrate gets neutral. The channel paper containing the insoluble issue was transferred to the first pot dried on a hot plate and touched off to steady weight. The deposit was cooled in reasonable desiccators for 30 min and weighed immediately. The level of corrosive

insoluble fiery remains was computed with reference to the air-dried powdered medication (Anonymous 2016).

(c) Determination of water soluble ash

Total ash was bubbled for 5 min with 25 ml of water and insoluble issue was gathered on an ashless channel paper. It was washed with boiling water and lighted for 15 min at a temperature not surpassing 450°C of every a mute heater. Contrast in weight of powder and weight of water insoluble issue gave the heaviness of water-dissolvable fiery debris. The level of water-dissolvable cinder was computed with reference to the air-dried powdered medication (Anonymous 2016).

4.3.3 DETERMINATION OF EXTRACTIVE VALUES

(**a) Determination of Alcohol-soluble Extractive value**

5 g of the air-dried powdered materials were macerated with 100 ml of liquor in a shut carafe for 6 h, shaking as often as possible at an interim of 1 h. It was then permitted to remain for 18 h and sifted quickly to keep any misfortune amid dissipation. 25 ml of the filtrate was dissipated to dryness in a porcelain dish and dried at 105°C to a steady weight. The level of liquor solvent extractive was computed with reference to the air-dried powdered medication (Anonymous 2016).

(b) Determination of water soluble extractive

4 g of the powdered material was macerated in 100 ml of water in a shut jar for 1 h and was shaken as often as possible. It was then bubbled delicately for 1 h on water shower, cooled and weighed and the weight was corrected. 25 ml of the filtrate was dissipated to dryness in a porcelain dish and dried at 105°C to a steady weight. The level of water-solvent extractive was computed with reference to the air-dried powered drug. (Anonymous 2002).

4.4 PHYTOCHEMICAL ANALYSIS

The dried powder of root, stem and leaves of *Capparis decidua* and fruits of *Citrullus colocynthis* was subjected to the preliminary phytochemical analysis for the presence of pharmacologically active compounds (Khanam et al, 2015).

4.4.1 TEST FOR PHENOLIC COMPOUNDS

Ferric chloride test: 1 g of powder was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. The appearance of bluish black color indicated the presence of phenolic compounds.

Lead acetate Test: To 3 ml of solution prepared by adding 1g of powder, 3 ml of lead acetate solution (10%) was added. The occurrence of white precipitates indicates the presence of phenols.

4.4.2 TEST FOR FLAVONOIDS

Alkaline reagent test- Few drops of dilute sodium hydroxide solution were added into the powder (1 g) to give intense yellow color which disappears after addition of dilute hydrochloride acid showed the presence of flavonoids.

Shinoda test - 1 g powdered stem was extracted with 10 ml of ethanol (95% v/v) for 15 min on a boiling water bath and filtered. To the filtrate, a small piece of magnesium ribbon and 3 to 4 drops of concentrated sulphuric acid were added. Development of red color indicated the presence of flavanones (Geissman 1955).

4.4.3 TEST FOR TERPENOIDS

Salkowski's test - The extract (1 g) of powdered drug was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Formation of the reddish-brown ring at the interface indicated the presence of terpenoids.

4.4.4 TEST FOR SAPONINS

Foam test - Powdered drug (1 g) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min using hands. The formation of two cm layer of foam layer indicated the presence of saponins.

4.4.5 TEST FOR ALKALOIDS

Wagner's test- About 1 g of powdered drug was shaken with few ml of dilute hydrochloric acid and filtered. Few drops of Wagner's reagent were added at the side of the test tube. The appearance of reddish-brown precipitate indicated the presence of alkaloids.

Dragendorff's test- 1 g of powder was extracted with 20 ml alcohol (95%) by refluxing for 15 min and filtered and the filtrate was evaporated to dryness. The residue was dissolved in 15 ml of 2 N H_2SO_4 and filtered. After making alkaline, the filtrate was extracted with chloroform. The residue left after evaporation was tested for the presence of alkaloids with Dragendorff's reagent. Development of orange colored precipitates indicated the presence of alkaloids (Sim 1969).

4.4.6 TEST FOR GLYCOSIDES

Keller-Kiliani's test - Powdered drug (1 g) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface is a feature of cardenolide deoxy sugar and appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

4.4.7 TEST FOR STEROIDS

Liebermann Burchard test - Powdered drug (1 gm) was dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The color of the upper layer changed to red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Harborne 1998).

4.4.8 TEST OF CARBOHYDRATES

Molisch test: To 2–3 ml of the aqueous. Powdered drug was added two drops of alpha-naphthol solution in alcohol, shaken and added conc. H_2SO_4 from the sides of the test tube. Violet ring was formed (Boxi et al, 2012).

Barfoed's test: Equal volumes of Barfoed's reagent and the powdered drug were mixed to form a solution. Heated for 1–2 min in a boiling water bath and cooled. Red color indicated the presence of monosaccharide's (Hossain et al, 2013).

4.4.9 TEST FOR TANNINS

Powdered drug (1 gm) dissolved in water in a test tube and diluted with chloroform and added acetic anhydride (1 ml). Finally, sulphuric acid (1 ml) was added carefully to the side of the test tube to the solution. A green color was formed which showed the presence of tannins.

Lead acetate Test: To 3 ml of solution prepared by adding 1 g of powder, 3 ml of lead acetate solution (10%) was added. The occurrence of white precipitates indicates the presence of phenols (Goyal et al, 2009).

4.5 EXPERIMENTAL STUDIES

Selection of doses

As per the previous experimental studies the dose of 100 mg/Kg, 200 mg/Kg of *Capparis decidua* and 50 mg/Kg, 100 mg/Kg of *Citrullus colocynthis* were administered to wistar rats (Mehrzadi et al, 2016).

4.5.1 PREPARATION OF TEST EXTRACTS FOR PHARMACOLOGICAL STUDIES

Hydroalcoholic extract of both the plants i.e. *Capparis decidua* and *Citrullus colocynthis* were prepared. The dried and powdered plant material (100 g) was Soxhlet extracted with water and ethyl alcohol (99.9%) in the ratio of 30:70 done for both the plant species for 48 hours. The extraction was carried out for 24 h at room temperature with mild shaking.

The extract was filtered and concentrated at 48˚C by keeping on a water bath and weight of residue was recorded. The percentage yield of hydroalcoholic extract of *Capparis decidua* was found to be 42.8% and *Citrullus colocynthis* as 38.5%. The collected extract was stored in a sterile container for further pharmacological studies. Hydroalcoholic extracts were suspended in distilled water prior to oral administration as per the dose, expressed as mg of extract per kg body weight of rat.

The following pharmacological activities have been explored in the present research work.

4.5.2 EVALUATION OF ANTI-DIARRHEAL ACTIVITY

Materials

Chemicals- Castor oil (Jayant Agro-Organics, Mumbai Maharashtra), Loperamide (Arene Life Sciences, Andhra Pradesh), Barium Sulfate (Oasis Fine Chem, Vadodara) and distilled water were used in this study.

Preparation of test extracts

Three hydroalcoholic extracts, viz. *Citrullus colocynthis* extract at the dose of 50 mg/Kg, 100 mg/Kg, and *Per se* group (only plant extract with 100 mg/Kg was administered). All the extracts were made in distilled water prior to administration, as per the dose, expressed as mg of extract per kg body weight of rat.

Preparation of standard drug

Solution of Loperamide was made in distilled water using and dose has been expressed as mg of powder per kg body weight of rat.

Selection of animals

Healthy adult untreated Wistar Albino rats of either sex with weight 100 to 200 g were selected. All the animals were housed in animal house under standard conditions having temperature (24 ± 1˚C), relative humidity (45-50%), and 12 hrs light and dark cycle fed

with standard food pellets and water ad-libitum. The animals were acclimatized to laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

4.5.3 CASTOR OIL INDUCED DIARRHEA MODEL (COID):

Experimental groups:

Group I: Normal control distilled water (2 ml/rat), p.o (n=6)

Group II: Disease control, p.o (n=6)

Group III: Standard drug Loperamide (2 mg/Kg) for the positive control group, p.o (n=6)

Group IV: *Citrullus colocynthis* extract at the dose of 50 mg/Kg, p.o (n=6)

Group V: *Citrullus colocynthis* extract at the dose of 100 mg/Kg, p.o (n=6)

Group VI: *Per se* group (only plant extract with 100 mg/Kg was administered)

Anti-diarrheal activity of *Citrullus colocynthis* extract was estimated with COID model as per Awouters et al, (1978) method. Wistar rats were divided into 6 groups by random selection and each group contained six rats. At first, extract and drugs were provided orally and after 1 h castor oil (2 ml/rat) was supplied for inducing diarrhea. Only distilled water (2 ml/rat) was supplied for the normal control group and standard drug Loperamide (2 mg/Kg) was provided for the positive control group. Disease control group were subjected with only castor oil. Treated Groups IV, V received *Citrullus colocynthis* extract at the dose of 50 mg/Kg and 100 mg/Kg respectively. While group VI is *per se* group only plant extract with 100 mg/Kg dose was administered. Separate cages were used for each rat and sheets of paper were placed below the cage for collection of fecal matters. The presence of stool with fluid material that stained the paper was placed beneath the cages indicated diarrhea. At every hour the numbers of both hard and soft pellets were counted up to 6 h period for each rat and finally moisture content of all feces was measured (Rahman et al, 2015).

The following parameters were evaluated for anti-diarrheal activity in all above groups: Latency time, defecation frequency (total number of stools in 6 hours), number of wet defecations, water content of feces, and weight of stool.

Water content of feces was expressed in terms of percentages using the formula:

$$Wc\ (\%) = \frac{Fw - Dw}{Fw} \quad X \quad 100$$

Where, Wc=Water content of feces; Fw=Fresh weight (g); Dw=Dry weight (g).

4.5.4 GASTROINTESTINAL MOTILITY TEST WITH BARIUM SULFATE MILK (BSM) MODEL FOR DIARRHEA

Experimental groups

Group I: Normal control distilled water (2 ml/rat), p.o (n=6)

Group II: Standard drug Loperamide (2 mg/Kg) for the positive control group, p.o (n=6)

Group III: *Citrullus colocynthis* extract at the dose of 50 mg/Kg, p.o (n=6)

Group IV: *Citrullus colocynthis* extract at the dose of 100 mg/Kg, p.o (n=6)

Group V: *Per se* group (only plant extract with 100 mg/Kg was administered)

BSM model was carried out by the method developed by Chatterjee (1993). By random selection Wistar rats (over night fasted) was divided into five groups each with six rats. Only distilled water of 2 ml/rat was supplied orally for Group I recognized as normal control group. Commercially available reference anti-diarrheal drug Loperamide at the dose of 2 mg/Kg was provided orally for Group II marked as positive control group. *Citrullus colocynthis* orally treated at dose of 50mg/Kg and 100 mg/Kg for Groups III, & IV respectively assigned as treated groups and group V as *Per se* group (only plant extract with 100 mg/Kg was administered). After 30 min, 2 ml of 10% barium sulfate solution was administered in all groups of rats. Rats were sacrificed after 30 min of

extract and drug administration. Finally, the distance travelled by BSM was measured and showed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction) (Rahman et al, 2015).

4.5.5 STATISTICAL ANALYSIS

The data were represented as a mean ± standard deviation (SD). Statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software (Kulkarni 1993).

4.6 EVALUATION OF ANTI-ARTHRITIC ACTIVITY

Materials

Chemicals- Freund's complete adjuvant (FCA) was obtained from Sigma-Aldrich Ltd. (USA). Diclofenac sodium was procured as gift sample from Afton Pharma, Gujarat, India. All reagents and chemicals used for study were belonged to analytical grade procured from approved organization.

Preparation of test extracts

Three hydroalcoholic extracts, viz. *Capparis decidua* extract at the dose of 100 mg/Kg, 200 mg/Kg, and *Per se* group (only plant extract with 200 mg/Kg was administered). All the extracts were made in distilled water prior to administration, as per the dose, expressed as mg of extract per kg body weight of rat.

Preparation of standard drug

Solution of Diclofenac sodium was made in distilled water using and dose has been expressed as mg of powder per kg body weight of rat.

Instruments- Plethysomograph, Vernier caliper, Eddy's Hot Plate apparatus, Rota rod apparatus, Centrifuge

Selection of animals

Healthy adult untreated female Wistar Albino rats with weight 100 to 200 g were selected. All the animals were housed in animal house under standard conditions having temperature (24 ± 1˚C), relative humidity (45-50%), and 12 h light and dark cycle fed with standard food pellets and water ad-libitum. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

4.6.1 FREUND'S COMPLETE ADJUVANT (FCA) INDUCED ARTHRITIS

Experimental groups

Group I: Normal control group administered with distilled water 1 ml/Kg p.o (non-arthritic), (n=6)

Group II: FCA injected arthritic control; (n=6)

Group III: Arthritic animals administered with Diclofenac Sodium (5 mg/Kg/day), (n=6)

Group IV: Arthritic animals administered with hydroalcoholic extracts of *Capparis decidua* (100 mg/Kg bw/day p.o), (n=6)

Group V: Arthritic animals administered with hydroalcoholic extracts of *Capparis decidua* (200 mg/Kg bw/day p.o), (n=6)

Group VI: *Per se* group (normal group where only plant extract with 200 mg/Kg will be administered p.o)

Arthritis was caused to all the groups of animals except vehicle control group using single intra-dermal injection of Freund's Complete Adjuvant (0.1 ml) containing 1 mg/ml mycobacterium tuberculosis H37Ra suspension in paraffin oil (sterile) into foot pad of the left hind paw of female rats. The rats were anesthetized by inhalation with ether

before and during FCA injection, as the highly viscous behavior of the FCA shows difficulty in injecting.

Treatment with *Capparis decidua*, Diclofenac sodium and vehicle was started from the 14th day after arthritis induction and continued for 28 days. The paw volume of all the animal groups was measured by plethysmograph at 1, 4, 10, 14, 17, 21, 24 and 28 after the injection of Freund's complete adjuvant (Bandwane et al, 2014 and Paval et al, 2009).

Anti-arthritic activity of *Capparis decidua* extract was evaluated on body weights changes, joint diameter, paw volume, pain threshold, fall off time and arthritic score on day 1, 4, 10, 14, 17, 21, 24 and day 28. On day 28 the animals anaesthetized by anesthetic ether and the blood was withdrawn using tail vein for the estimation of various biochemical parameters and hematological parameters (Argentieria et al, 2012).

4.6.2 ARTHRITIC SCORE

The morphological feature of the arthritis like redness, swelling and erythema were monitored by set visual criteria like-

Normal paw= 0, mild erythema and swelling of digits = 1, erythema and swelling of the digits = 2, severe erythema and swelling = 3, gross deformity and inability to use the limb = 4 on respective days (Wood et al, 1969 and Valiollah et al, 2009).

4.6.3 PAW VOLUME

The left hind paw volumes of all animals were measured just before FCA injection on day 0 and thereafter at different time intervals till day 28 using a plethysmometer. The change in paw volume was measured as the difference between the final and initial paw volumes. The joint diameters of left hind paw were measured using a Vernier caliper on the above-mentioned testing days after induction of arthritis (Argentieria et al, 2012).

4.6.4 ANTI-NOCICEPTIVE ACTIVITY

The device comprises of a hot plate on which the rats were set for testing (Eddy's Hot Plate Method). Pain threshold was evaluated using latency for pain effect (withdrawal of any paw) for all groups (Valiollah et al, 2009).

4.6.5 MOTOR IN-COORDINATION TEST

Motor in-coordination was investigated by Rota-rod assembly. Rats were set on the turning pole of instrument and the time taken for the falling of rats from the roller was recorded (Choudhary et al, 2014).

4.6.6 BODY WEIGHT RECORDING

Body weight was recorded on day 0 just before FCA injections and thereafter on day 1, 4, 10, 14, 17, 21, 24 and day 28 (Asquith et al, 2009).

4.6.7 HAEMATOLOGICAL AND SERUM PARAMETERS

On day 28, hematological parameters like white blood cell (WBC) count, red blood cell (RBC) count, platelets (PLT), hemoglobin (Hb), and Erythrocyte sedimentation rate (ESR) were determined by usual standardized laboratory method.

4.6.8 BIOCHEMICAL PARAMETERS

On day 28, rat’s blood were withdrawn using tail vein and serum will be used for the estimation of alanine aminotransferase (ALT), serum aspartate transaminase (AST), total protein levels, and alkaline phosphatase (ALP) (Bihani et al, 2014).

4.6.9 ESTIMATION OF SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

Principle:

As per the method described by Teitz (1976) glutamic pyruvic transaminase catalyses the reaction between alpha-ketoglutaric acid and alanine gives L-glutamic acid and Pyruvic acid. Pyruvic acid, in the presence of lactate dehydrogenase (LDH), reacts with NADH

giving lactic acid and NAD. The rate of NADH consumption is determined photometrically and is directly proportional to the GPT activity in the sample.

Reagents: Reagent I: Buffer reagent

Reagent II: Enzyme reagent (LDH)

Sample: Serum or Plasma.

Reagent preparation: 4 ml of reagent I was mixed with 1 ml of reagent II.

Procedure: Following quantities were pipette out into test tubes:

Sample 100 µl

Reagent 1000 µl

The contents were mixed well and allowed to stand for 1 min. at 37°C. The decrease in absorbance per minute during 3 min. was measured at 340 nm and ΔA/min was determined.

Calculation: ΔA/min. × 1746 = U/L ALT

ΔA = Difference in absorbance

U/L = Units per liter

4.6.10 ESTIMATION OF SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT)

Principle:

As per the method described by Teitz (1976) Aspartate transaminase catalyses the reaction between alpha-ketoglutaric acid and L-aspartate gives glutamate and oxaloacetate. Oxaloacetate, in the presence of malate dehydrogenase (MDH), reacts with NADH giving malate and NAD. The rate of NADH decrease is determined photometrically and is directly proportional to the GOT activity in the sample.

Reagents: Reagent I: Buffer reagent

Reagent II: Enzyme reagent (MDH)

Sample: Serum or plasma.

Reagent preparation: 4 ml of reagent I was mixed with 1 ml of reagent II.

Procedure: Following quantities were pipette out into test tubes: The contents were mixed well and allowed to stand for 1 min. at 37°C. The decrease in absorbance per minute during 3 min was measured at 340 nm and ΔA/min was determined.

Calculation: ΔA/min. × 1746 = U/L AST

ΔA = Difference in absorbance

U/L = Units per liter

4.6.11 ESTIMATION OF ALKALINE PHOSPHATASE (ALP)

Principle:

As per the method described by Young, (1973) p-Nitrophenyl phosphate is converted to p-nitrophenol and phosphate by alkaline phosphatase. The increase of absorption at 405 nm is proportional to the alkaline phosphatase concentration in the sample.

Reagents: Reagent I: Buffer reagent

Reagent II: Substrate reagent (p-nitrophenyl phosphate)

Sample: Serum or plasma.

Reagent preparation: 4 ml of reagent I was mixed with 1 ml of reagent II.

Procedure: Following quantities were pipette out into test tubes:

The contents were mixed well and incubated for 1 min at 37°C. The increase in absorbance every 30 sec was measured for 2 min at 405 nm and ΔA/min was determined.

Calculation: ΔA/min × 2720 = U/L Alkaline phosphatase

4.6.12 ESTIMATION OF TOTAL PROTEIN (COLORIMETRIC TEST - BIURET METHOD)

Principle:

As per the method described by Gournall (1949) cupric ions, in an alkaline medium, interact with protein peptide bonds results in the formation of a violet-colored complex. The absorption of the colored complex is proportional to the concentration of protein in the sample.

Reagents: Reagent I : Biuret reagent (1% Copper sulphate and 40% Sodium hydroxide)

Protein standard: 6 g/dl (store at 2 to 8°C)

Sample: Serum, heparinised plasma or EDTA plasma.

Reagent preparation: The reagents were ready to use.

Procedure: Following quantities were pipette out into test tubes:

	Blank	**Standard solution**	**Test solution**
Sample	-	-	20 µl
Protein Standard	-	20 µl	-
Reagent I	1000 µl	1000 µl	1000 µl

The contents were mixed well and incubated for 10 min at 20 to 25°C. Absorbance of the sample (At) and standard (As) were measured against reagent blank at 565 nm.

Calculation: At / As × Conc. of standard = g/dl Protein

4.6.13 STATISTICAL ANALYSIS:

The data were represented as a mean ± standard deviation (SD). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test where $P<0.001$ was considered statistically significant using Graph Pad Prism version 5.03 software (Kulkarni 1993).

4.7 EVALUATION OF WOUND HEALING ACTIVITY

Materials

Chemicals- Povidone iodine ointment Cipla India, distilled water, ketamine

Preparation of test extracts

Two hydroalcoholic extracts, viz. *Citrullus colocynthis* extract at the concentration of 5% and 10% were prepared by incorporating in the simple ointment using oleaginous base. All the extracts were administered topically.

Preparation of standard drug

Povidone iodine ointment was used as a standard drug for topical administration in Wistar rats.

Selection of animals

Healthy adult untreated female albino rats of Wistar strain of either sex weighing 100 to 200 g were selected. All the animals were housed in animal house under standard conditions: temperature (24 ± 1˚C), relative humidity (45-50%), 12 h (light) and 12 hrs (dark) cycle and fed with standard food pellets and water ad libitum. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

4.7.1 EXCISION WOUND MODEL

Experimental groups

Group I: normal control group (distilled water 1ml/Kg p.o), (n= 6)

Group II: standard drug treated group (5 % Povidone iodine ointment topically), (n= 6)

Group III: hydro-alcoholic extract of *Citrullus colocynthis* extract ointment (5% topically), (n= 6)

Group IV: hydro-alcoholic extract of *Citrullus colocynthis* extract ointment (10% topically), (n= 6)

First, the animals were anesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. Back of six rats in each group was depilated and the areas was cleaned with 70% alcohol under Ketamine (0.15 cc) anesthesia. Excision wound was inflicted by cutting away a 500 mm^2 full thickness of skin from a predetermined area. After completion of wounding process, the wound part was well washed with normal saline solution. Rat's wounds were left undressed to the open environment. The percentage wound closure, epithelization time and scar area on complete epithelization was measured. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound, i.e. the measurement of wound area on graph paper were expressed as unit (mm^2) (Sharma et al, 2012 and Rouf et al, 2007).

4.7.2 INCISION WOUND MODEL

Experimental groups

Group I: Normal control group (distilled water 1 ml/Kg p.o), (n= 6)

Group II: Standard drug treated group (5% Povidone iodine ointment topically), (n= 6)

Group III: Hydro-alcoholic extract of *Citrullus colocynthis* extract ointment (5% topically), (n= 6)

Group IV: Hydro-alcoholic extract of *Citrullus colocynthis* extract ointment (10% topically), (n= 6)

Four groups of animals containing six in each group were taken. The animals were anaesthetized under light ether anesthesia. One full thickness paravertebral incision of 6 cm length was made including the cutaneous muscles of the depilated back of each rat. After the incision was made, the parted skin was kept together and stitched with sutures, 1 cm apart. The continuous threads on both wound edges were tightened for good adaptation of wound and it was left undressed. All the groups were administered daily for 10 days. No ligature was used for stitching. The stitches were removed after 8 days and the tensile strength of the wounds was determined on 10th day (Ratnasooriya et al, 2008).

4.7.3 TENSIOMETER AND DETERMINATION OF TENSILE STRENGTH

Rigidity of wound speaks to the adequacy of wound mending. Typically twisted recuperating operators advance the picking up of elasticity. Rigidity (the power required to open the recuperating skin) is utilized to quantify the culmination of mending. Tensiometer comprises of a 6-12 inch wooden board with one arm of 4 inch since quite a while ago, settled on each side of the conceivable longest separation of the board. The board was set at the edge of a table. A pulley with bearing was mounted on the highest point of one arm. A gator clip with 1 cm width was tied on the tip of another arm by an angling line (20 lb test monofilament) such that the cinch could achieve the center of the board. Another gator clasp was tied on a more drawn out angling line with 1-l polyethylene bottle on the opposite end. One day before playing out the trial (estimation of elasticity) the sutures were expelled from the sewed injuries of rats after recuperation and rigidity was estimated as takes after. On the ninth day in the wake of injuring the sutures were expelled and the rigidity was estimated on tenth day. For estimating the rigidity the rats were again anesthetized and each rodent was put on a heap of towels on the center of the board. The measure of the towels could be balanced in such a path in this way, to the point that the injury was on an indistinguishable level from the tips of the arms. The clasps were then painstakingly clipped on the skin of the contrary edges of the injury at a separation of 0.5 cm far from the injury. The more extended bits of the angling

line were set on the pulley lastly to the polyethylene bottle. The situation of the board was balanced with the goal that the jug gets a quick and consistent rate of water from a substantial supply, until the point that the injury started to open. The measure of water in the polyethylene sack was weighed and likened as the rigidity of the injury. The rigidity actuated by the concentrate and by Povidone iodine balm treated injuries was contrasted and the control (Kirtikar 1933).

4.7.4 STATISTICAL ANALYSIS

The data were represented as a mean ± standard deviation (SD). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software (Kulkarni 1993).

4.8 EVALUATION OF APHRODISIAC ACTIVITY

Materials

Chemicals-Sildenafil citrate was procured from the Cadila Pharmaceuticals Limited, Ahmadabad, and Gujarat, India as a generous gift. Ethinyl oestradiol and progesterone were purchased from Sigma Chemical Co. (USA).

Preparation of test extracts

Three hydroalcoholic extracts, viz. *Capparis decidua* extract at the dose of 100 mg/Kg, 200 mg/Kg, and *Per se* group (only plant extract with 200 mg/Kg was administered). All the extracts were made in distilled water prior to administration, as per the dose, expressed as mg of extract per kg body weight of rat.

Preparation of standard drug

Solution of Sildenafil citrate was made in distilled water using and dose has been expressed as mg of powder per kg body weight of rat.

Selection of animals

Healthy adult untreated male and female albino rats of Wistar strain of either sex weighing 100 to 200g were selected. All the animals were housed in animal house under standard conditions: temperature (24 ± 1˚C), relative humidity (45-50%), 12 h (light) and 12 hrs (dark) cycle and fed with standard food pellets and water ad libitum. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

4.8.1 SEXUAL BEHAVIOR STUDY

Experimental groups

Animals were randomly divided into five groups with six animals per group and were orally administered the following:

Groups 1: Normal control group (distilled water 1 ml/Kg p.o) (n=6)

Group 2: Standard group (treated with Sildenafil citrate 5 mg/Kg p.o), (n=6)

Group 3: Hydro-alcoholic extract of *Capparis decidua* treated group (100 mg/Kg body weight), (n=6)

Group 4: Hydro-alcoholic extract of *Capparis decidua* treated group (200 mg/Kg body weight), (n=6)

Group 5: *Per se* group (normal group where only plant extract with 200 mg/Kg will be administered p.o), (n=6)

Thirty male rats were randomly divided into five groups of 6 rats each and were orally administered the following: Group 1 (control), 1 ml/kg of distilled water orally in sexually active male rats; group 2 (standard), solution of Sildenafil citrate 5 mg/Kg body weight orally in sexually sluggish male rats; groups 3 and 4, received solution of the

hydroalcoholic extract of *Capparis decidua* at 100, 200 mg/Kg body weight orally in sexually sluggish male rats; group 5 (*Per se*) received solution of the hydroalcoholic extract of *Capparis decidua* at 200 mg/Kg body weight orally only to note the effect of extract only in sexually active male rats. Oral administration was carried out using a metal oropharyngeal cannula. Five rats in each group were monitored for sexual behavior after their daily doses on days 0, 7, 14, 21 28.

Prior to the drug treatment, the male rats were trained separately with normal adult female rat for sexual experience. At that point, the male rats were partitioned into sexually active and sexually inactive groups, in view of their copulatory conduct. A male was considered sexually dynamic when it endeavored to mount any female brought into the cage. The normal mountings in ordinary male rats were observed to be 4-10 of every 5 min. The animal showing below 4 mounts was considered as inactive. Sexually inactive male rats were selected for extract treated groups and standard group in the present study. The female sexual behavior is restricted to the estrous phase, that agrees with ovulation and during this time animal is said to be in heat. The estrous female stirs sexual enthusiasm for male rat by physical changes in the genital district and the creation of pheromones. These are sexual fragrances found in rats that deliver sensational sex-chasing conduct in rats. The female rats react to each mount with a lordosis reaction. This reaction happens when the female is responsive to mounting male and comprises of an angling of the back to a curved position with deviation along the side and the neck extended. The female rat with estrous cycle was affirmed by vaginal spread technique. A dropper with a drop of distilled water was brought into the rats's vagina and the discharges were gathered and were seen under microscope. Estrous cycle was affirmed when half or a greater amount of the cells were cornified. The sexually inactive male rats were divided into three groups and each contains five animals while control group and *per se* group includes sexually active male rats. Standard group (Sildenafil citrate at 5 mg/Kg p.o), hydroalcoholic extract of *Capparis decidua* at a dose of 100mg/Kg (p.o.) group and hydroalcoholic extract of *Capparis decidua* at a dose of 200mg/Kg (p.o.) treated groups includes the sexually inactive rats. The female animals were artificially brought into oestrus (heat) as the female rats allow mating only during the estrus phase. They were administered suspension of Ethinyl oestradiol orally at the dose of 100

µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The exceedingly responsive female (in estrous stage) was brought into the home cage of the male rats and the accompanying male sexual behavioral parameters were recorded amid a time of 30 min: Latency (time) of first mount, number of mounts, latency of first intromission, number of intromission, latency of ejaculation (time from intromission to ejaculation), number of ejaculations. All the groups were tested for copulatory behavior on 0, 7th, 14th, 21st and 28th day. The following parameters were evaluated during the 28 days study period. Singh et al, (2013)

(A) Male sexual behavioral study

1) Mount Latency (ML)
2) Intromission latency (IL)
3) Ejaculation latency (EL)
4) Mount frequency (MF)
5) Intromission frequency (IF)

4.8.2 PARAMETERS OF APHRODISIAC ACTIVITY

I. Mount frequency

Mounting is defined as the climbing of one animal by another usually from the posterior end with the intention of introducing one organ into another. Mount may also be operationally defined as the male assuming the copulatory position but failing to achieve intromission. Mount Frequency (MF) is therefore defined as the number of mounts without intromission from the time of introduction of the female until ejaculation.

II. Intromission frequency

Intromission is the introduction of one organ or parts into another. e.g. the penis into the vagina. Intromission Frequency (IF) is therefore defined as the number of intromissions from the time of introduction of the female until ejaculation.

III. Mount latency

Mount latency (ML) is defined as the time interval between the introduction of the female and the first mount by the male.

IV. Intromission latency

Intromission latency (IL) is the time interval from the time of introduction of the female to the first intromission by the male. This is usually characterized by pelvic thrusting, and springing dismounts.

V. Ejaculatory latency

Ejaculation is the act of ejecting semen brought about by a reflex action that occurs as the result of sexual stimulation. Ejaculatory latency (EL) is defined as the time interval between the first intromission and ejaculation. This is usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity or reduced activity.

4.8.3 ESTIMATION OF SERUM TESTOSTERONE

Blood samples were collected from retro-orbital plexus before treatment, 30 min after administration of plant extracts on 0 day, 7th, 14th, 21st, 28th days and 7 days after withdrawal of treatment. Serum was separated upon centrifugation at 3000 rpm for 20 min (Asuntha et al, 2014 and Kabir et al, 2016).

4.8.4 STATISTICAL ANALYSIS

The data were represented as a mean ± standard deviation (SD). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software (Kulkarni 1993).

Chapter 5

Results

5.1 DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS

The powder of *Capparis decidua* root, stem and leaf was studied for loss on drying, ash values and extractive values (Table 4).

Table 4: Physico-chemical parameters of *Capparis decidua*

S. No.	Quality parameters	Values (%±SD w/w)
1.	**Loss on drying**	40.16±0.04%
2.	**Ash value**	
	Total ash	7.14±0.02%
	Acid insoluble ash	0.62±0.24%
	Water soluble ash	6.01±0.15 %
3.	**Extractive value**	
	Water soluble extractive	18±0.41%
	Alcohol Soluble extractive	8.51±0.23%

The powder of *Citrullus colocynthis* fruits was studied for loss on drying, ash values and extractive values (Table 5).

Table 5: Physico-chemical parameters of *Citrullus colocynthis*

S. No.	Quality parameters	Values (%±SD w/w)
1.	**Loss on drying**	35.24±0.16%
2.	**Ash value**	
	Total ash	6.31±0.09%
	Acid insoluble ash	0.54±0.25%
	Water soluble ash	7.06±0.46%
3.	**Extractive value**	
	Water soluble extractive	17.09±0.11%
	Alcohol Soluble extractive	9.32±0.10%

5.2 PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* AND *CITRULLUS COLOCYNTHIS*

Preliminary qualitative phytochemical analysis of hydroalcoholic extract of *Capparis decidua* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates.

Table 6: Representation of phytochemical analysis of *Capparis decidua* plant extract.

S.No.	Phytoconstituents	Tests	Present or Absent
1.	Phenolic compounds	Ferric chloride test Lead acetate test	Present
2.	Flavonoids	Alkaline reagent test Shinoda test	Present
3.	Terpenoids	Salkowski's test	Present
4.	Saponins	Foam test	Present
5.	Alkaloids	Wagner's test Dragendorff's test	Present
6.	Cardiac glycosides	Keller-Kiliani's test	Present
7.	Steroids	Liebermann Burchard test	Present
8.	Carbohydrates	Molisch test Barfoed's test	Present
9.	Tannins	Ferric chloride test Lead acetate test	Present

Preliminary qualitative phytochemical analysis of hydroalcoholic extract of *Citrullus colocynthis* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates.

Table 7: Representation of phytochemical analysis of *Citrullus colocynthis* plant extract.

S.No.	Phytoconstituents	Tests	Present or Absent
1.	Phenolic compounds	Ferric chloride test Lead acetate test	Present
2.	Flavonoids	Alkaline reagent test Shinoda test	Present
3.	Terpenoids	Salkowski's test	Present
4.	Saponins	Foam test	Present
5.	Alkaloids	Wagner's test Dragendorff's test	Present
6.	Cardiac glycosides	Keller-Kiliani's test	Present
7.	Steroids	Liebermann Burchard test	Present
8.	Carbohydrates	Molisch test Barfoed test	Present
9.	Tannins	Ferric chloride test Lead acetate test	Present

Evaluation of anti-diarrheal activity

5.3 EFFECT OF *CITRULLUS COLOCYNTHIS* (OBTAINED FROM JAIPUR DISTRICT) EXTRACT ON CASTOR OIL INDUCED DIARRHEA (COID) MODEL

The results about the anti-diarrheal effect of Loperamide and hydroalcoholic extract of *Citrullus colocynthis* in COID on Wistar rats are shown in Table 8. The results indicated that both the doses of *Citrullus colocynthis* extract (100 mg/Kg and 50 mg/Kg) showed protection against COID model. The hydroalcoholic extract of *Citrullus colocynthis* at 100 mg/Kg showed highly significant results such as prolonged the latency time, reduced the defecation frequency, number of wet defecations, the weight of stool and water content of feces when compared with the disease control group ($P<0.001$). In addition, this dose of plant extract had shown results comparable to Loperamide while at a dose of 50 mg/Kg the results were same as the significant value of ($P<0.01$). *Per se* group showed a highly significant result ($P<0.001$) on all the parameters discussed above as compared to disease control group and it contributes to the beneficial effects of *Citrullus colocynthis* extract in the treatment of diarrhea.

5.4 EFFECT OF *CITRULLUS COLOCYNTHIS* (OBTAINED FROM JAIPUR DISTRICT) EXTRACT ON BARIUM SULFATE MILK (BSM) MODEL

The results of the gastrointestinal motility test with BSM of hydroalcoholic extract of *Citrullus colocynthis* and Loperamide on Wistar rats have been shown in Table 9. The treatment with standard drug Loperamide and with all the doses of hydroalcoholic extract of *Citrullus colocynthis* significantly inhibited the gastrointestinal motility of rats. The percentages of inhibition of 100 mg/Kg, 50 mg/Kg, and *per se* groups compared to control group was 26.57%, 17.30%, 23.74% respectively. While standard group exhibited 38.89% inhibition.

One more anti-diarrheal activity was done in the *Citrullus colocynthis* plant procured from Jodhpur, Rajasthan and prepared the hydroalcoholic extract of fruits of *Citrullus colocynthis* and was done to compare the difference between the anti-diarrheal potential of two plants of *Citrullus colocynthis* obtained from different regions.

The results of the anti-diarrheal effect of *Citrullus colocynthis* plant procured from Jodhpur has been shown in Table 10 and 11.

5.5 EFFECT OF *CITRULLUS COLOCYNTHIS* (OBTAINED FROM JODHPUR DISTRICT) EXTRACT ON CASTOR OIL INDUCED DIARRHEA (COID) MODEL

The results about the anti-diarrheal effect of Loperamide and hydroalcoholic extract of *Citrullus colocynthis* in COID on Wistar rats are shown in Table 10. The results indicated that both the doses of *Citrullus colocynthis* extract (100 mg/Kg and 50 mg/Kg) showed protection against COID model which shows that the fruit extract prepared of *Citrullus colocynthis* plant from both the regions i.e Jaipur and Jodhpur are prominent in terms of anti-diarrheal activity. The hydroalcoholic extract of *Citrullus colocynthis* at 100 mg/Kg showed highly significant results such as increased the latency time, decreased the defecation frequency, number of wet defecations, the weight of stool and water content of feces when compared with the disease control group ($P<0.001$). In addition, this dose of plant extract had shown results comparable to Loperamide while at a dose of 50 mg/Kg the results were same as the significant value of $P<0.001$. *Per se* group showed a highly significant result ($P<0.001$) on all the parameters discussed above as compared to disease control group and it contributes to the beneficial effects of *Citrullus colocynthis* extract in the treatment of diarrhea. It could be attributed from the comparative study of *Citrullus colocynthis* obtained from two regions that the plant taken from Jodhpur region showed significant % inhibition of water content as compared to plant taken from Jaipur region. Here both the doses of *Citrullus colocynthis* extract i.e. 100 mg/Kg and 50 mg/Kg showed significant results ($P<0.001$).

5.6 EFFECT OF *CITRULLUS COLOCYNTHIS* (OBTAINED FROM JODHPUR DISTRICT) EXTRACT ON BARIUM SULFATE MILK (BSM) MODEL

The results of the gastrointestinal motility test with BSM of hydroalcoholic extract of *Citrullus colocynthis* and Loperamide on Wistar rats have been shown in Table 11. The treatment with standard drug Loperamide and with all the doses of hydroalcoholic extract of *Citrullus colocynthis* significantly inhibited the gastrointestinal motility of rats. The percentages of inhibition of 100 mg/Kg, 50 mg/Kg, and *per se* groups compared to control group was 23%, 11.17%, 22.4% respectively. While standard group exhibited 33.24% inhibition. Here both the doses of *Citrullus colocynthis* extract i.e. 100 mg/Kg and 50 mg/Kg showed significant results ($P<0.001$).

Table 8: Effect of hydroalcoholic extract of *Citrullus colocynthis* obtained from Jaipur district on COID model in Wistar rats.

Groups	Dose (mg/Kg)	Latency Time (min)	Defecation Frequency in 6 hrs.	% Inhibition of defecation	No. of wet defecation in 6 hrs.	% Inhibition of defecation	Wt. of stool (gm)	Wt. of wet stool (gm)	Water content of faeces (%)	% Inhibition of water content
Normal control (Distilled water)	2 ml/rat	181±1.78	4±1.26		1.33±0.51		0.13±0.02	.02±.008	4.19±1.90	
Disease control	2 ml/rat	107.33 ±5.50$^{\#\#}$	20.33±2.16$^{\#\#}$		12.66±2.16$^{\#\#}$		0.63±.10$^{\#\#}$	0.57±.08$^{\#\#}$	87.60±2.80$^{\#\#}$	
Loperamide	2 mg/Kg	304.16 ±4.26***	5.66±1.63***	72.16	2.66±0.81***	78.98	0.13±.04***	0.10±.04	00±00***	100
CCE	50 mg/Kg	117.83±2.40**	16±2.09***	21.29	9.5±1.04**	24.96	0.51±.05**	0.41±.02	80.91±1.56***	07.63
	100 mg/Kg	233.66±4.76***	9.66±2.16***	52.48	5±1.78***	60.5	0.32±.02***	0.23±.09	57.51±3.07***	34.34
Per se	100 mg/Kg of CCE	200.66±6.15***	5.83±0.75***	71.32	2±0.89***	84.2	0.27±.02***	0.06±.01	14.72±3.67***	83.19

Values are expressed as mean±SD (n=6); $^{\#\#}$P<0.001 refers to significant difference compared normal control with disease control; *:P<0.05; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

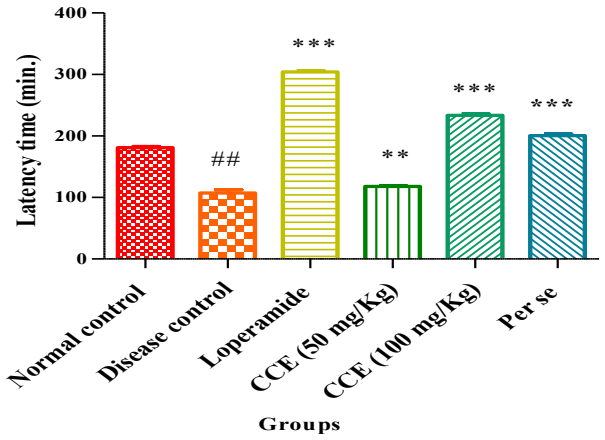


Figure 8: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on latency time (min.). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

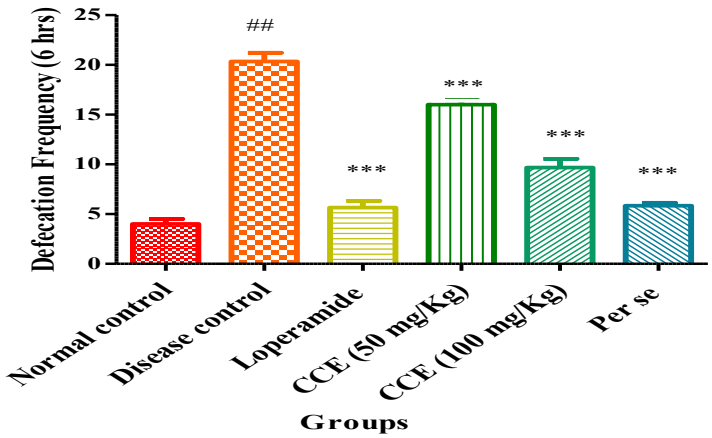


Figure 9: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on defecation frequency (h). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

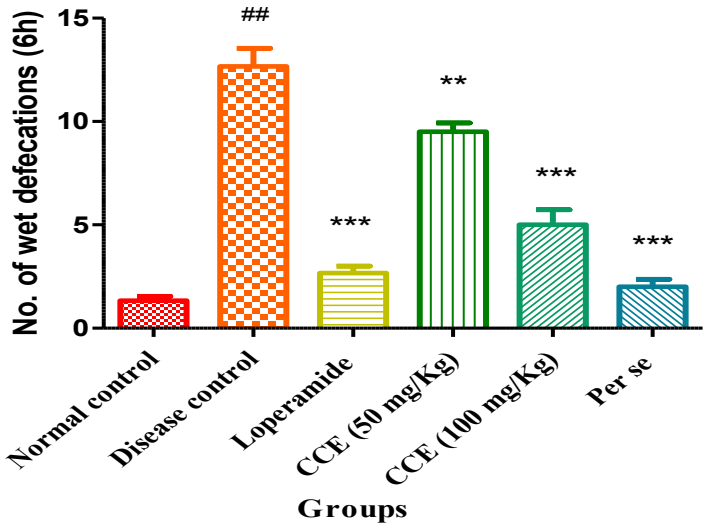


Figure 10: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on no. of wet defecations (h). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

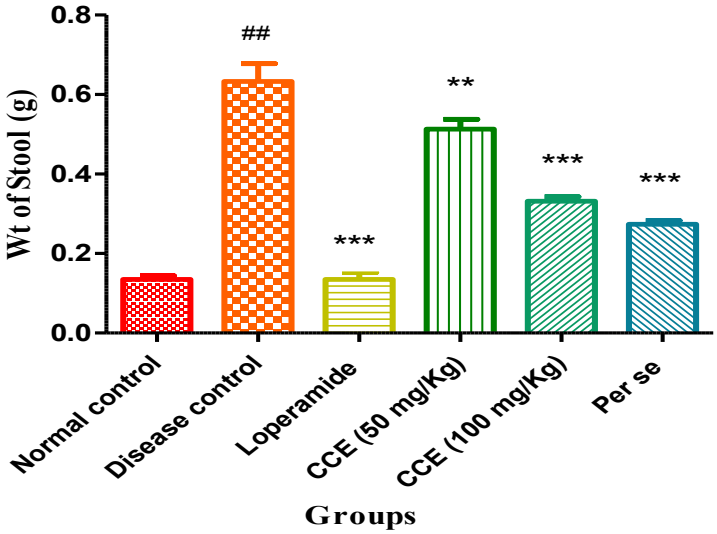


Figure 11: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on wt. of stool (g). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

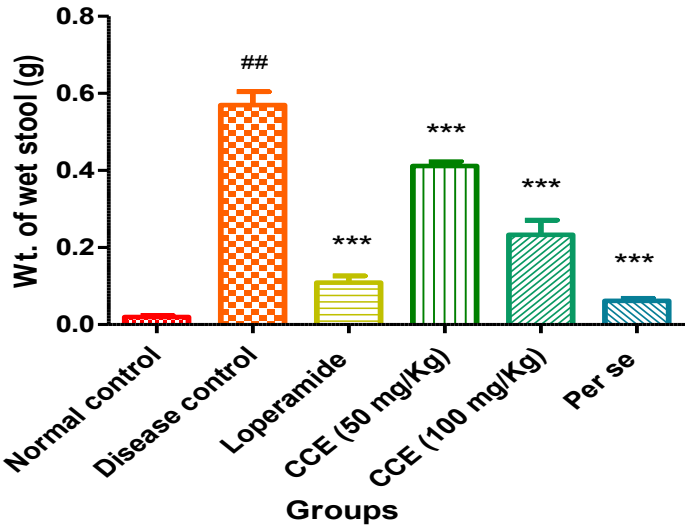


Figure 12: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on wt. of wet stool (g). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

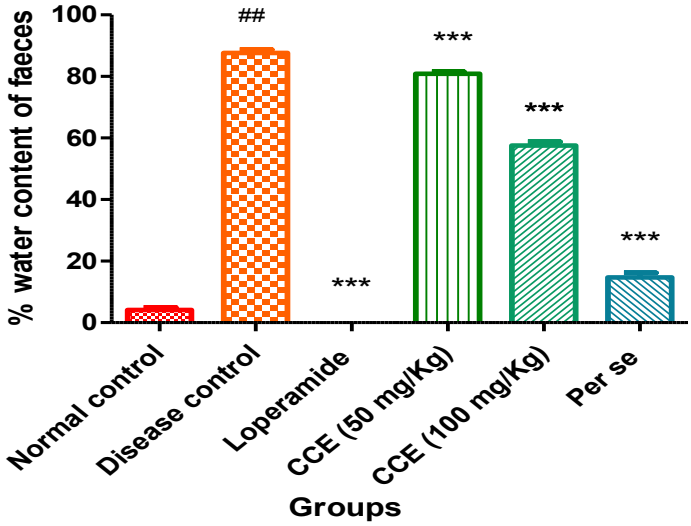


Figure 13: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on % water content of feces. Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

Table 9: Effects of hydroalcoholic extract of *Citrullus colocynthis* (obtained from Jaipur district) on gastrointestinal motility with Barium Sulfate milk model on rats.

Group	Dose (mg/Kg)	Length of GIT (cm)	Distance passed by $BaSO_4$ (cm)	$BaSO_4$ Transverse (%)	Inhibition (%)
Control	2 ml/rat	119.24±2.31	67.89±0.76	56.93	
Loperamide	2 mg/Kg	112.16±1.47	39.03±0.90	34.79***	38.89
CCE	100 mg/Kg	99.95±1.03	41.78±0.66	41.8***	26.57
	50 mg/Kg	106.74±1.54	50.26±0.86	47.08***	17.3
Per se	100 mg/Kg of CCE	103.10±1.07	44.76±1.22	43.41***	23.74

Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

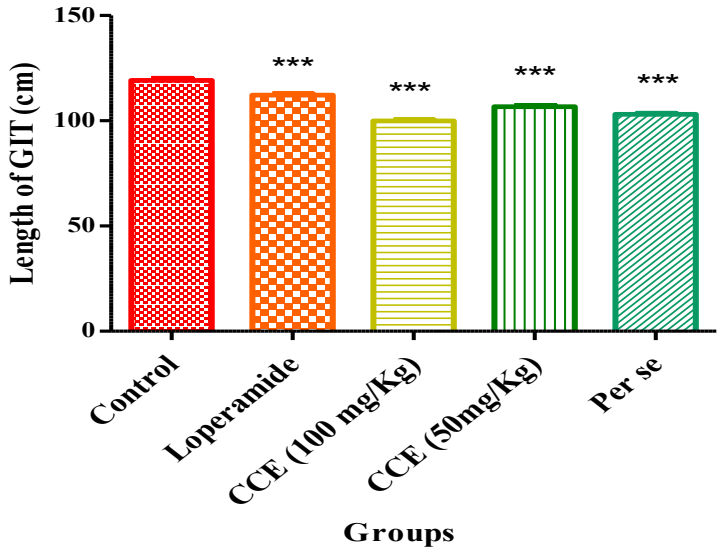


Figure 14: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on length of GIT (cm). Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

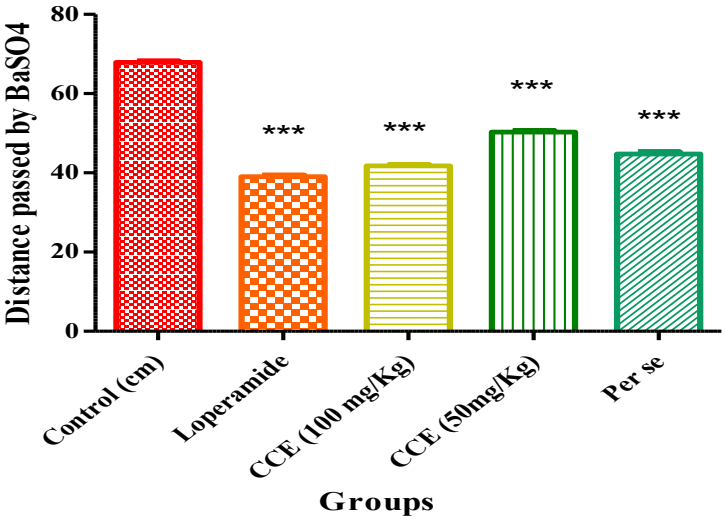


Figure 15: Effect of *Citrullus colocynthis* (obtained from Jaipur district) extract on distance passed by $BaSO_4$. Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

Table 10: Effect of hydroalcoholic extract of *Citrullus colocynthis* obtained from Jodhpur district on COID model in Wistar rats.

Groups	Dose (mg /Kg)	Latency Time (min)	Defecation Frequency in 6 hrs.	% Inhibition of defecation	No. of wet defecation in 6 hrs.	% Inhibition of defecation	Wt. of stool (gm)	Wt. of wet stool (gm)	Water content of faeces (%)	% Inhibition of water content
Normal control (Distilled water)	2 ml/rat	181.66±2.73	3.5±1.04		1±0.63		0.13±0.02	0.01±.008	4.75±1.69	
Disease control	2 ml/rat	108.66±5.35$^{\#\#}$	21.16±2.04$^{\#\#}$		10.83± 1.47$^{\#\#}$		0.53±.06$^{\#\#}$	0.48±.05$^{\#\#}$	87.60±2.80$^{\#\#}$	
Loperamide	2 mg/Kg	305.66 ±4.76***	5±1.41***	76.37	2.16±0.75***	80.05	0.13±.03***	0.10±0.02	00±00***	100
CCE	50 mg/Kg	119.16±1.94**	13.83± 1.94***	34.64	7.83± 1.16***	27.7	0.42±.03***	0.37±.02	81.06± 1.78***	7.46
	100 mg/Kg	238.83±2.92***	8.83±2.13***	58.27	4±1.26***	63.06	0.29±.02***	0.18±.03	56.93± 3.28***	35.01
Per se	100 mg/Kg of CCE	197.5±7.17***	5.66±0.81***	73.25	1.5±0.54***	86.14	0.24±.03***	.05±.008	14.13± 3.94***	83.86

Values are expressed as mean±SD (n=6); $^{\#\#}$P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

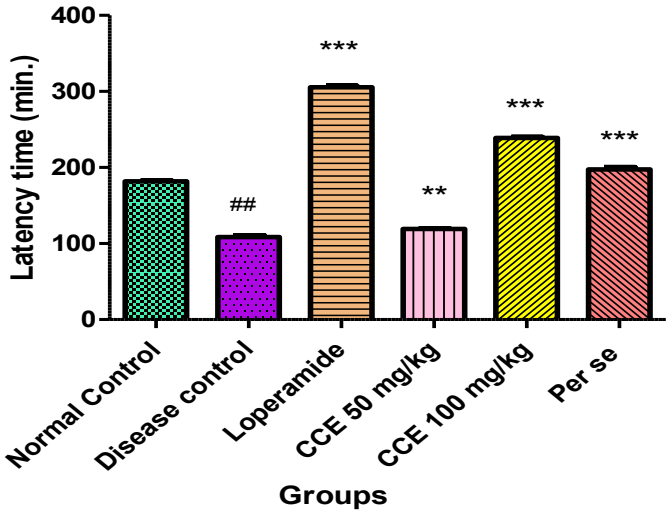


Figure 16: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on latency time (min.). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

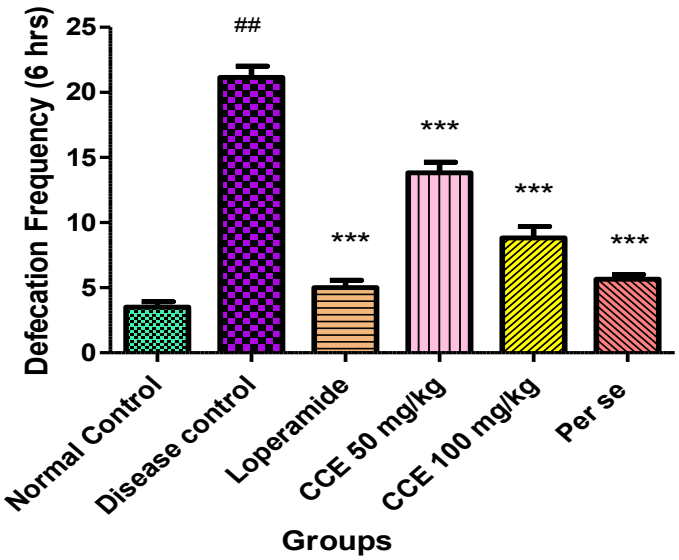


Figure 17: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on defecation frequency (h). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

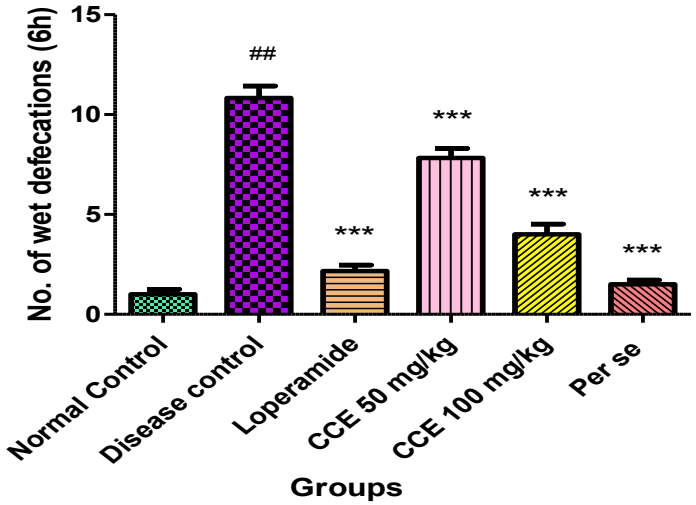


Figure 18: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on no. of wet defecations (h). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

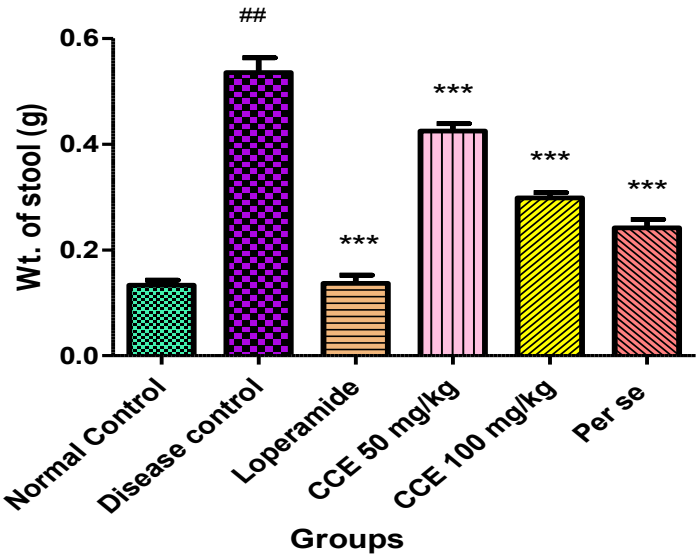


Figure 19: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on wt. of stool (g). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

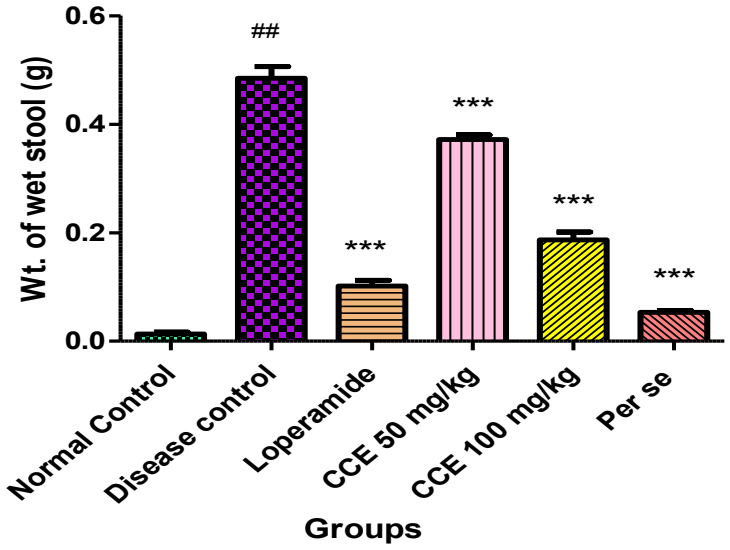


Figure 20: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on wt. of wet stool (g). Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

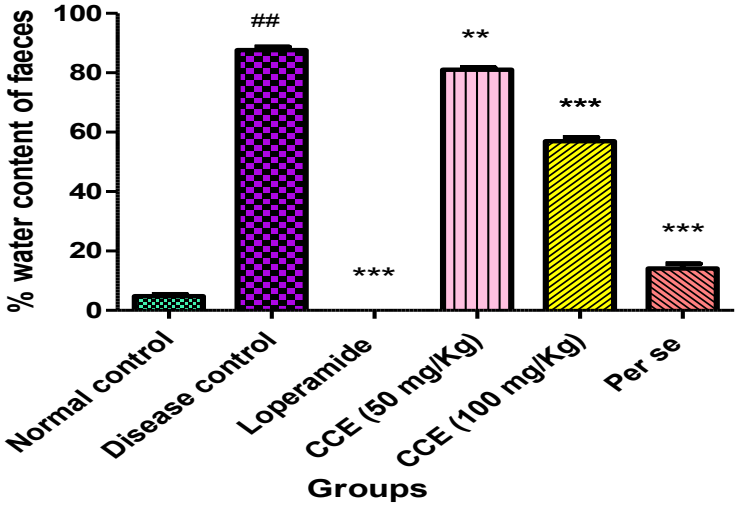


Figure 21: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on %water content of feces. Values are expressed as mean±SD (n=6); ##P<0.001 refers to significant difference compared normal control with disease control; **:P<0.01; ***:P<0.001 refers to significant difference compared disease control with Loperamide and extract treated groups.

Table 11: Effects of hydroalcoholic extract of *Citrullus colocynthis* obtained from Jodhpur district on gastrointestinal motility with Barium Sulfate milk model on rats.

Group	Dose (mg/Kg)	Length of GIT (cm)	Distance passed by $BaSO_4$ (cm)	$BaSO_4$ Transverse (%)	Inhibition (%)
Control	2 ml/rat	120±2.50	66.31±0.84	55.25	
Loperamide	2 mg/Kg	109.3±4.13	40.31±1.36	36.88***	33.24
CCE	100 mg/Kg	97.04±3.69	41.24±0.87	42.49***	23
	50 mg/Kg	104.91±1.74	51.17±1.02	48.77***	11.7
Per se	100 mg/Kg of CCE	102.68±1.34	43.98±1.77	42.83***	22.4

Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

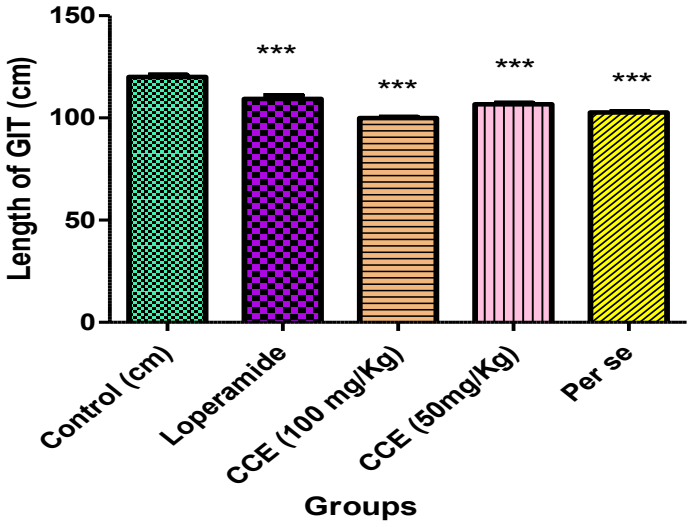


Figure 22: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on length of GIT (cm). Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

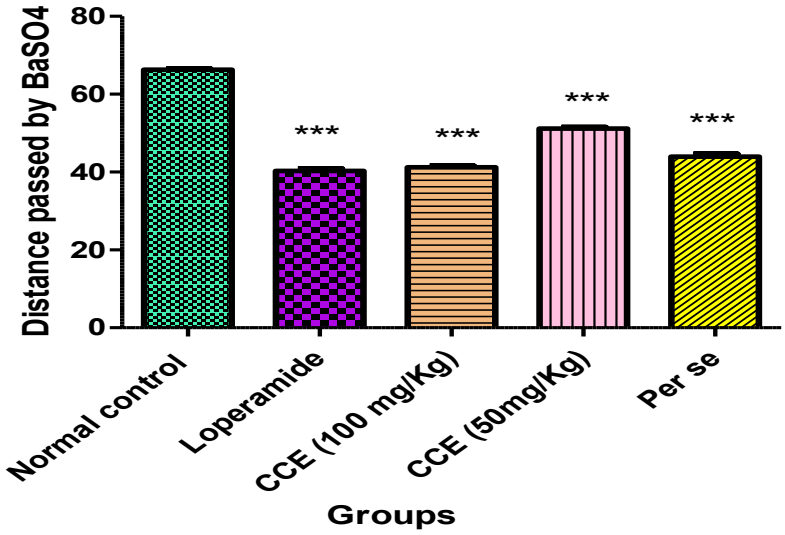


Figure 23: Effect of *Citrullus colocynthis* (obtained from Jodhpur district) extract on distance passed by $BaSO_4$. Values are expressed as mean±SD (n=6); **:P<0.01; ***:P<0.001 refers to significant difference compared control with Loperamide and extract treated groups.

Evaluation of antiarthritic activity

5.7 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR DISTRICT) ON ARTHRITIC SCORE

All the groups of animals administered with FCA started showing signs of clinical inflammation i.e. swelling and rigidity in one or more hind paws. The first manifestation of disease was erythema of one or more ankle joints followed by involvement of the metatarsal and interphalangeal joints. There was an initial development in the manifestations of inflammation from day 1 of administration to day 14, followed by a brief decrease in the inflammatory signs from day 14 to 28. A dose dependent decrease in inflammation was seen at *Capparis decidua* (200mg/Kg; P<0.001), 100 mg/Kg (P<0.01) and Diclofenac treated group from day 14 to day 28 as compared to FCA treated group (Figure 24).

Table 12: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on arthritic score in FCA induced arthritic model on rats.

Treatment	**Arthritic score**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	0	0	0	0	0	0	0	0	0
FCA (1 mg/ml)	0	3.83±2.31$^{\#\#}$	3±2.09$^{\#\#}$	3.83±1.47$^{\#\#}$	4±1.26$^{\#\#}$	4±1.67$^{\#\#}$	3.83±1.47$^{\#\#}$	1.47±0.6$^{\#\#}$	3.83±1.47$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	0	2.5±0.54	2.16±1.16	3.16±1.16	4±0.63	1.66±1.21**	1.33±1.36***	0.75±0.3***	0.83±0.75***
***Capparis decidua* (100 mg/Kg)**	0	1.66±0.51	2.33±0.81	3.33±1.63	4±1.26	2.16±0.98*	2±0.63*	0.75±0.3**	1.83±0.75**
***Capparis decidua* (200 mg/Kg)**	0	2.5±1.04	2.66±0.81	3.66±1.21	4±1.09	1.83±0.98**	1.66±0.81**	0.51±0.21***	0.83±0.4***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	0	0	0	0	0	0	0	0	0

Effect of *Capparis decidua* on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}$P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

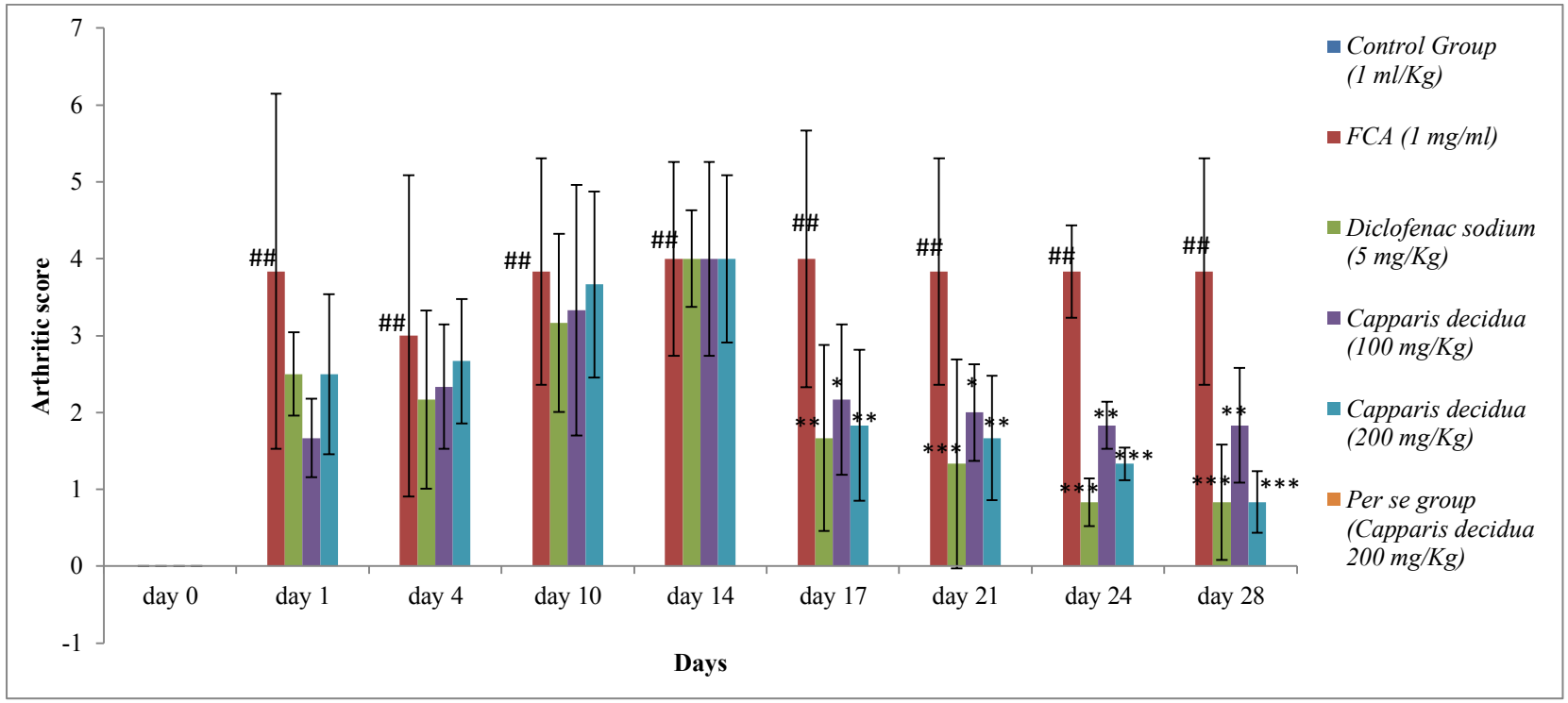


Figure 24: Effect of *Capparis decidua* (obtained from Jaipur) on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. ##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

5.8 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR DISTRICT) ON PAW VOLUME

There was significant (P<0.001) increase in paw volume of all the rats treated with FCA compared to control groups rats. Hydroalcoholic extract of *Capparis decidua* (100and 200 mg/Kg) significantly (P<0.001) lowered the paw volume from day 14 onwards as compared to FCA control group. *Per se* group also showed significant (P<0.001) reduction in paw volume from day 4 onwards till 28th day. *Capparis decidua* extract at 100 mg/Kg was less effective initially (P< 0.05) till 14th day but thereafter showed more significant result (P<0.001). Diclofenac 5 mg/Kg showed significant (P<0.001) reduction in paw volume from day 4 onwards. **(Figure 25 and Figure 39)**

Table 13: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on paw volume (ml) in FCA induced arthritic model on rats.

Treatment	**Paw volume (ml)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	0.9±0.03	0.86±0.05	0.86±0.04	0.89±0.05	0.9±0.04	0.91±0.03	0.92±0.02	0.92±0.04	0.92±0.05
FCA (1 mg/ml)	1.13±0.02	1.15±0.02	1.16±0.01$^{\#\#}$	1.2±0.02$^{\#\#}$	1.29±0.03$^{\#\#}$	1.51±0.09$^{\#\#}$	1.56±0.06$^{\#\#}$	1.68±0.06$^{\#\#}$	1.84±0.06$^{\#\#}$
Diclofenac sodium (5 mg/kg)	1.03±0.02	1.03±0.01	1.05±0.01***	1.08±0.01***	1.15±0.02***	1.1±0.05***	0.79±0.05***	0.73±0.04***	0.66±0.03***
***Capparis decidua* (100 mg/kg)**	1.08±0.01	1.09±0.02	1.11±0.02*	1.13±0.06*	1.21±0.04***	1.16±0.05***	0.83±0.03***	0.79±0.05***	0.74±0.02***
***Capparis decidua* (200 mg/kg)**	1.03±0.02	1.04±0.02	1.06±0.02***	1.08±0.04***	1.19±0.02***	1.14±0.05***	0.8±0.04***	0.82±0.03***	0.81±0.03***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	0.86±0.02	0.88±0.01	0.92±0.03***	0.95±0.03***	0.96±0.01***	0.91±0.03***	0.89±0.06***	0.83±0.03***	0.83±0.03***

Effect of *Capparis decidua* on Paw volume test in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA. **P < 0.01 as compared to FCA. *P < 0.05 as compared to FCA.

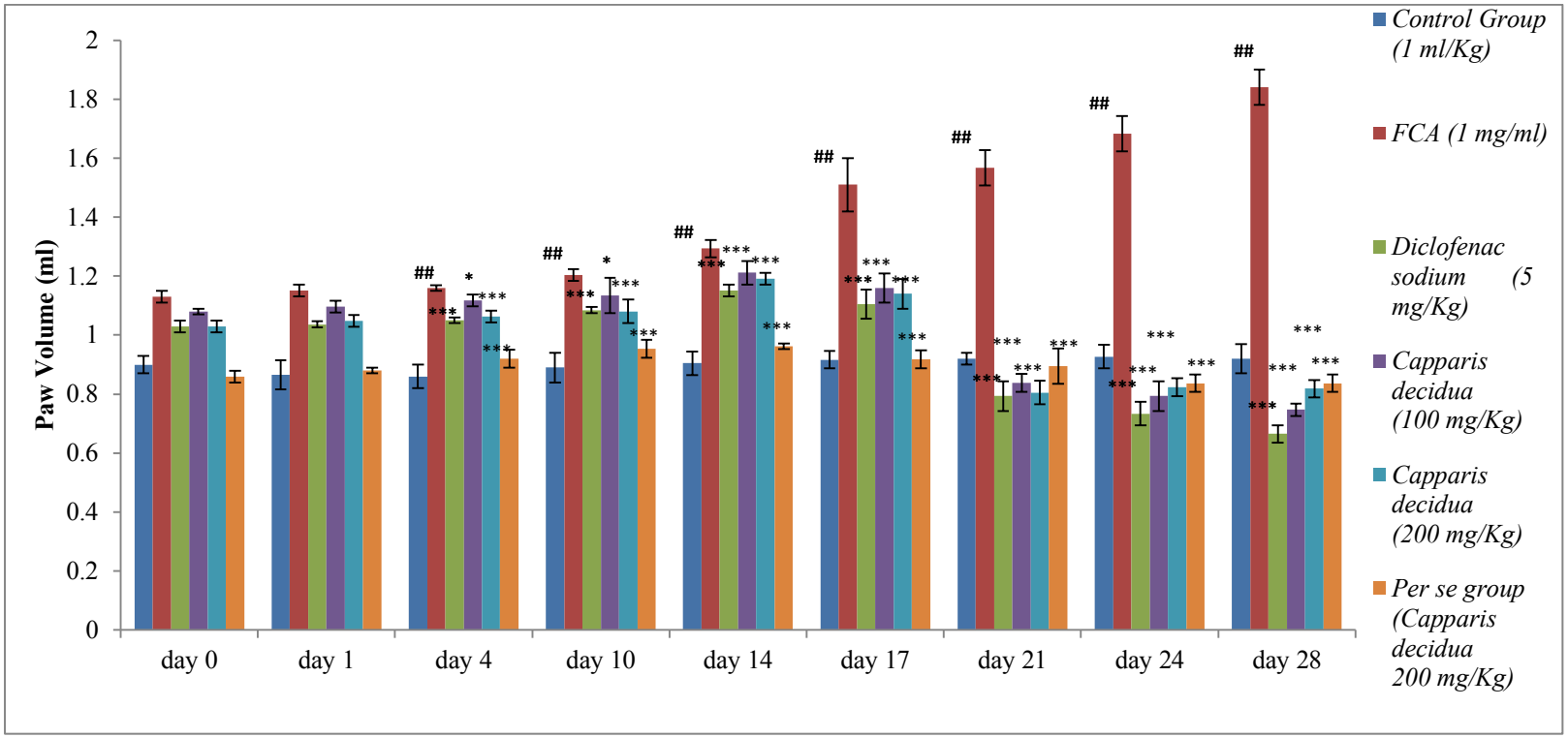


Figure 25. Effect of *Capparis decidua* (obtained from Jaipur) on Paw volume test in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA. **$P < 0.01$ as compared to FCA. *$P < 0.05$ as compared to FCA.

5.9 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR DISTRICT) ON NOCICEPTIVE THRESHOLD

There was consistent decrease in paw withdrawal threshold observed in FCA group rats compared to control animals and pain threshold was observed to be lowest on day 28. *Capparis decidua* treated (100 and 200 mg/Kg), Diclofenac treated group significantly ($P<0.001$) increased the pain threshold from day 14 to day 28, whereas *per se* group also showed significant ($P<0.001$) result in the pain threshold response as compared to FCA group animals (Figure 26).

Table 14: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on pain threshold (nociceptive threshold) in FCA induced arthritic model on rats.

Treatment	**Pain threshold (sec)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	8.8±0.49	8.8±0.49	8.62±0.63	8.76±0.37	8.68±0.45	8.44±0.31	8.68±0.55	8.65±0.39	8.78±0.36
FCA (1 mg/ml)	7.86±0.77	6.74±0.63$^{\#\#}$	5.93±0.7$^{\#\#}$	5.7±0.4$^{\#\#}$	4.84±0.28$^{\#\#}$	3.95±0.32$^{\#\#}$	3.76±0.37$^{\#\#}$	3.49±0.28$^{\#\#}$	3.21±0.19$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	7.78±0.81	7.27±0.13	7.21±0.13***	6.93±0.25***	6.4±0.37***	6.52±0.35***	7.68±0.45***	7.96± 0.22***	7.67± 0.21***
***Capparis decidua* (100 mg/Kg)**	7.73±0.74	6.97±0.33	6.96±0.3**	6.47±0.35*	6.28±0.41***	6.29±0.39***	6.31±0.38***	6.34± 0.39***	6.45±0.4***
***Capparis decidua* (200 mg/Kg)**	7.95±0.4	7.02±0.26	7.01±0.33**	6.54±0.43*	6.01±0.62***	6.26±0.44***	7.28±0.58***	7.47± 0.52***	7.47± 0.41***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	8.2±0.74	8.2±0.74	8.2±0.74***	8.03±0.7***	7.95±0.54***	8.02±0.55***	7.9±0.44***	8.08± 0.28***	8.14± 0.16***

Effect of *Capparis decidua* (obtained from Jaipur) on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA. **$P < 0.01$ as compared to FCA. *$P < 0.05$ as compared to FCA.

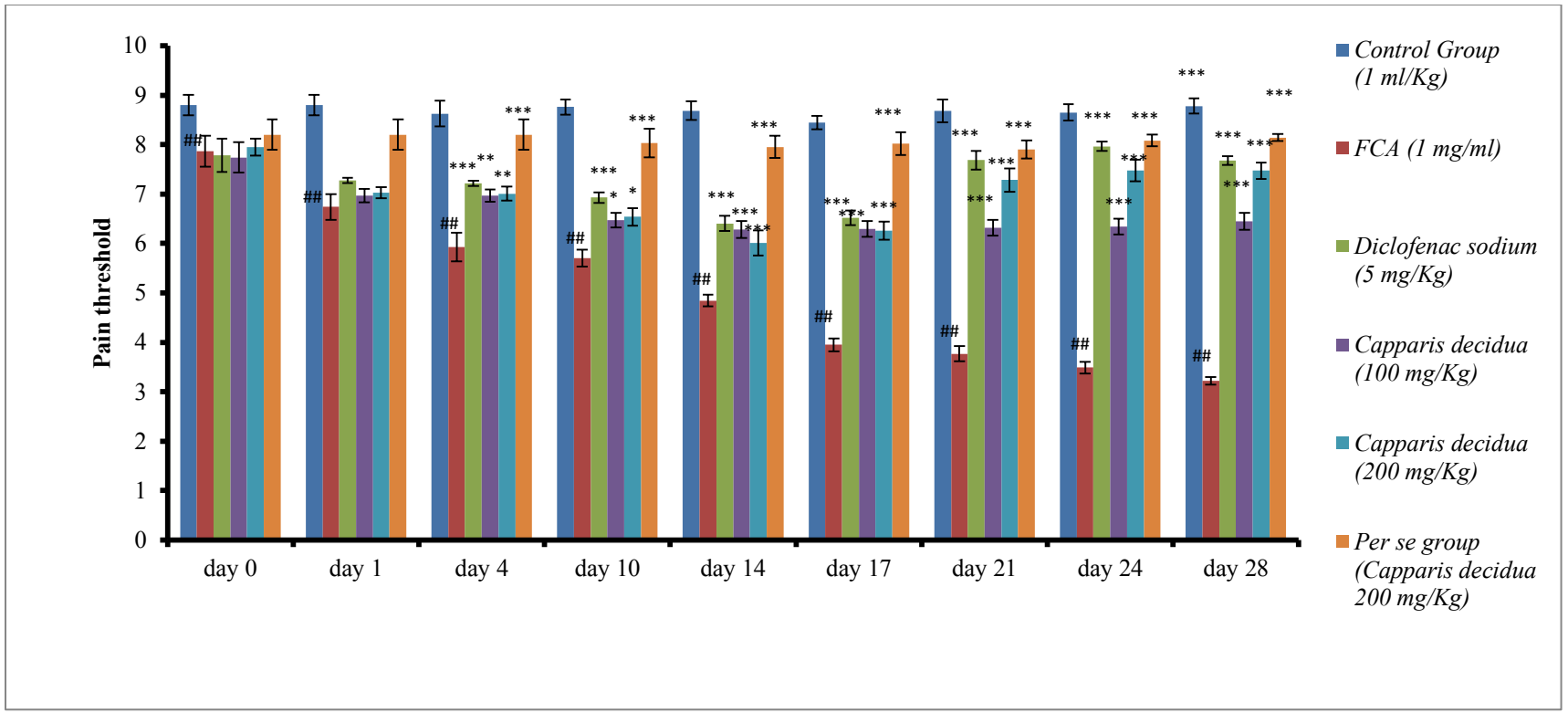


Figure 26: Effect of *Capparis decidua* (obtained from Jaipur) on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA. **$P < 0.01$ as compared to FCA. *$P < 0.05$ as compared to FCA.

5.10 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR) ON FALL OF TIME

Average fall off time in Rota rod test was determined for the assessment of motor in-coordination. Administration of FCA results in the decrease in fall off time in the FCA treated group as compared to the control group. *Capparis decidua* treated (100 and 200 mg/Kg), significantly (P<0.001) increased fall off time from day 14 till day 28 as compared to the FCA control group while Diclofenac (5 mg/Kg) treated group also showed significant (P<0.001) increase in fall off time but lesser than *Capparis decidua* (200 mg/Kg) as compared to FCA group animals (Figure 27).

Table 15: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on fall of time (muscle grip strength) in FCA induced arthritic model on rats.

Treatment	**Muscle grip strength (Fall of time in sec.)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	58±0.63	57.98±0.43	58.09±0.56	58±0.69	58±0.69	58.11±0.6	57.47±0.57	57.64±1.05	56.3±2.24
FCA (1 mg/ml)	57.33±0.98	40.88±0.38$^{\#\#}$	31.5±1.4$^{\#\#}$	22.78±0.8$^{\#\#}$	12.71±0.91$^{\#\#}$	20.45±2.35$^{\#\#}$	24.85±2.3$^{\#\#}$	24.32± 2.56$^{\#\#}$	20.86±1.03$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	57.25±0.93	48.23±0.32***	47.39±0.6***	44.05± 0.97***	47.21± 3.03***	45.68± 2.99***	44.66± 2.47***	44.09± 2.53***	44.87± 2.64***
***Capparis decidua* (100 mg/Kg)**	56.38±1.01	42.28±0.36*	35.43±0.75***	26.75± 1.51***	25.17± 2.95***	25.35± 2.94**	28.8±2.08**	30.82± 3.95**	30.45± 3.52***
***Capparis decidua* (200 mg/Kg)**	57.3±0.86	46.68±1.1***	43.11±0.58***	44.48± 0.74***	45.08± 1.66***	45.06± 1.33***	47.3±1.2***	47.39± 1.19***	47.16± 2.22***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	57.25±1.26	56.16±1.55***	55.75±1.55***	55.41± 1.44***	54.66± 2.25***	55±2.09***	54.66± 2.5***	56.5± 1.51***	56.5± 1.51***

Effect of *Capparis decidua* (obtained from Jaipur) on fall of time in motor incoordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}$P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

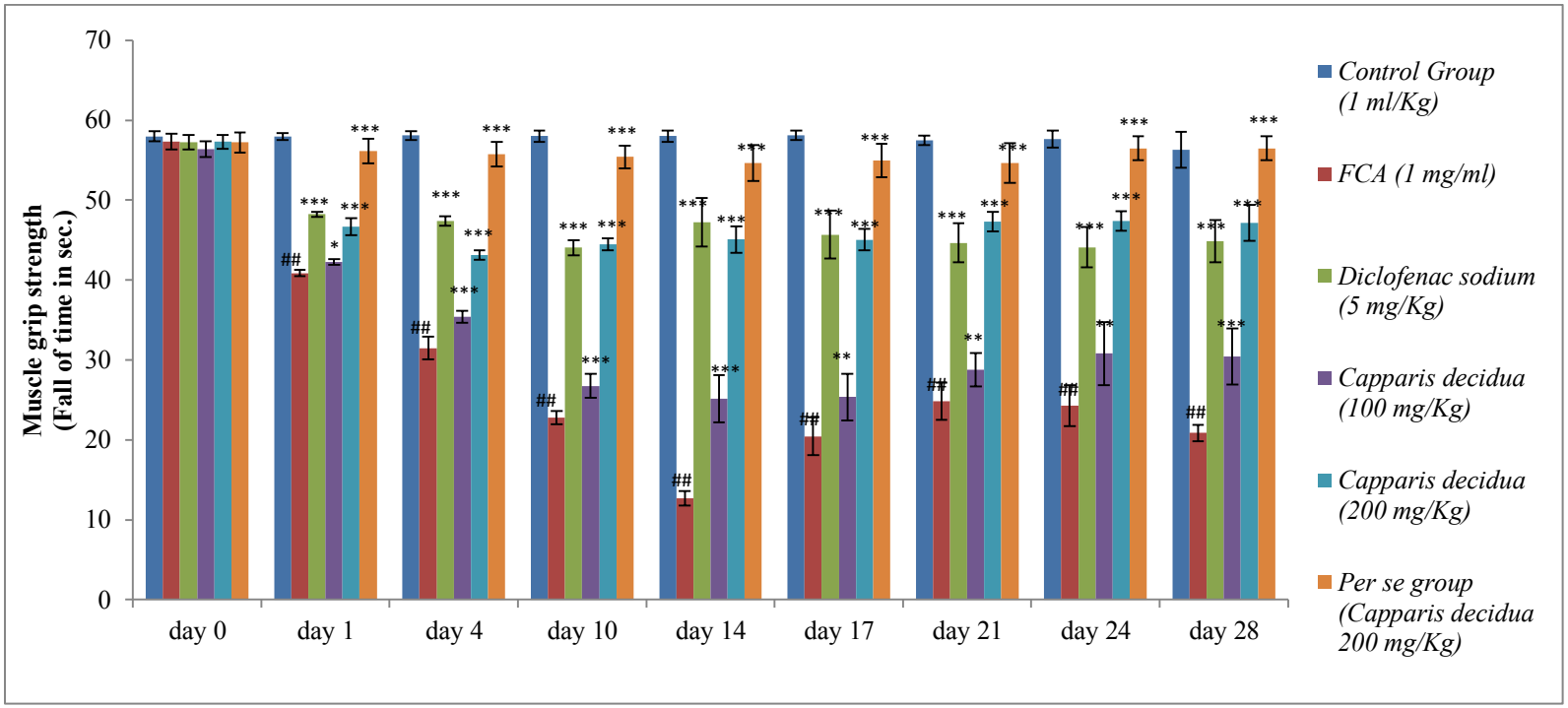


Figure 27: Effect of *Capparis decidua* (obtained from Jaipur) on fall of time in motor in-coordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.D (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}P<0.001$ as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

5.11 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR) ON BODY WEIGHT

The rats in the FCA treated group lost body weight as compared with the *Capparis decidua* extract treated and Diclofenac treated groups. The body weight of *Capparis decidua* at 100 mg/Kg, 200mg/Kg and *per se* significantly (P<0.001) increased from day 17th onwards till day 28th as compared to FCA treated group rats. While Diclofenac treated groups also showed the significant result (P<0.001) from day 17th onwards till day 28th as compared to FCA treated group rats. The effect produced by *Capparis decidua* extract at 100 mg/Kg and 200 mg/Kg produced a similar result as seen in a Diclofenac-treated group on days 17, 21, 24 and 28**. (Figure 28)**

Table 16: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on body weight in FCA induced arthritic model on rats.

Treatment	**Body weight**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	176±3.46	176±4.14	176.5±4.23	177.33±4.88	178.83±4.4	179.66±5.5	181.83±5.19	183.83±5.07	188±6.26
FCA (1 mg/ml)	170.66±1.96	169.5±2.58	164.66±4.13$^{\#\#}$	164.33±3.07$^{\#\#}$	160.5±2.88$^{\#\#}$	158.5±2.88$^{\#\#}$	157.66±3.9$^{\#\#}$	151.66± 3.01$^{\#\#}$	150.5±4.67$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	167.33±3.14	166.66±3.44	168±3.4	165.5±2.81	163.33±2.58	170.66± 5.6***	173.33±5.6	176.83± 4.3***	180.5±4.6***
***Capparis decidua* (100 mg/Kg)**	167±2.82	166.5±2.88	166.5±4.76	164.66±3.77	162.33±2.73	169.66± 5.31***	172.16±4.8	175.83± 3.31***	178.33± 2.1***
***Capparis decidua* (200 mg/Kg)**	168.66±2.25	168.33±1.63	167.5±3.61	165.16±2.78	163±1.89	171.16± 2.71***	173.5±2.3	176.5± 2.42***	180.16± 1.1***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	163.16±1.16	162.66±1.03	164.66±3.77	166.16±3.25	168.66±2.33	171.16± 2.85***	173.66±1.63	176.16± 2.92***	178.83± 2.6***

Effect of *Capparis decidua* on body weight (g) in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. $^{\#\#}$P<0.001 as compared to control. ***P < 0.001 as compared to FCA treated.

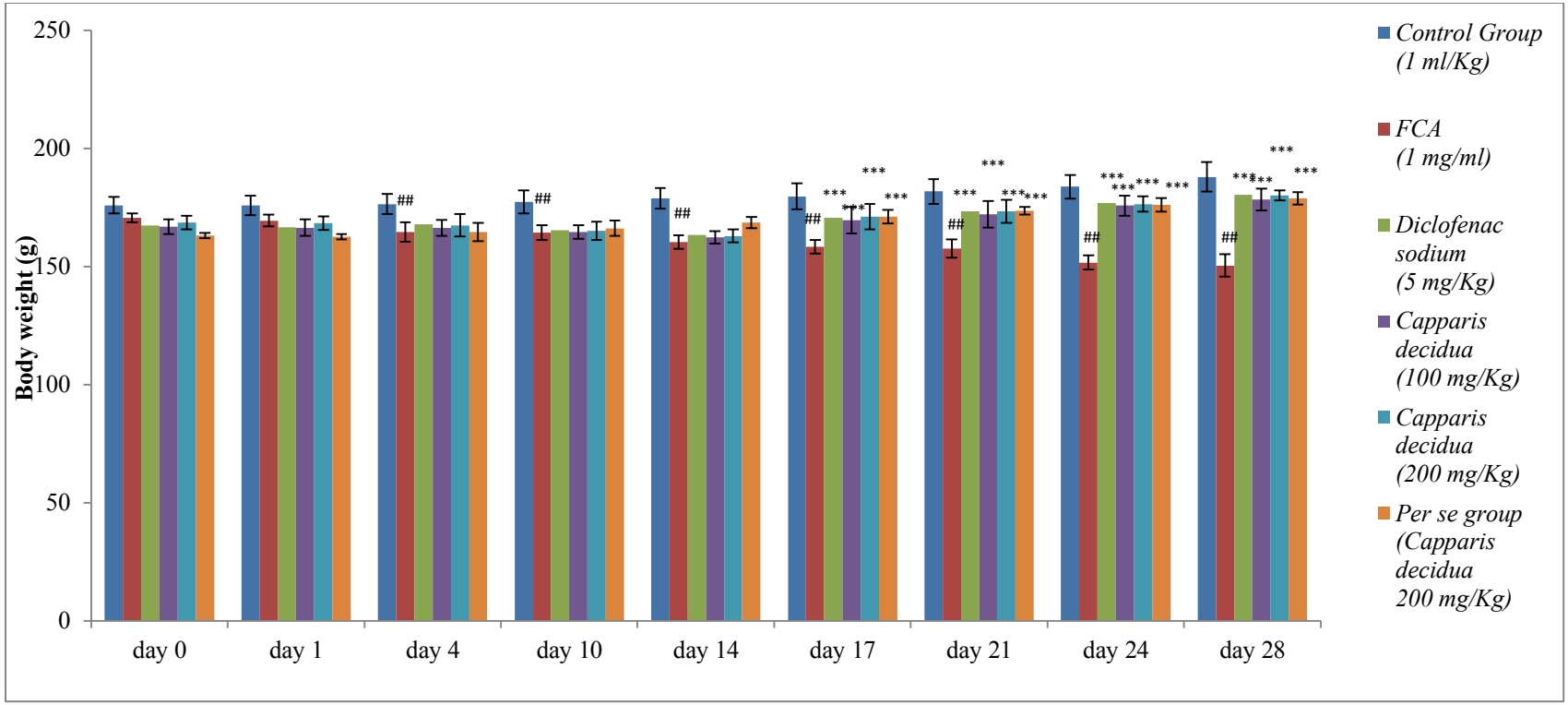


Figure 28: Effect of *Capparis decidua* (obtained from Jaipur) on body weight (g) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. $^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

5.12 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR DISTRICT) ON PAW JOINT DIAMETER.

There was significant (P<0.001) increase in joint diameter of rats of all the groups from day 1 till day 14 treated with FCA compared to control group. *Capparis decidua* (100 and 200 mg/Kg) significantly (P<0.01 and P<0.001, respectively), decreased the joint diameter from day 14 till day 28 as compared to FCA group. Diclofenac (5 mg/Kg) treated group also showed significant reduction in paw diameter as compared to FCA group rats **(Figure 29).**

Table 17: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on paw joint diameter in FCA induced arthritic model on rats.

Treatment	**Paw Joint Diameter (mm)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23	3.46±0.23
FCA (1 mg/ml)	4.11±0.25	4.6±0.15	4.7±0.15$^{\#\#}$	4.9±0.15$^{\#\#}$	6.7±0.15$^{\#\#}$	5.7±0.15$^{\#\#}$	5.21±0.18$^{\#\#}$	4.8±0.15$^{\#\#}$	4.8±0.15$^{\#\#}$
Diclofenac sodium (5 mg/kg)	4.08±0.51	4.13±0.25	4.23±0.25	4.43±0.25	6.23±0.25	5.23±0.25**	4.68±0.26**	4.23±0.25**	4.23±0.25***
***Capparis decidua* (100 mg/kg)**	3.86±0.43	4.08±0.14	4.18±0.14	4.36±0.13	6.18±0.14	5.18±0.14**	4.63±0.17**	4.3±0.2***	4.3±0.2**
***Capparis decidua* (200 mg/kg)**	3.88±0.27	3.98±0.29	4.08±0.29	4.3±0.28	6.11±0.27	5.08±0.27***	4.6±0.35***	4.18±0.29***	4.18±0.29***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	3.9±0.28	3.9±0.28	3.9±0.28	3.9±0.28	3.9±0.28	3.9±0.28***	3.9±0.28***	3.9±0.28	3.9±0.28***

Effect of *Capparis decidua* on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}$P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

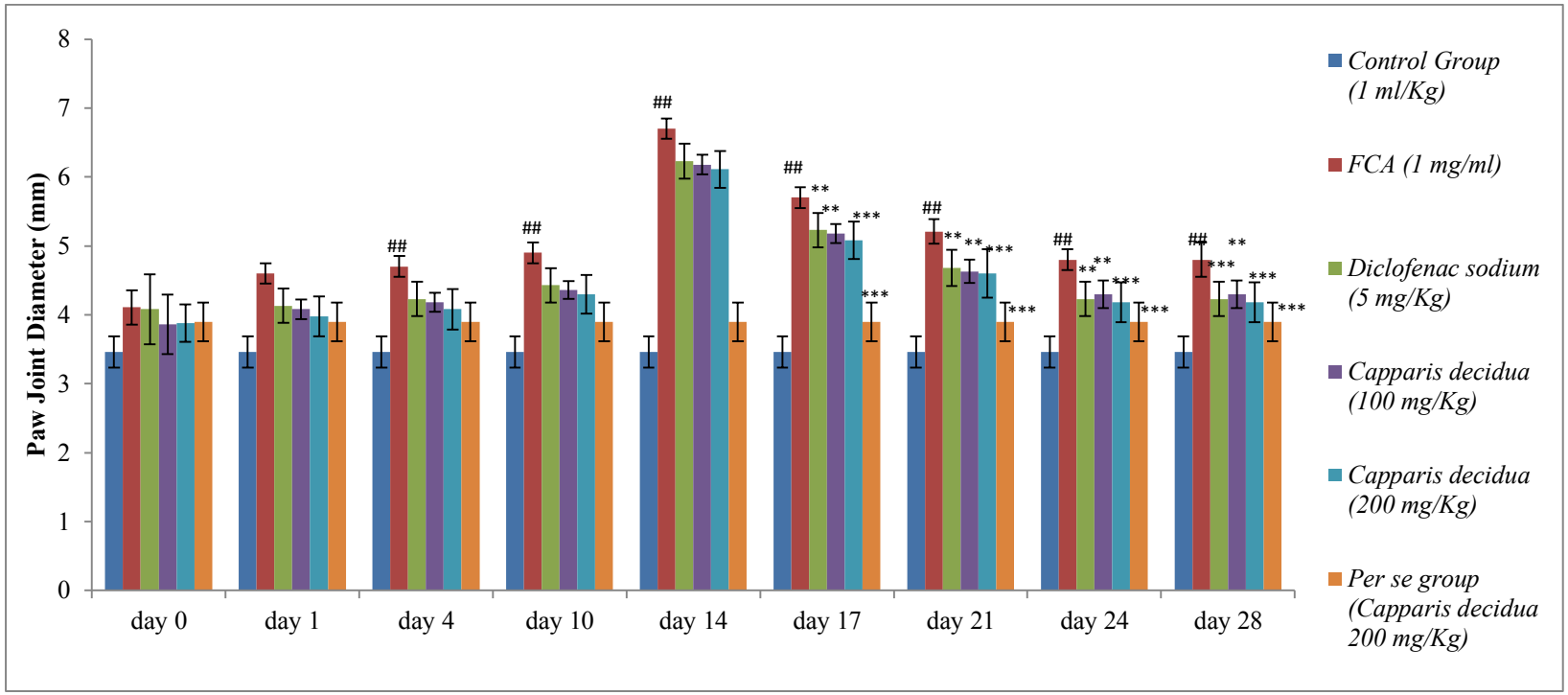


Figure 29: Effect of *Capparis decidua* (obtained from Jaipur) on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}P<0.001$ as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

5.13 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR) ON HEMATOLOGICAL PARAMETERS

The significant increase in levels of platelets (P<0.001), ESR count (P<0.001) and WBC (P<0.001) and significant decrease in levels of RBC (P<0.001) and Hb (P<0.001) were observed in FCA group as compared to normal control group indicating a stimulation of immune response towards FCA in arthritic rats. Treatment with hydroalcoholic extract of *Capparis decidua* (100 & 200 mg/Kg), *per se* group significantly (P<0.001) inhibited the stimulation of immune response towards FCA by decreasing blood WBC, ESR, and increasing Hb and RBC compared to FCA treated group. Diclofenac sodium treated rats also showed significant result (P<0.001) by reducing the WBC, ESR count and platelet and increasing hemoglobin and RBC levels **(Figure 30-34).**

Table 18: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on RBC count, WBC count, Platelet count, Hemoglobin count, ESR count in FCA induced arthritic model on rats.

Treatment	**RBC count (x 1000000 cells/mm3)**	**WBC count (x 1000/ml)**	**Platelet count (x 1000 cells/mm3)**	**Hb count (g/dL)**	**ESR count (mm/hr)**
Control Group (1 ml/Kg)	8.09±0.13	7.95±0.36	198±11.04	14.76±0.31	3.9±1.32
FCA (1 mg/ml)	4.03±0.65$^{\#\#}$	13.53±0.96$^{\#\#}$	443.33±12.11$^{\#\#}$	9.33±0.19$^{\#\#}$	9.65±0.64$^{\#\#}$
Diclofenac sodium (5 mg/kg)	7.13±0.33***	8.55±0.3***	275±18.97***	13.15±0.45***	3.76±0.21***
***Capparis decidua* (100 mg/kg)**	6.04±0.07***	11.86±0.99**	241.66±8.16***	11.82±0.09***	4.95±0.12***
***Capparis decidua* (200 mg/kg)**	8.3±0.41***	9.13±0.37***	213.83±7.78***	12.79±0.25***	4.16±0.11***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	7.88±0.31***	7.26±0.51***	197±10.91***	13.88±0.39***	3.7±0.37***

Effect of *Capparis decidua* on RBC count test in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA treated.

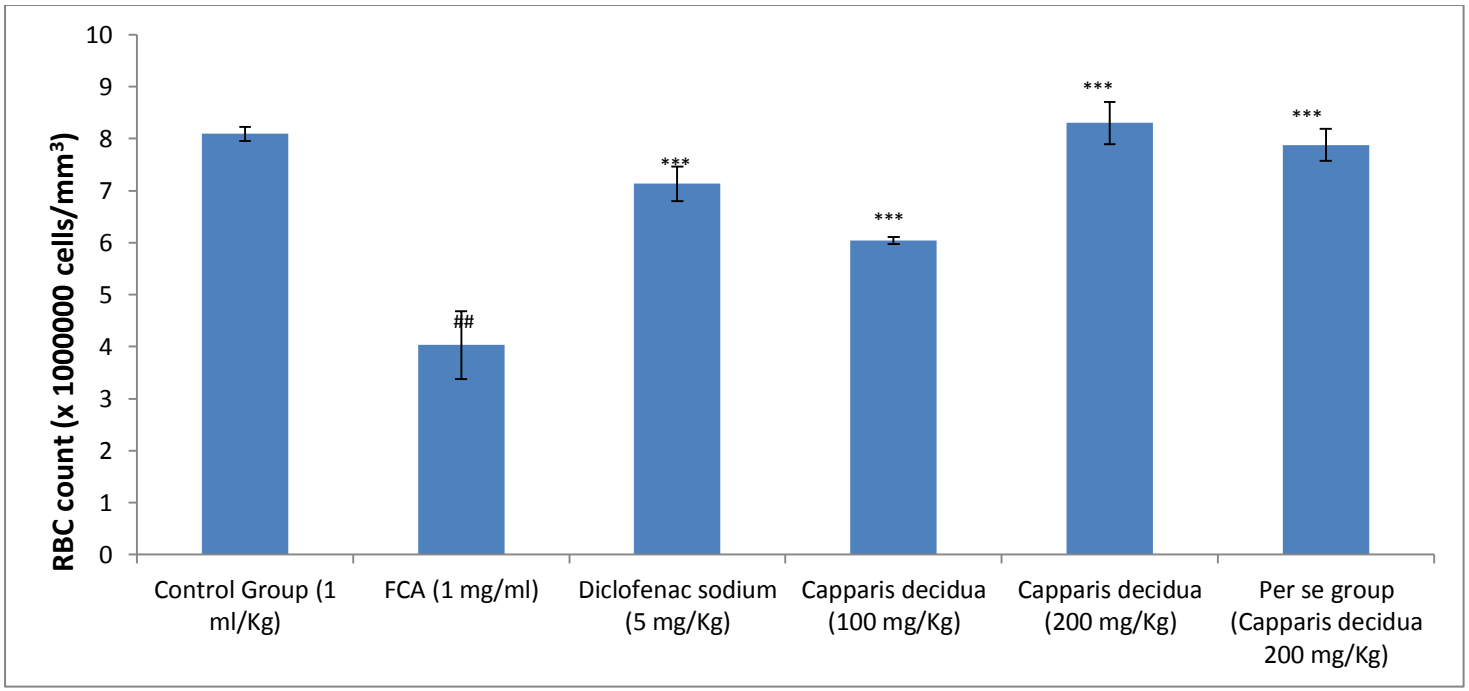


Figure 30: Effect of *Capparis decidua* (obtained from Jaipur) on RBC count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

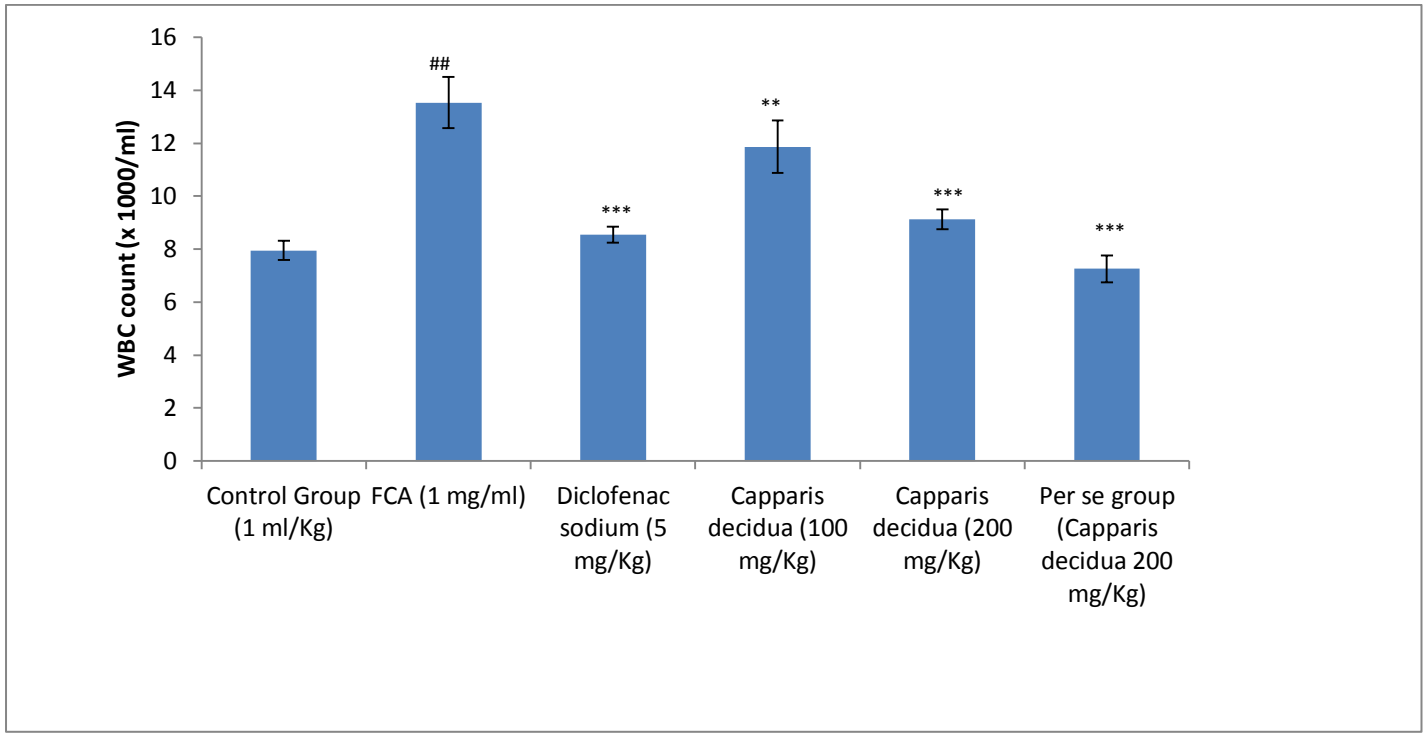


Figure 31: Effect of *Capparis decidua* (obtained from Jaipur) on WBC count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

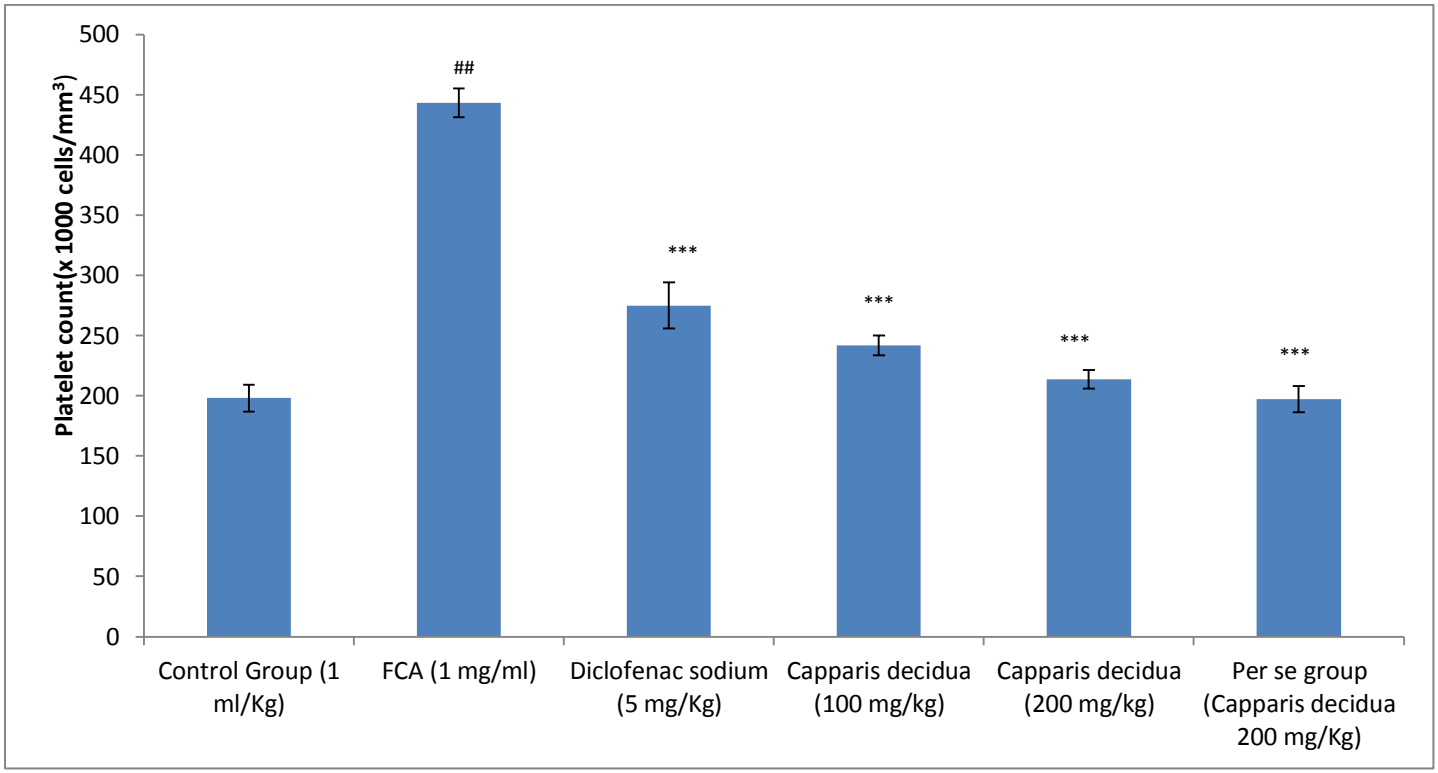


Figure 32: Effect of *Capparis decidua* (obtained from Jaipur) on Platelet count (x 1000 cells/mm3) test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

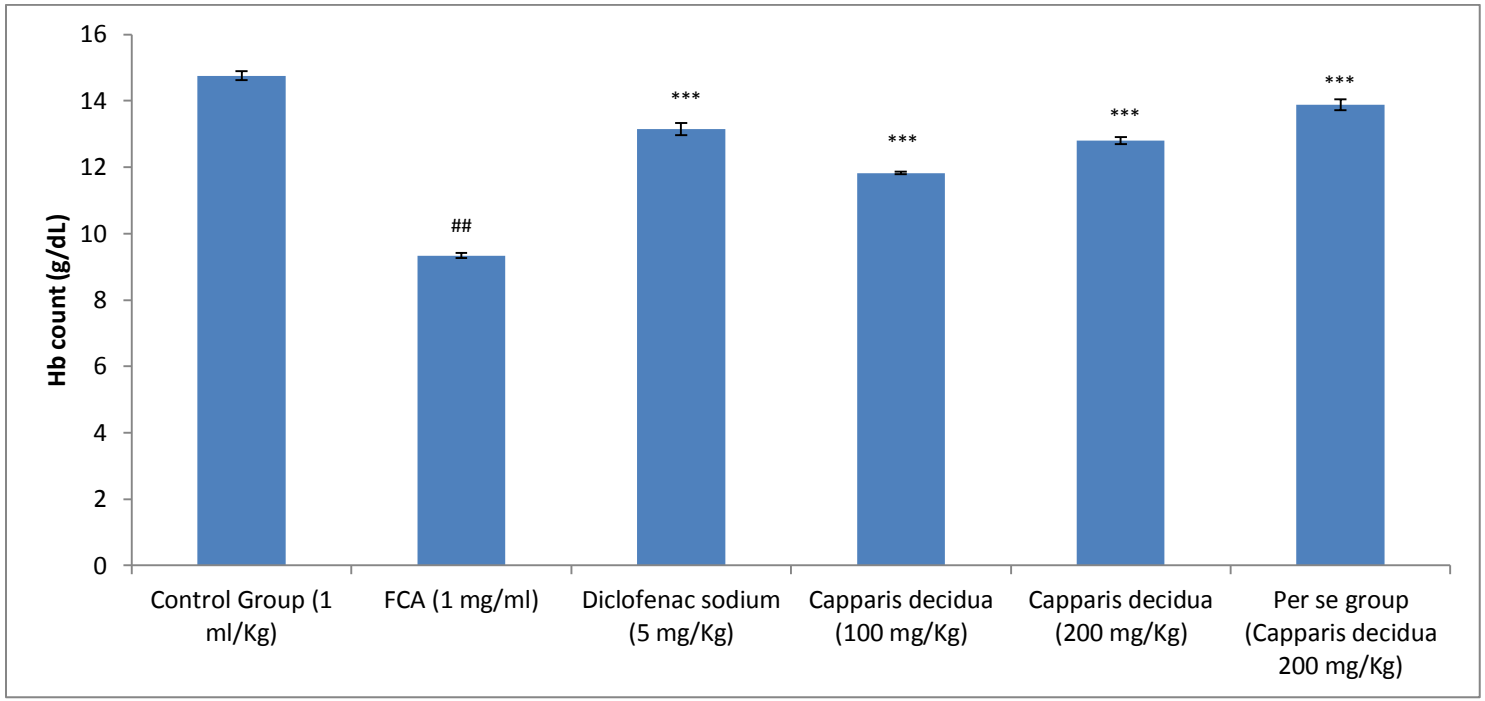


Figure 33: Effect of *Capparis decidua* (obtained from Jaipur) on Hemoglobin count (g/dL) count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

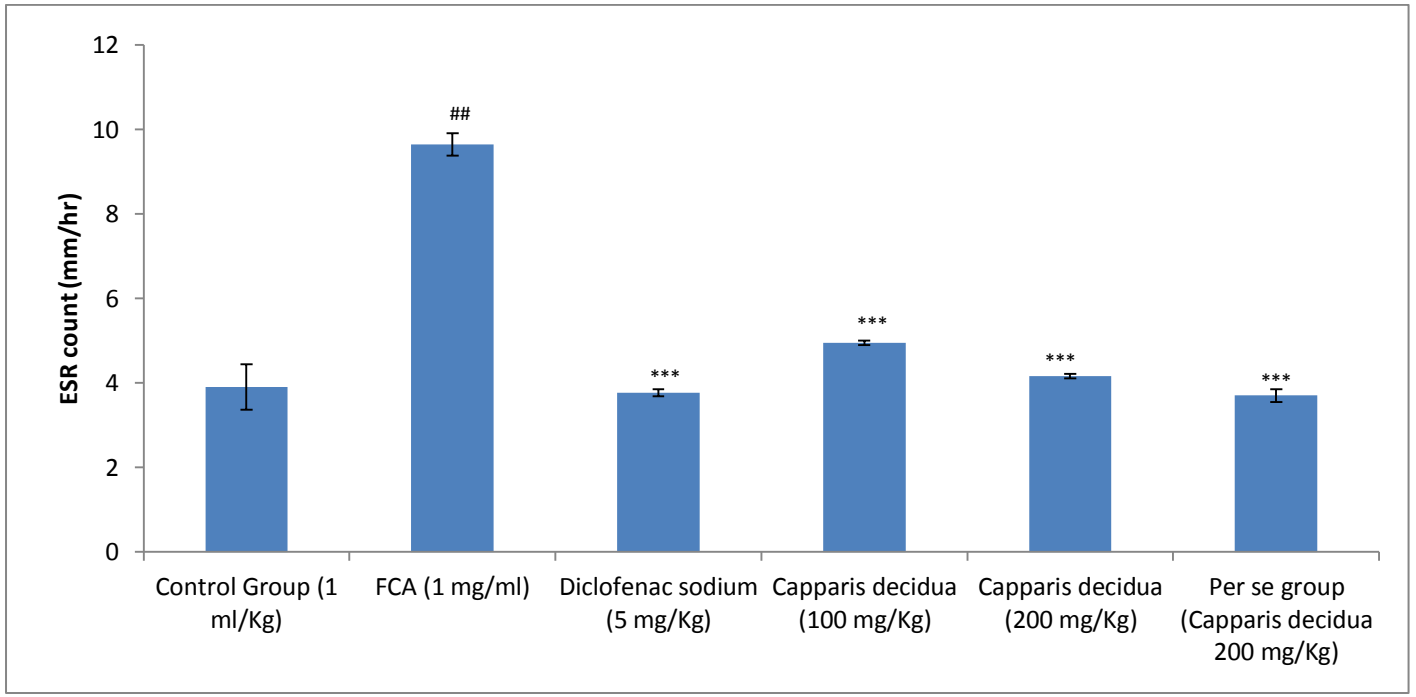


Figure 34: Effect of *Capparis decidua* (obtained from Jaipur) on ESR count mm/hr test in FCA-induced arthritic rats. Data are expressed as mean ± S.D (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

5.14 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JAIPUR DISTRICT) BIOCHEMICAL PARAMETERS

As a result of FCA-induced arthritis, the serum levels of AST, ALT and ALP were increased significantly (P<0.001) and total protein level was decreased significantly (P<0.001) in FCA group. These enzyme levels were altered by treatment with *Capparis decidua* (100 and 200 mg/Kg), and Diclofenac (5 mg/Kg) group. The level of AST, ALT and ALP were significantly (P<0.001) decreased by treatment with *Capparis decidua* (100 and 200 mg/Kg), and Diclofenac 5 mg/Kg and the level of total protein was significantly (P<0.001) increased in *Capparis decidua* (100 mg/Kg; P<0.01, 200mg/Kg; P<0.001) and Diclofenac group (P<0.001) as compared to FCA group (Figures 35-38).

Table 19: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jaipur) on AST, ALP, ALT, Total protein level in FCA induced arthritic model on rats.

Treatment	**AST/SGOT (IU/L)**	**ALP (IU/L)**	**ALT/SGPT (IU/L)**	**Total protein (g/dL)**
Control Group (1 ml/Kg)	74.33±3.55	67.33±3.26	48.5±2.88	6.56±0.22
FCA (1 mg/ml)	154.66±6.68$^{\#\#}$	167.83±3.31$^{\#\#}$	153.16±3.54$^{\#\#}$	5.01±0.14$^{\#\#}$
Diclofenac sodium (5 mg/kg)	88.83±2.13***	83.66±2.16***	56.5±4.23***	6.4±0.16***
***Capparis decidua* (100mg/kg)**	96.16±2.31***	97.16±1.47***	103.16±9.66***	5.38±0.14***
***Capparis decidua* (200mg/kg)**	91.83±1.47***	90±1.41***	63.33±5.04***	5.57±0.31***
***Per se* group (*Capparis decidua* 200mg/Kg)**	78.5±5.31***	83.33±2.94***	47.16±4.95***	6.14±0.13***

Effect of *Capparis decidua* on biochemical paremeters in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA treated.

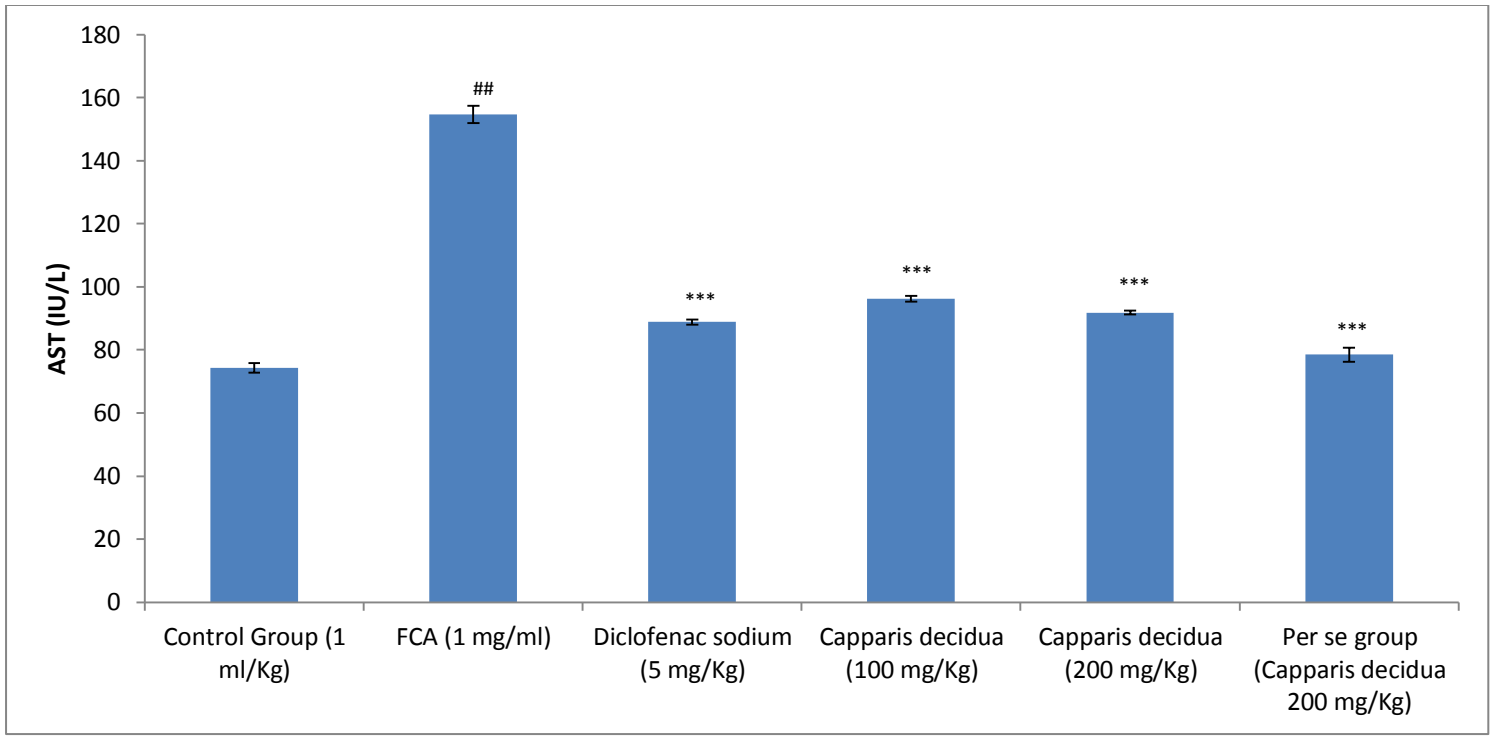


Figure 35: Effect of *Capparis decidua* (obtained from Jaipur) on AST (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

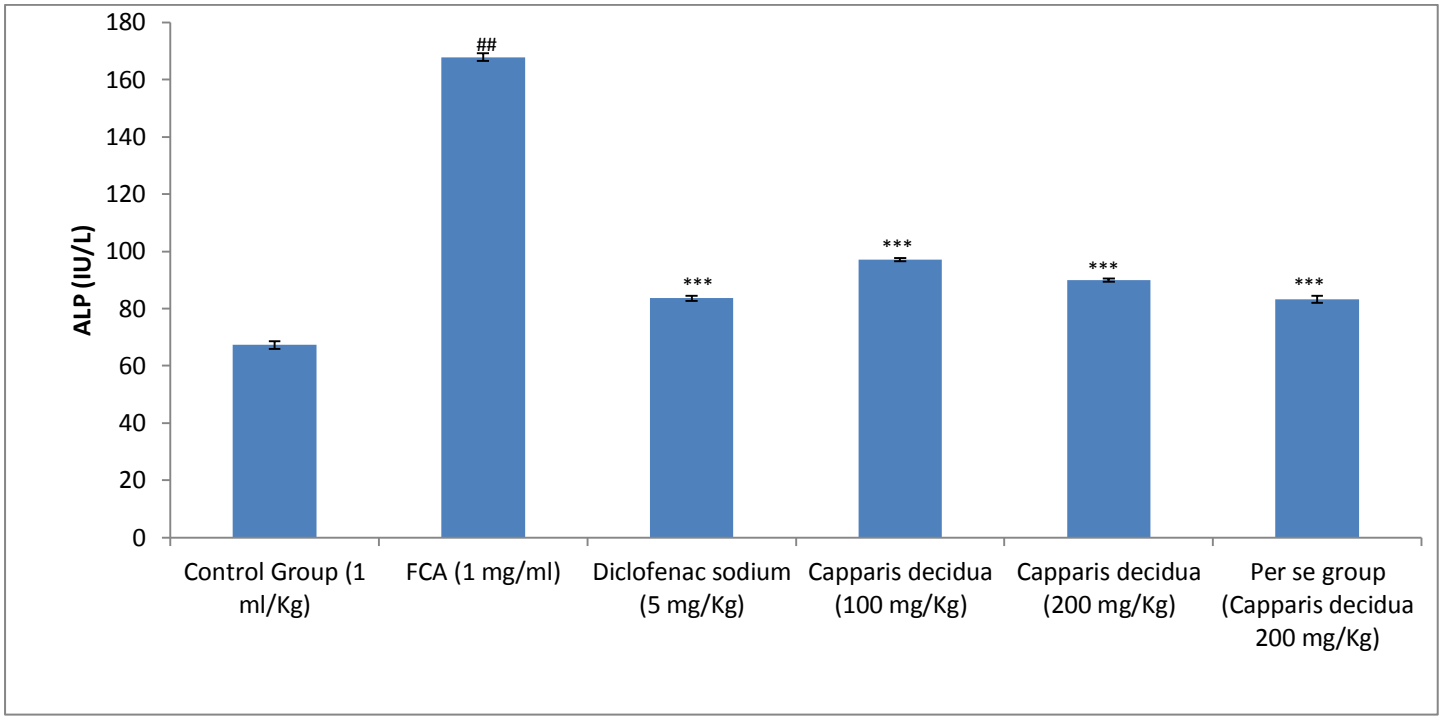


Figure 36: Effect of *Capparis decidua* (obtained from Jaipur) on ALP (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

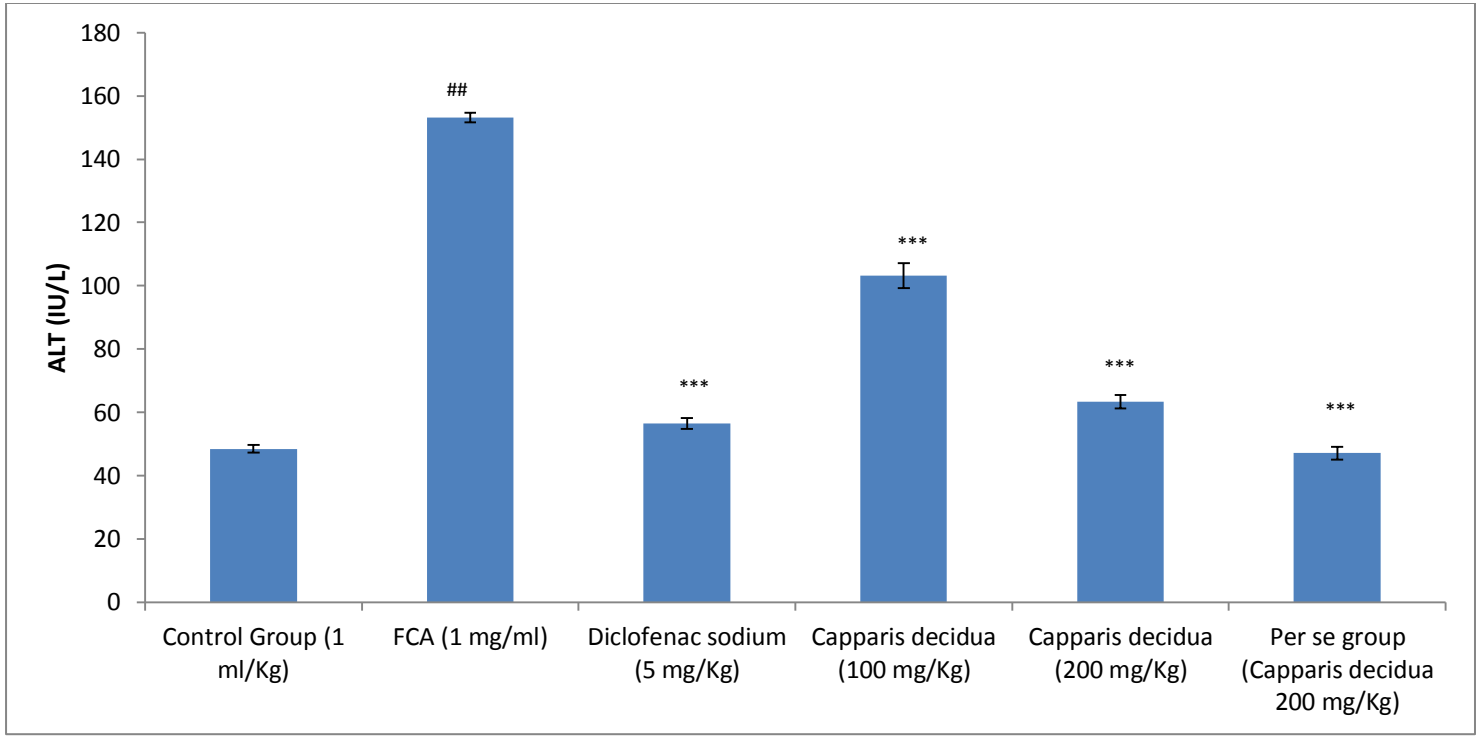


Figure 37: Effect of *Capparis decidua* (obtained from Jaipur) on ALT (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA

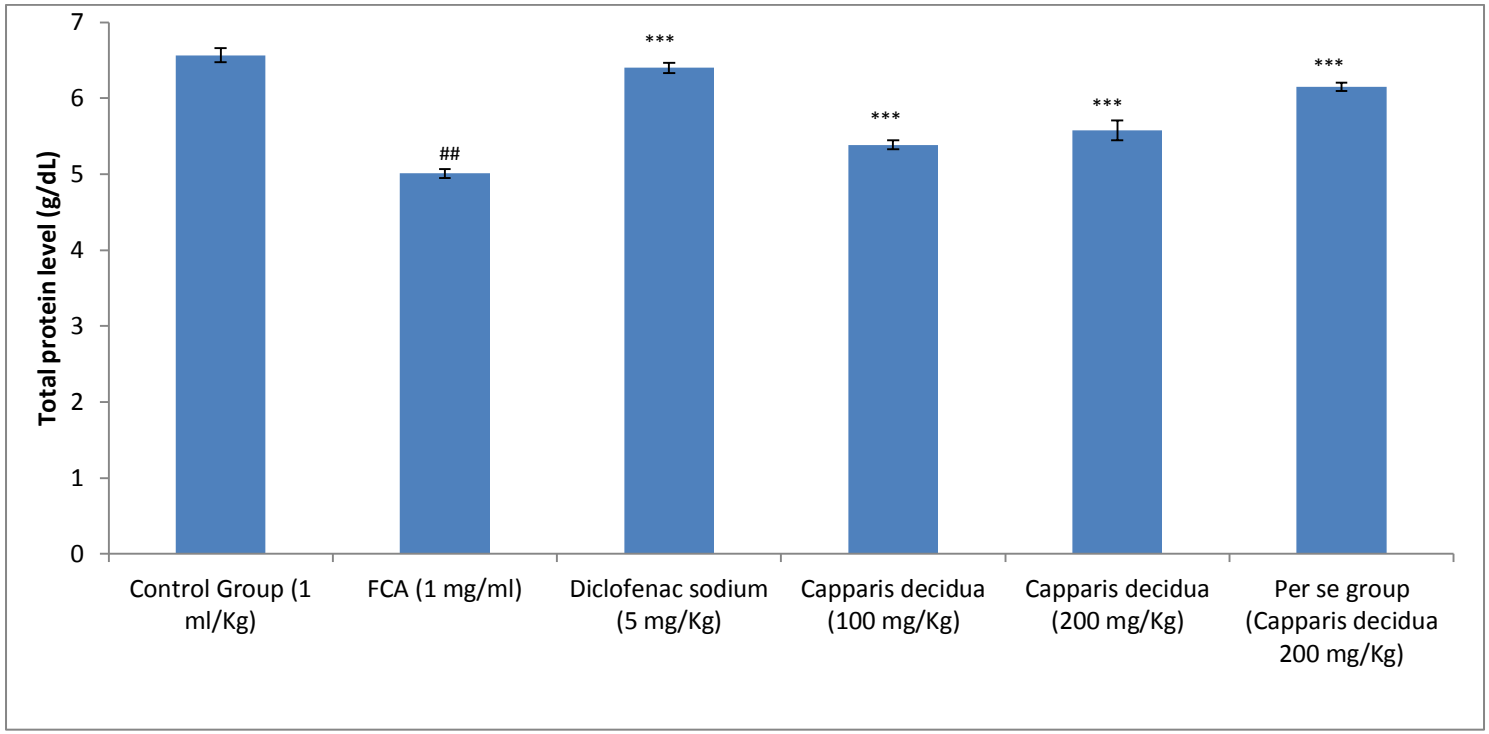
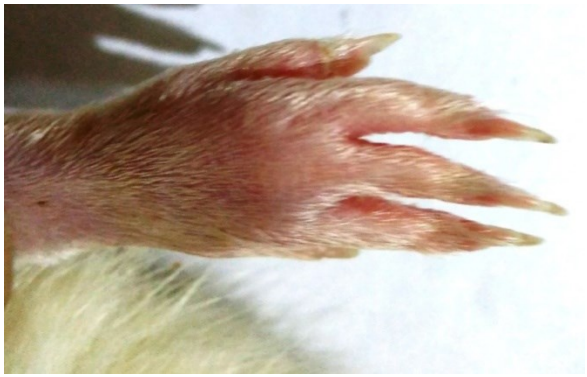
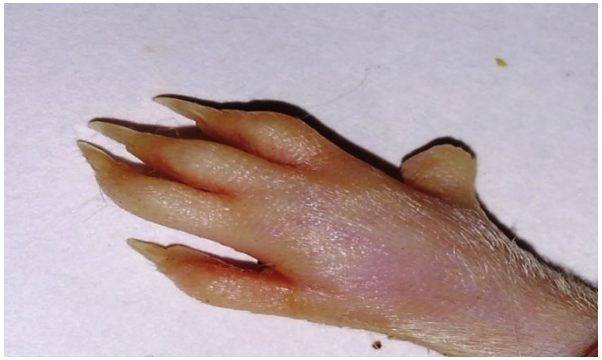


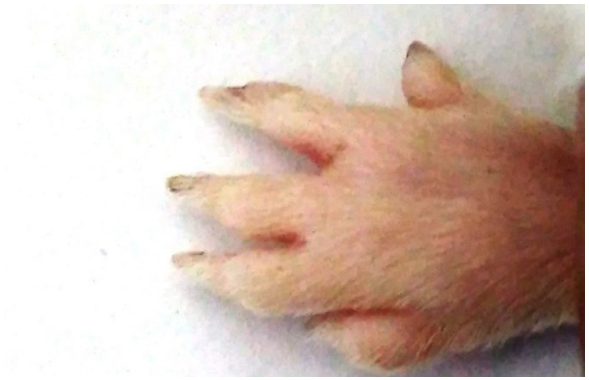
Figure 38: Effect of *Capparis decidua* (obtained from Jaipur) on Total Protein Level (g/dL) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.



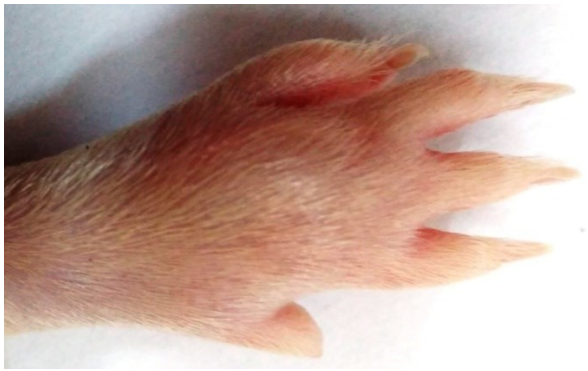
Day 0



Day 1

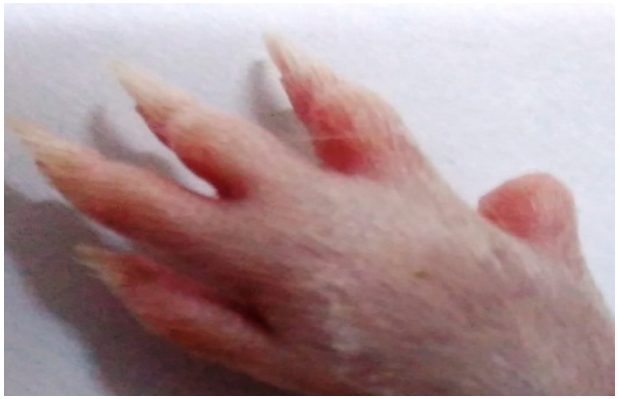


Day 4

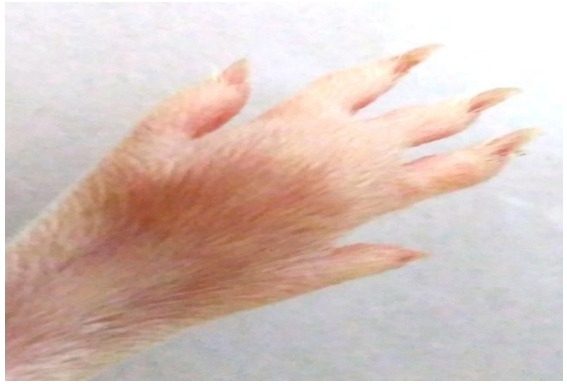


Day 10

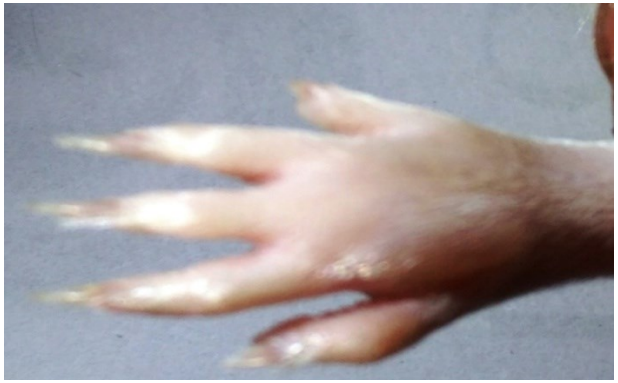
Figure 39: Representation of paw swelling in FCA induced arthritic rats showing varying degree of swelling in rats on days 0, 4, 10, 14, 17, 21, 24, 28.



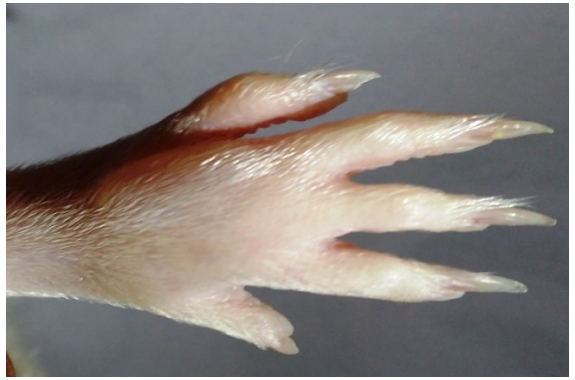
Day 14



Day 17



Day 24



Day 28

Figure 39: Representation of paw swelling in FCA induced arthritic rats showing varying degree of swelling in rats on days 0, 4, 10, 14, 17, 21, 24, 28.

Evaluation of anti-arthritic activity

5.15 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON ARTHRITIC SCORE

All the groups of animals administered with FCA started showing signs of clinical inflammation i.e. swelling and rigidity in one or more hind paws. The first manifestation of disease was erythema of one or more ankle joints followed by involvement of the metatarsal and interphalangeal joints. There was an initial development in the manifestations of inflammation from day 1 of administration to day 14, followed by a brief decrease in the inflammatory signs from day 14 to 28. A dose dependent decrease in inflammation was seen at *Capparis decidua* (200mg/Kg; P<0.001), 100 mg/Kg (P<0.01) and Diclofenac treated group (P<0.001) from day 17 to day 28 as compared to FCA treated group **(Figure 40).**

Table 20: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on arthritic score in FCA induced arthritic model on rats.

Treatment	**Arthritic score**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	0	0	0	0	0	0	0	0	0
FCA (1 mg/ml)	0	2±0.63	2.16±0.75$^{\#\#}$	3±0.63$^{\#\#}$	3.33±0.81$^{\#\#}$	4±0.63$^{\#\#}$	4.33±0.51$^{\#\#}$	4.33±0.51$^{\#\#}$	4.33±0.51$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	0	1.66±0.51	1.66±0.51	2.66±0.51	3.66±0.51	2±0.63***	1.5±0.54***	1±0***	0.5±0.54***
***Capparis decidua* (100 mg/Kg)**	0	1.83±0.75	1.83±0.75	3±0.89	3.83±1.16	2.66±0.81**	1.83±0.4**	1.66±0.81**	1.33±0.51***
***Capparis decidua* (200 mg/Kg)**	0	1.66±0.51	1.83±0.75	2.83±0.4	3.66±0.51	2.33±0.51***	1.66±0.51***	1.33±0.51***	0.66±0.51***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	0	0	0	0	0	0	0	0	0

Effect of Capparis decidua on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. $^{\#\#}$P<0.001 as compared to control group. ***P<0.001 as compared to FCA group. **P<0.01 as compared to FCA group. *P<0.05 as compared to FCA group.

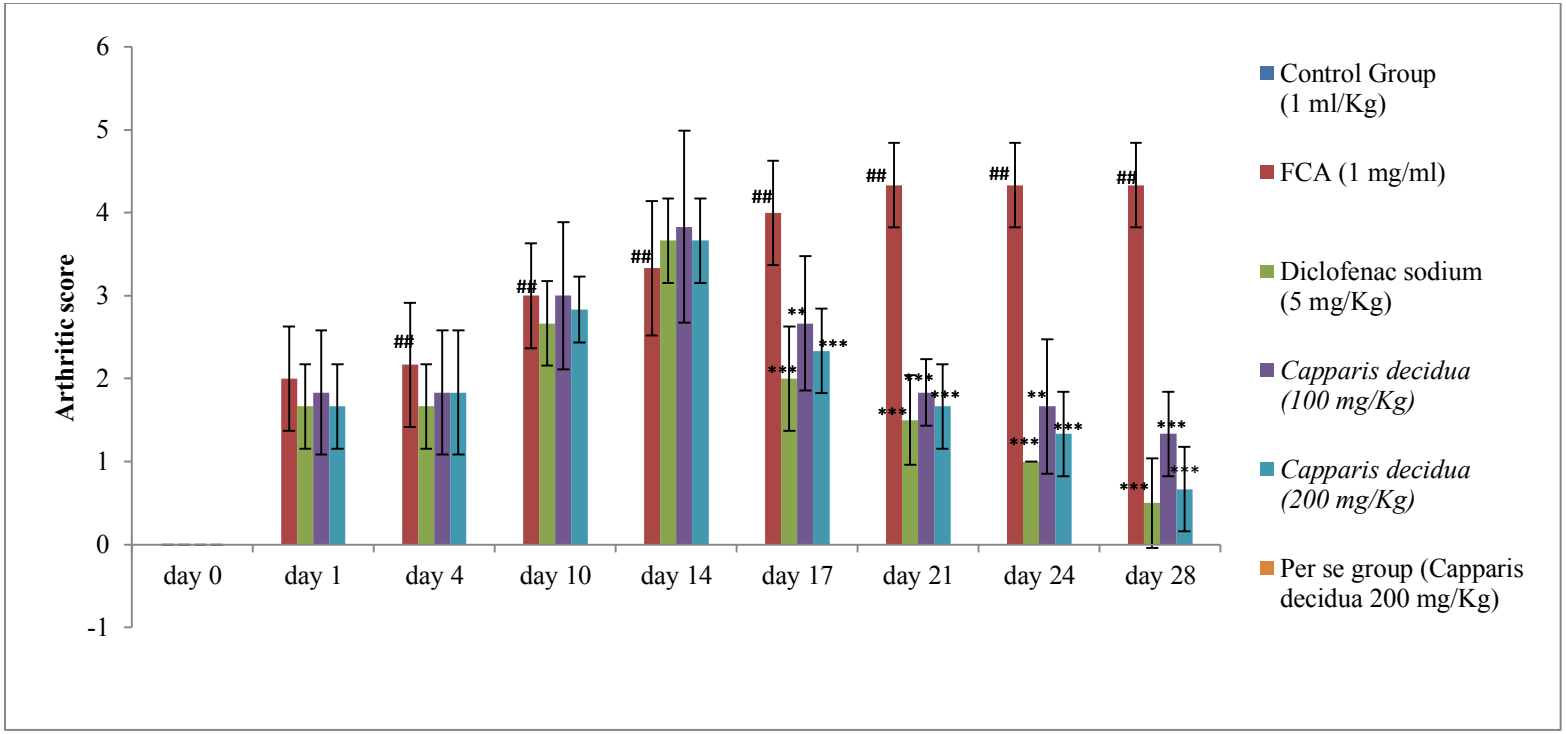


Figure 40: Effect of *Capparis decidua* (obtained from Jodhpur) on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison. ##P<0.001 as compared to control group. ***P<0.001 as compared to FCA group. **P<0.01 as compared to FCA group. *P<0.05 as compared to FCA group.

5.16 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON PAW VOLUME

There was significant (P<0.001) increase in paw volume of all the rats treated with FCA compared to control groups rats. Hydroalcoholic extract of *Capparis decidua* (100and 200 mg/Kg) significantly (P<0.001) lowered the paw volume from day 14 onwards as compared to FCA control group. *Per se* group also showed significant (P<0.001) reduction in paw volume from day 4 onwards till 28th day. *Capparis decidua* extract at 100 mg/Kg was effective initially on day 4 (P< 0.01) while on day 10 to day 28 showed more significant result (P<0.001). Diclofenac 5 mg/Kg showed significant (P<0.001) reduction in paw volume from day 4 onwards. **(Figure 41 and Figure 55)**

Table 21: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on paw volume (ml) in FCA induced arthritic model on rats.

Treatment	**Paw volume**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	0.9±0.03	0.86±0.05	0.87±0.03	0.88±0.05	0.9±0.05	0.89±0.04	0.87±0.04	0.89±0.05	0.88±0.05
FCA (1 mg/ml)	1.14±0.02	1.16±0.02	1.17±0.01$^{\#\#}$	1.21±0.02$^{\#\#}$	1.3±0.03$^{\#\#}$	1.43±0.03$^{\#\#}$	1.52±0.02$^{\#\#}$	1.65±0.02$^{\#\#}$	1.76±0.04$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	1.04±0.02	1.04±0.01	1.06±0.02***	1.09±0.01***	1.14±0.02***	1.11±0.07***	0.98±0.03***	0.84±0.14***	0.82±0.01***
***Capparis decidua* (100 mg/Kg)**	1.09±0.01	1.1±0.02	1.11±0.02**	1.12±0.01***	1.14±0.02***	1.13±0.02***	1.03±0.07***	0.93±0.08***	0.92±0.06***
***Capparis decidua* (200 mg/Kg)**	1.03±0.02	1.05±0.01	1.06±0.02***	1.07±0.02***	1.1±0.03***	1.1±0.04***	0.98±0.07***	0.88±0.1***	0.87±0.06***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	0.86±0.02	0.87±0.01	0.92±0.03***	0.91±0.03***	0.89±0.06***	0.92±0.03***	0.9±0.05***	0.87±0.05***	0.88±0.05***

Effect of *Capparis decidua* on Paw volume test in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA. **P < 0.01 as compared to FCA. *P < 0.05 as compared to FCA.

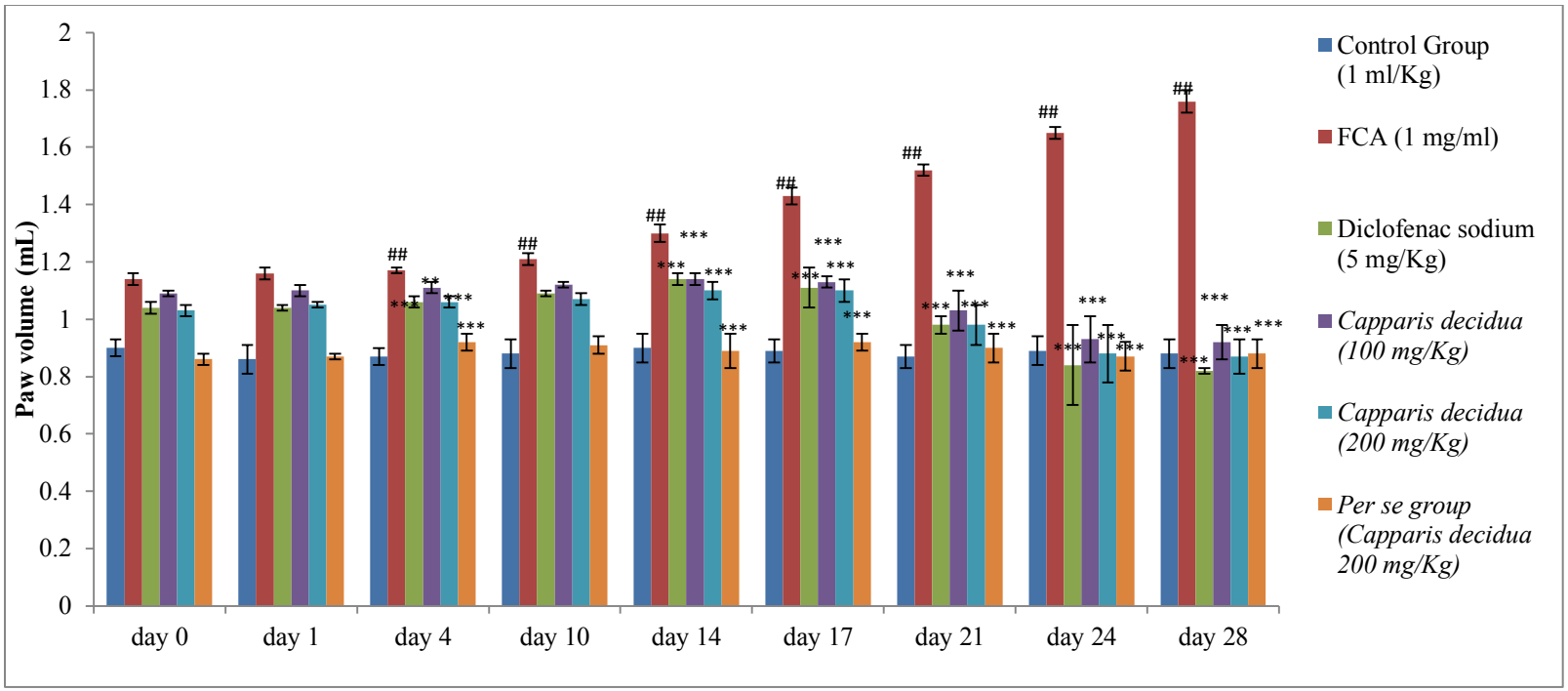


Figure 41: Effect of *Capparis decidua* (obtained from Jodhpur) on Paw volume test in FCA-induced arthritic rats. Data are expressed as mean ± SD (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA. **$P < 0.01$ as compared to FCA. *$P < 0.05$ as compared to FCA.

5.17 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON NOCICEPTIVE THRESHOLD

There was consistent decrease in paw withdrawal threshold observed in FCA group rats compared to control animals and pain threshold was observed to be lowest on day 28. *Capparis decidua* treated (100 and 200 mg/Kg), Diclofenac treated group significantly (P<0.001) increased the pain threshold from day 14 to day 28, whereas *per se* group also showed significant (P<0.001) result in the pain threshold response as compared to FCA group animals **(Figure 42).**

Table 22: Effect of hydroalcoholic extract of *Capparis deciduas* (obtained from Jodhpur) on pain threshold (nociceptive threshold) in FCA induced arthritic model on rats.

Treatment	**Pain Threshold**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	8.80±.47	8.8±0.47	8.78±0.49	8.77±0.37	8.65±0.43	8.47±0.3	8.72±0.6	8.68±0.41	8.95±0.62
FCA (1 mg/ml)	7.81±0.85	6.86±0.6$^{\#\#}$	5.94±0.7$^{\#\#}$	5.72±0.42$^{\#\#}$	4.84±0.28$^{\#\#}$	3.98±0.3$^{\#\#}$	3.79±0.33$^{\#\#}$	3.52±0.25$^{\#\#}$	3.26±0.17$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	7.76±0.72	7.33±0.13	7.23±0.13	6.95±0.29***	6.39±0.36***	6.55±0.39***	7.7±0.42***	7.99±0.19***	7.74±0.22***
***Capparis decidua* (100 mg/Kg)**	7.74±0.74	7.01±0.35	6.99±0.31	6.46±0.41*	6.26±0.38***	6.3±0.38***	6.35±0.36***	6.38±0.36***	6.51±0.36***
***Capparis decidua* (200 mg/Kg)**	7.96±0.48	7.02±0.24	7.02±0.32	6.72±0.32**	5.99±0.62***	6.34±0.42***	7.31±0.57***	7.5±0.55***	7.5±0.43***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	8.11±0.66	7.71±0.44	8.4±0.68	8.11±0.73***	7.95±0.54***	8.07±0.57***	7.88±0.43***	8.11±0.31***	8.13±0.17***

Effect of *Capparis decidua* on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA. **P < 0.01 as compared to FCA. *P < 0.05 as compared to FCA.

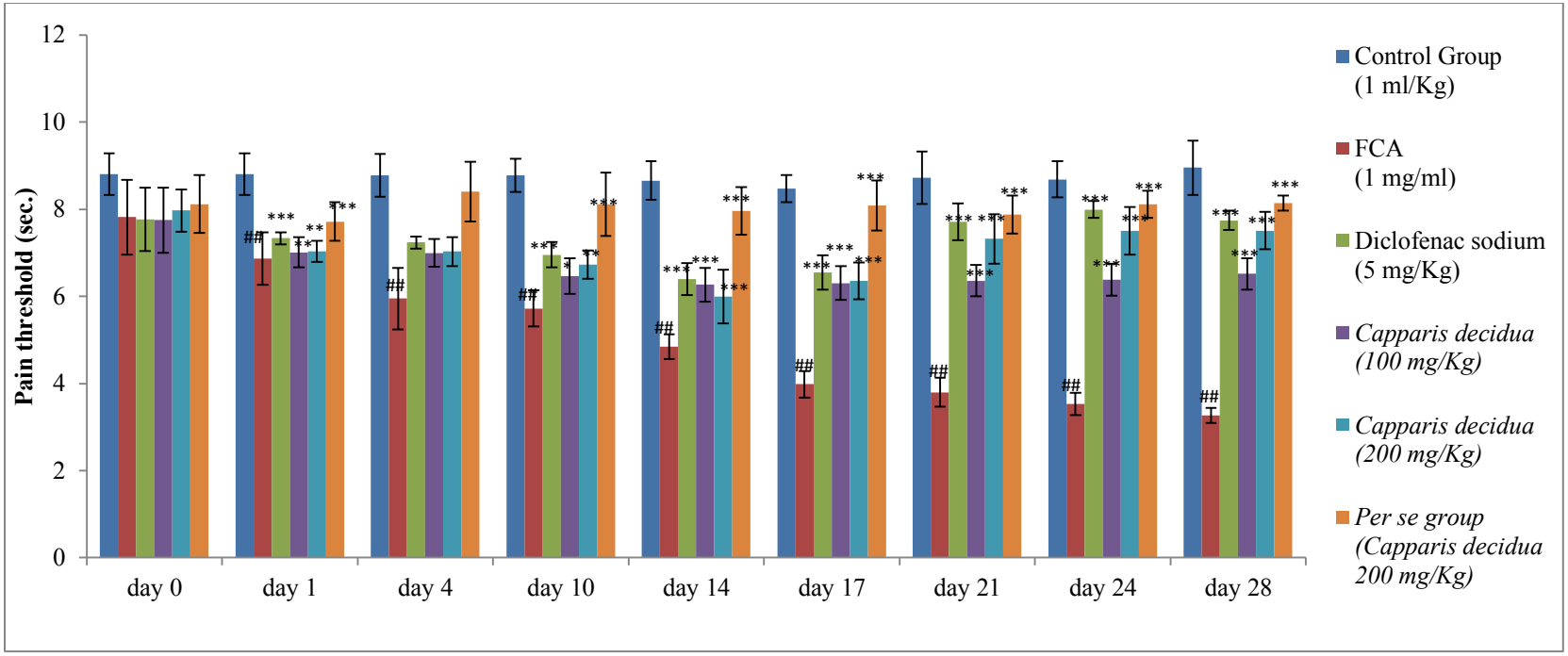


Figure 42: Effect of *Capparis decidua* (obtained from Jodhpur) on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA. **$P < 0.01$ as compared to FCA. *$P < 0.05$ as compared to FCA.

5.18 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON FALL OF TIME

Average fall off time in Rota rod test was determined for the assessment of motor in-coordination. Administration of FCA results in the decrease in fall off time in the FCA treated group as compared to the control group. *Capparis decidua* treated (100 and 200 mg/Kg), significantly (P<0.001) increased fall off time from day 14 till day 28 as compared to the FCA control group while Diclofenac (5 mg/Kg) treated group also showed significant (P<0.001) increase in fall off time but lesser than *Capparis decidua* (200 mg/Kg) as compared to FCA group animals **(Figure 43).**

Table 23: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on fall of time (muscle grip strength) in FCA induced arthritic model on rats.

Treatment	**Muscle grip strength (Fall of time in sec.)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	56.83±0.75	57.73±0.97	57.17±1.12	56.84±1.86	57.02±1.59	56.36±1.65	56.64±1.5	56.83±1.72	56.97±1.78
FCA (1 mg/ml)	56.16±1.47	51±3.09 ##	42.66±1.86 ##	31.88±1.6##	23.35±1.9##	22.06±1.58 ##	24.21±1.62 ##	24.82±2.08 ##	22.93±0.95 ##
Diclofenac sodium (5 mg/Kg)	57±1.89	52.33±1.86	47.39±0.81 ***	39.71±0.9**	35.9±3.11**	36.1±2.53**	42.66±2.81 ***	43.68±1.14 ***	44.43±2.77 ***
***Capparis decidua* (100 mg/Kg)**	56.05±1.41	51.16±1.6	44.85±2.08	36.46±1.9**	30.2±1.26**	30.48±1.26 ***	33.76±1.77 ***	34.2±1.88**	34.41±1.48 ***
***Capparis decidua* (200 mg/Kg)**	57.21±0.73	50.16±1.32	43.6±1.93	37.1±2.68**	33.21±1.6**	33.4±1.59**	35.66±1.86 ***	37.03±2.66 ***	37.53±2.14 ***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	56.8±1.51	56.51±1.42	55.41±1.44	56.38±1.54 ***	54.66±1.96 ***	54.83±2.31 ***	56.16±2.31 ***	55.83±2.85 ***	56.5±1.51**

Effect of Capparis decidua on fall of time in motor incoordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.D (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

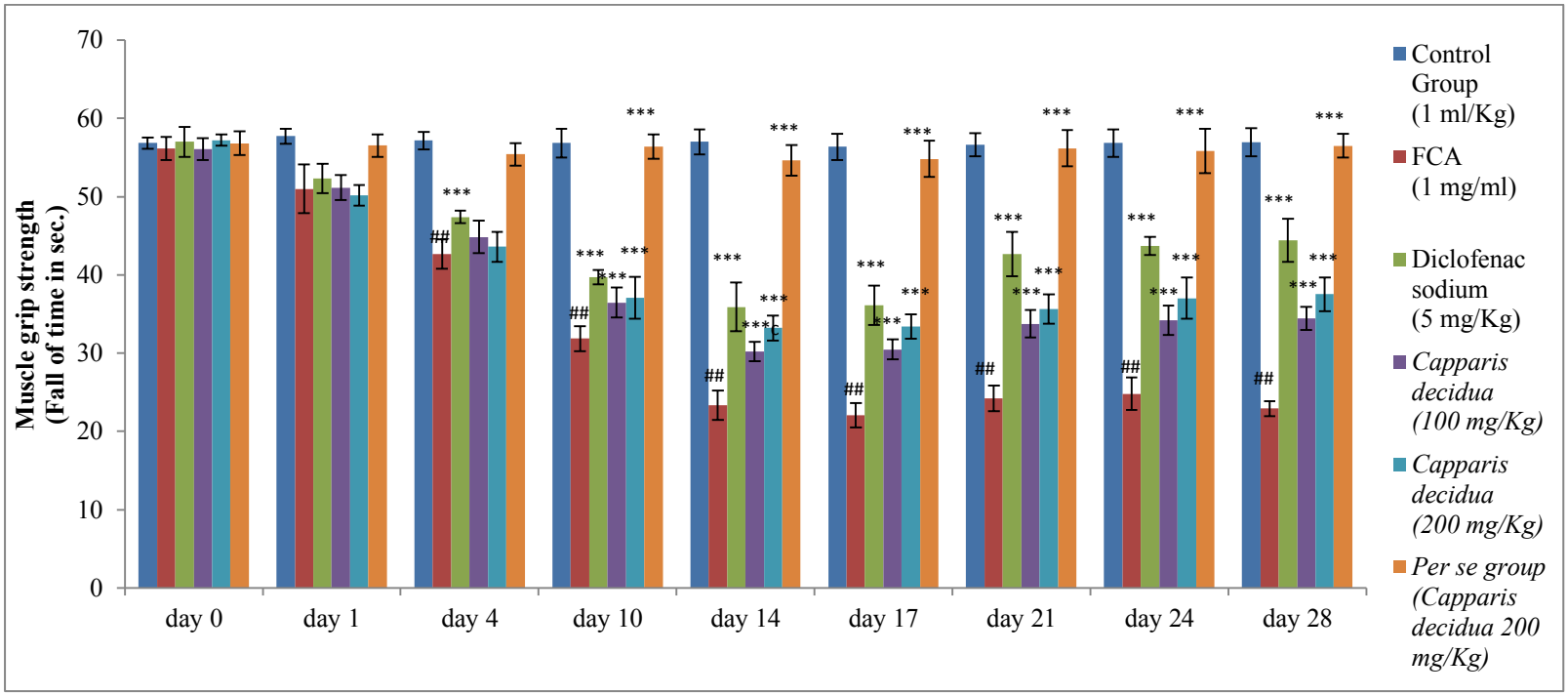


Figure 43: Effect of *Capparis decidua* (obtained from Jodhpur) on fall of time in motor in-coordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.D (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

5.19 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON BODY WEIGHT

The rats in the FCA treated group lost body weight as compared with the *Capparis decidua* extract treated and Diclofenac treated groups. The body weight of *Capparis decidua* at 100 mg/Kg, 200 mg/Kg and *per se* significantly (P<0.001) increased from day 17th onwards till day 28th as compared to FCA treated group rats. While Diclofenac treated groups also showed the significant result (P<0.001) from day 17th onwards till day 28th as compared to FCA treated group rats. The effect produced by *Capparis decidua* extract at 100 mg/Kg and 200 mg/Kg produced a similar result as seen in a Diclofenac-treated group on days 17, 21, 24 and 28**.** **(Figure 44)**

Table 24: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on body weight in FCA induced arthritic model on rats.

Treatment	**Body weight**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	177±3.89	176±4.14	175.33±4.11	177.5±4.5	178.16±3.54	178.16±3.07	178.16±4.7	178.16±4.27	185±4.38
FCA (1 mg/ml)	172.33±1.63	170.66±1.5	166.83±2.22	166.83±2.71$^{\#\#}$	158.66±1.96$^{\#\#}$	158.66±2.33$^{\#\#}$	158.66±3.54$^{\#\#}$	158.66±2.58$^{\#\#}$	148.66±2.16$^{\#\#}$
Diclofenac sodium (5 mg/Kg)	168.16±2.31	167.33±2.73	166.66±3.77	166.66±4.03	162.33±2.94	162.33±3.55***	162.33±4.03***	162.33±4.76***	179.5±3.5***
***Capparis decidua* (100 mg/Kg)**	169±3.57	168.33±2.58	166.33±4.41	166.33±4.08	163.16±3.12	163.16±3.93***	163.16±3.43***	163.16±3.01***	176.5±3.61***
***Capparis decidua* (200 mg/Kg)**	170±2.28	169.16±2.22	167.16±3.18	167.16±3.01	164.5±3.67	164.5±2.73***	164.5±1.86***	164.5±2.42***	179.5±2.42***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	164.16±1.47	164.16±1.47	164.66±3.77	165±4	168.16±2.56	168.16±1.78***	168.16±1.72***	168.16±3.14***	175.33±3.88***

Effect of *Capparis decidua* on body weight (g) in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. $^{\#\#}$P < 0.001 as compared to control.***P < 0.001 as compared to FCA treated

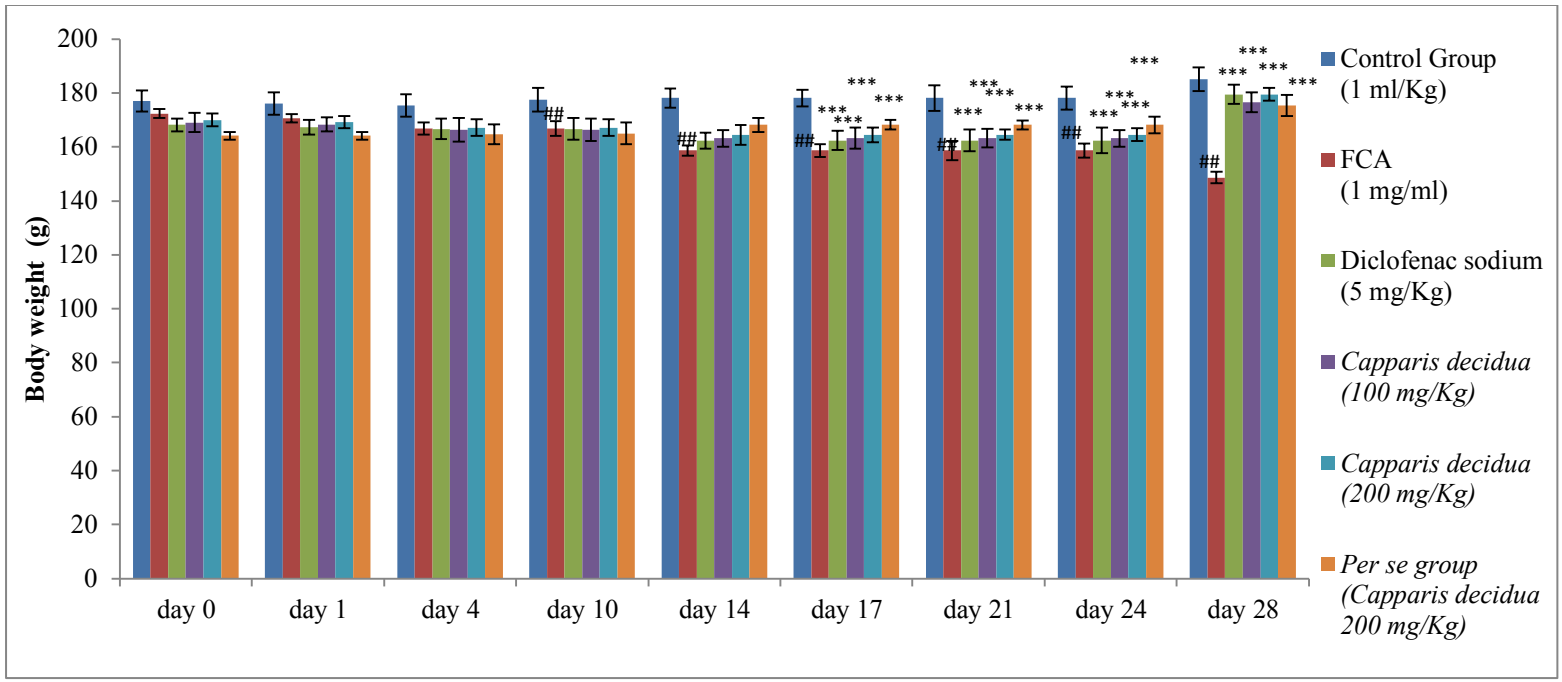


Figure 44: Effect of *Capparis decidua* (obtained from Jodhpur) on body weight (g) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. $^{\#\#}P < 0.001$ as compared to control.. ***$P < 0.001$ as compared to FCA treated

5.20 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR DISTRICT) ON PAW JOINT DIAMETER

There was significant (P<0.001) increase in joint diameter of rats of all the groups from day 1 till day 14 treated with FCA compared to control group. *Capparis decidua* (100 and 200 mg/Kg) significantly (P<0.01 and P<0.001, respectively), decreased the joint diameter from day 14 till day 28 as compared to FCA group. Diclofenac (5 mg/Kg) treated group also showed significant reduction in paw diameter as compared to FCA group rats **(Figure 45).**

Table 25: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on paw joint diameter in FCA induced arthritic model on rats.

Treatment	**Paw Joint diameter (mm)**								
	day 0	**day 1**	**day 4**	**day 10**	**day 14**	**day 17**	**day 21**	**day 24**	**day 28**
Control Group (1 ml/Kg)	3.36±0.23	3.36±0.23	3.4±0.2	3.43±0.2	3.43±0.2	3.45±0.23	3.45±0.2	3.46±0.35	3.46±0.23
FCA (1 mg/ml)	4.01±0.25	4.4±0.26	4.66±0.15$^{\#\#}$	4.8±0.24$^{\#\#}$	6.2± 0.85$^{\#\#}$	6.03±0.18$^{\#\#}$	5.48±0.29$^{\#\#}$	5±0.27$^{\#\#}$	5±0.27$^{\#\#}$
Diclofenac sodium (5 mg/kg)	3.98±0.51	4.05±0.16	4.18±0.22	4.25±0.1	5.91± 0.51	5.25±0.22***	4.66± 0.2***	4.13±0.2**	4.11± 0.21***
***Capparis decidua* (100 mg/Kg)**	3.76±0.43	3.95±0.12	4.13±0.13	4.28±0.0	5.95±0.5	5.28±0.39**	4.8±0.36**	4.41± 0.23**	4.39±0.25**
***Capparis decidua* (200 mg/Kg)**	3.76±0.25	3.83±0.24	4.05±0.29	4.21±0.24	6±0.16	5.25±0.32***	4.58± 0.27***	4.1± 0.25***	4.08± 0.27***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	3.83±0.2	3.85±0.2	3.8±0.29	3.8±0.29	3.88±0.3	3.86±0.25***	3.88± 0.27***	3.9± 0.28***	3.86± 0.25***

Effect of *Capparis decidua* on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

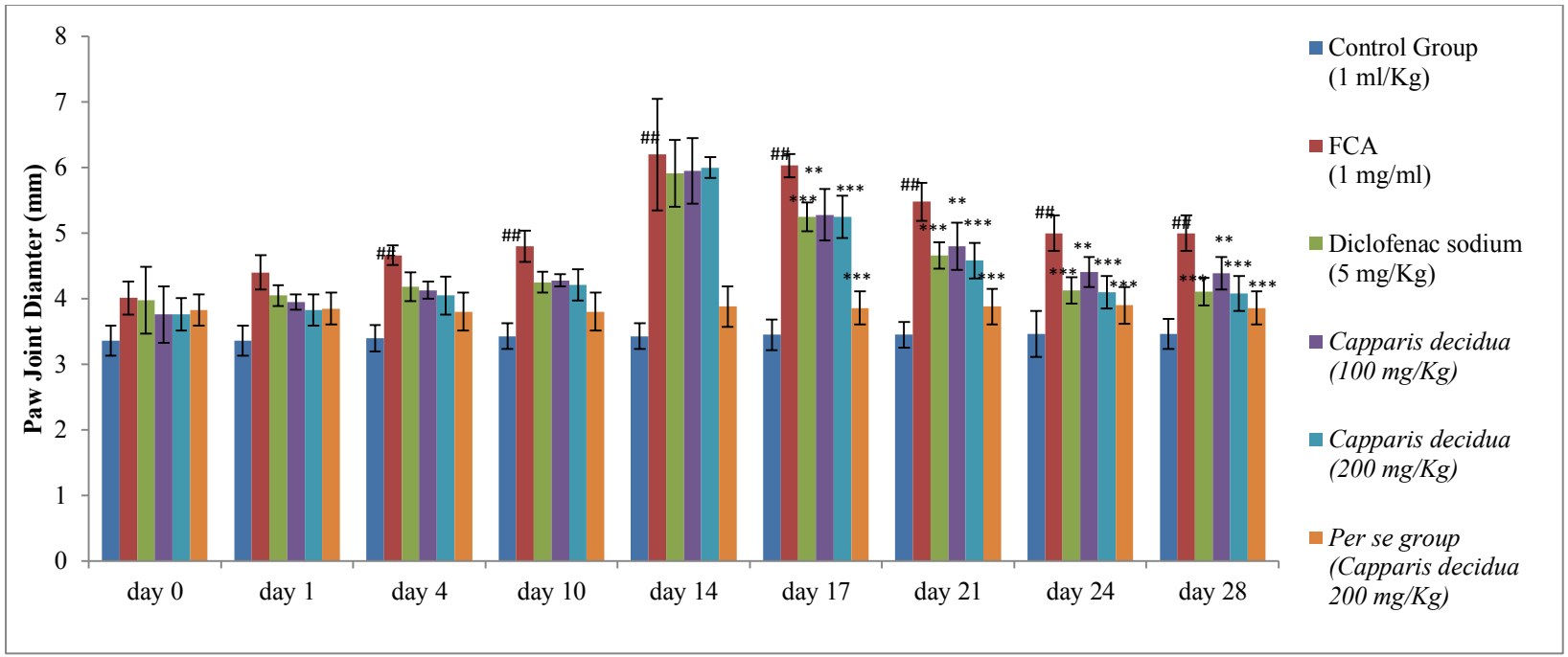


Figure 45: Effect of *Capparis decidua* (obtained from Jodhpur) on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

5.21 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR) ON HEMATOLOGICAL PARAMETERS

The significant increase in levels of platelets (P<0.001), ESR count (P<0.001) and WBC (P<0.001) and significant decrease in levels of RBC (P<0.001) and Hb (P<0.001) were observed in FCA group as compared to normal control group indicating a stimulation of immune response towards FCA in arthritic rats. Treatment with hydroalcoholic extract of *Capparis decidua* (100 & 200 mg/Kg), *per se* group significantly (P<0.001) inhibited the stimulation of immune response towards FCA by decreasing blood WBC, ESR, and increasing Hb and RBC compared to FCA treated group. Diclofenac sodium treated rats also showed significant result (P<0.001) by reducing the WBC, ESR count and platelet and increasing hemoglobin and RBC levels. **(Figure 46-50)**

Table 26: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on RBC count, WBC count, Platelet count, Hemoglobin count, ESR count in FCA induced arthritic model on rats.

Treatment	**RBC count (x 1000000 cells/mm^3)**	**WBC count (x 1000/ml)**	**Platelet count (x 1000 cells/ mm^3)**	**Hb count (g/dL)**	**ESR count (mm/hr)**
Control Group (1 ml/Kg)	8.75±0.32	9.46±0.6	260.83±27.64	15.11±1.9	3.25±0.16
FCA (1 mg/ml)	3.21±0.44$^{\#\#}$	14.48±0.73$^{\#\#}$	541.66±44.45$^{\#\#}$	8.96±0.86$^{\#\#}$	9.28±0.44$^{\#\#}$
Diclofenac sodium (5 mg/kg)	7.66±0.3***	9.2±0.3***	353.83±45.24***	14.16±0.66***	3.62±0.17***
***Capparis decidua* (100 mg/Kg)**	6.41±0.41***	12.15±0.95***	258.83±30.07***	11.05±0.5**	4.61±1.01***
***Capparis decidua* (200 mg/Kg)**	7.51±0.24***	10.05±0.68***	286.66±12.51***	12.88±0.87***	4.21±0.08***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	8.63±0.83***	8.55±1.22***	225.83±21.48***	14.43±1.4***	3.67±0.28***

Effect of *Capparis decidua* on hematological parameters in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA treated.

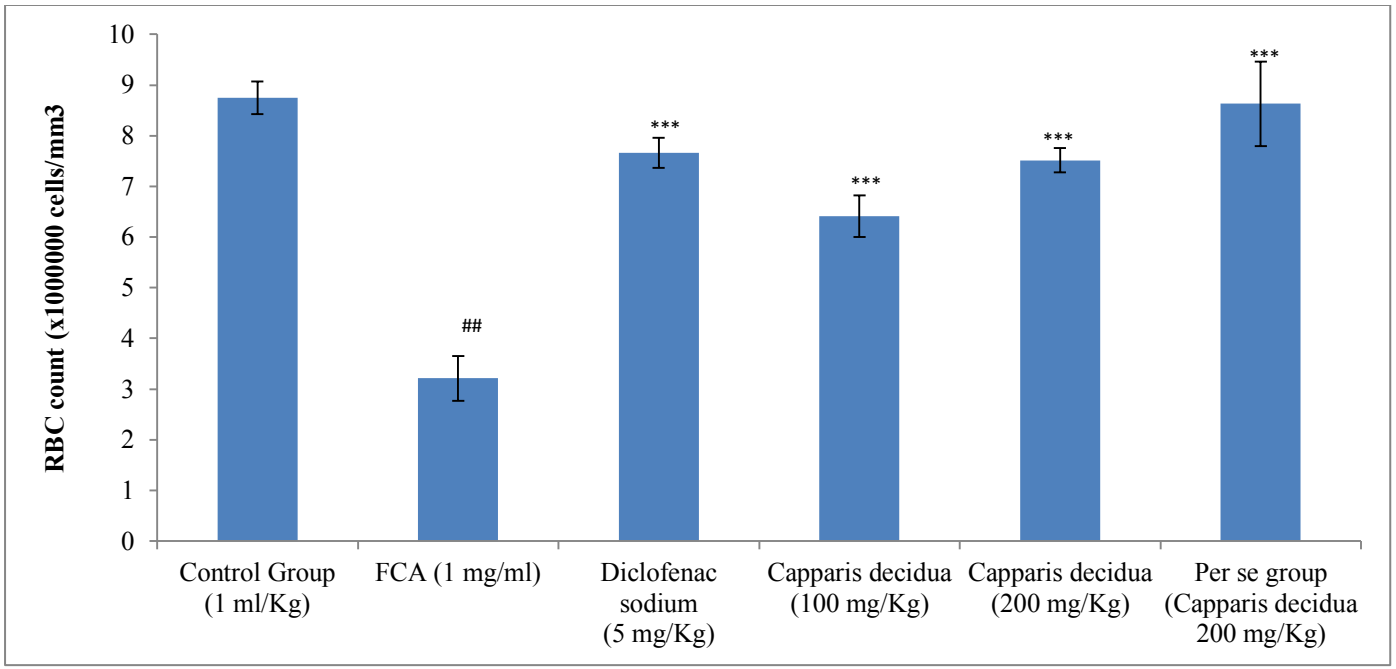


Figure 46: Effect of *Capparis decidua* (obtained from Jodhpur) on RBC count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

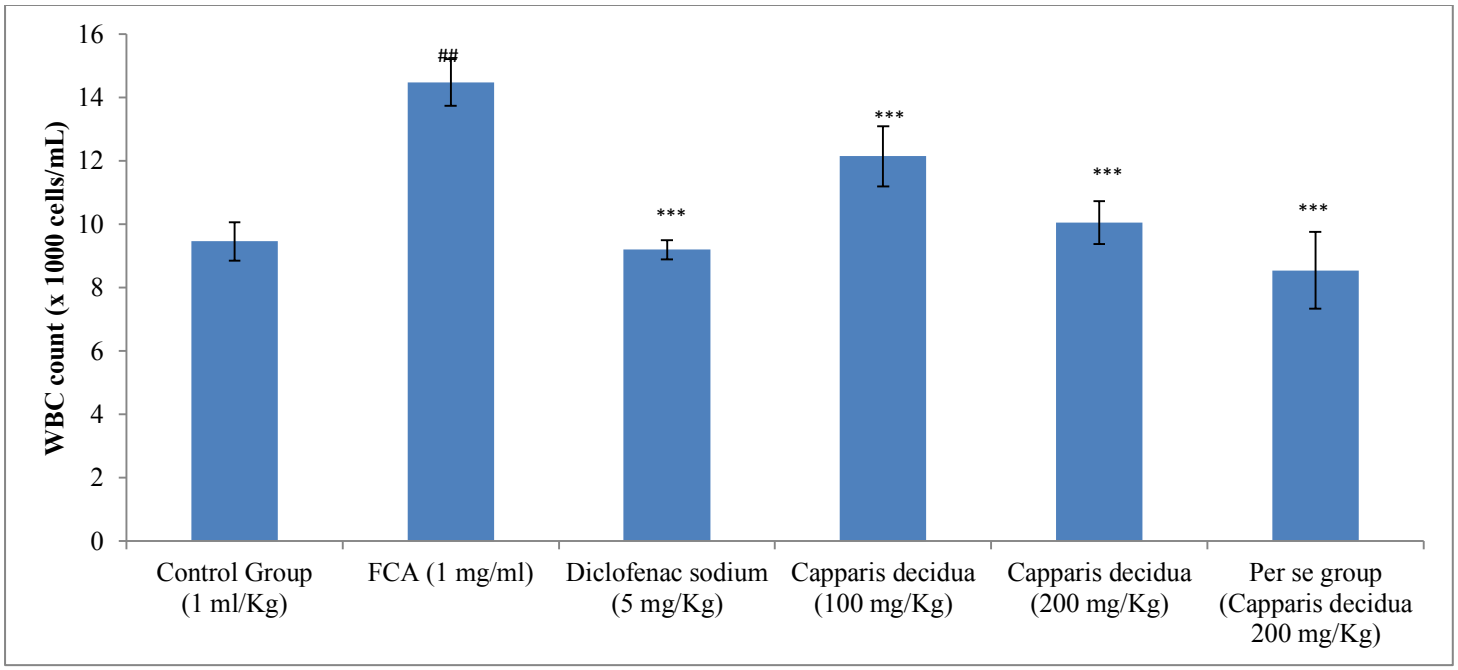


Figure 47: Effect of *Capparis decidua* (obtained from Jodhpur) on WBC count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

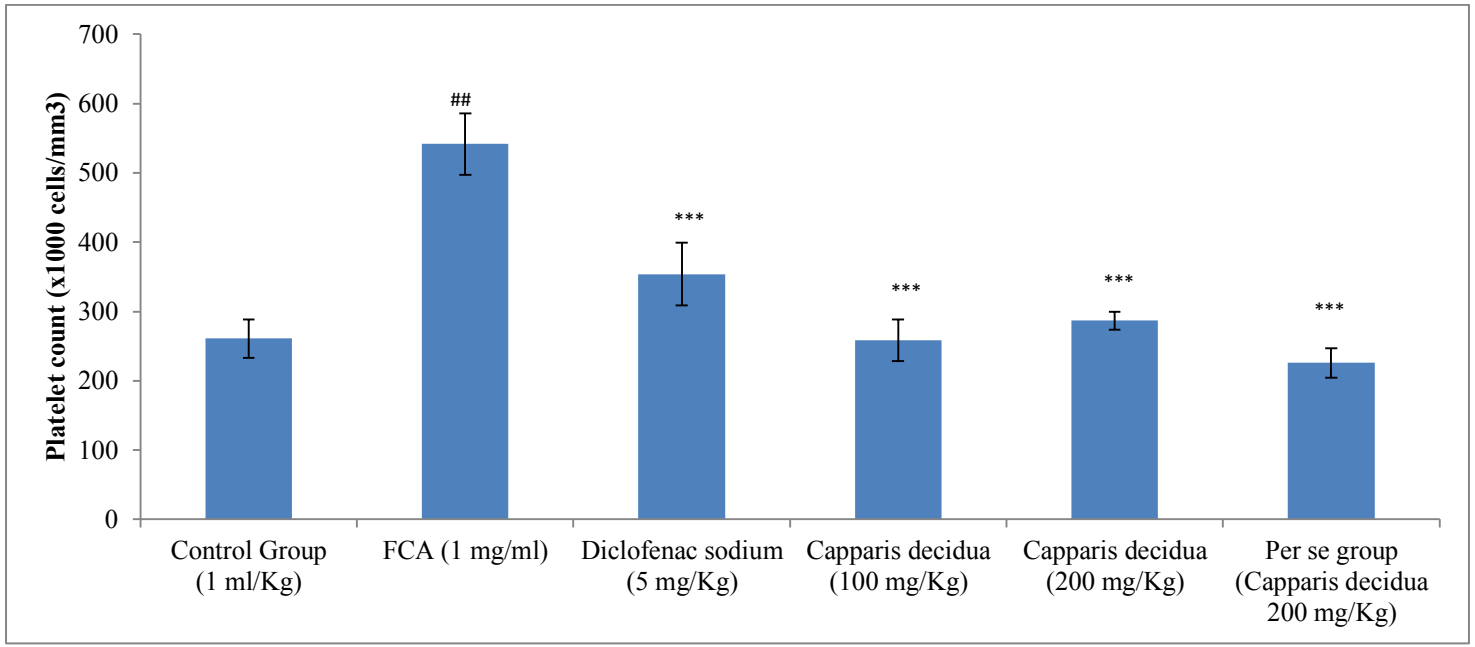


Figure 48: Effect of *Capparis decidua* (obtained from Jodhpur) on Platelet count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

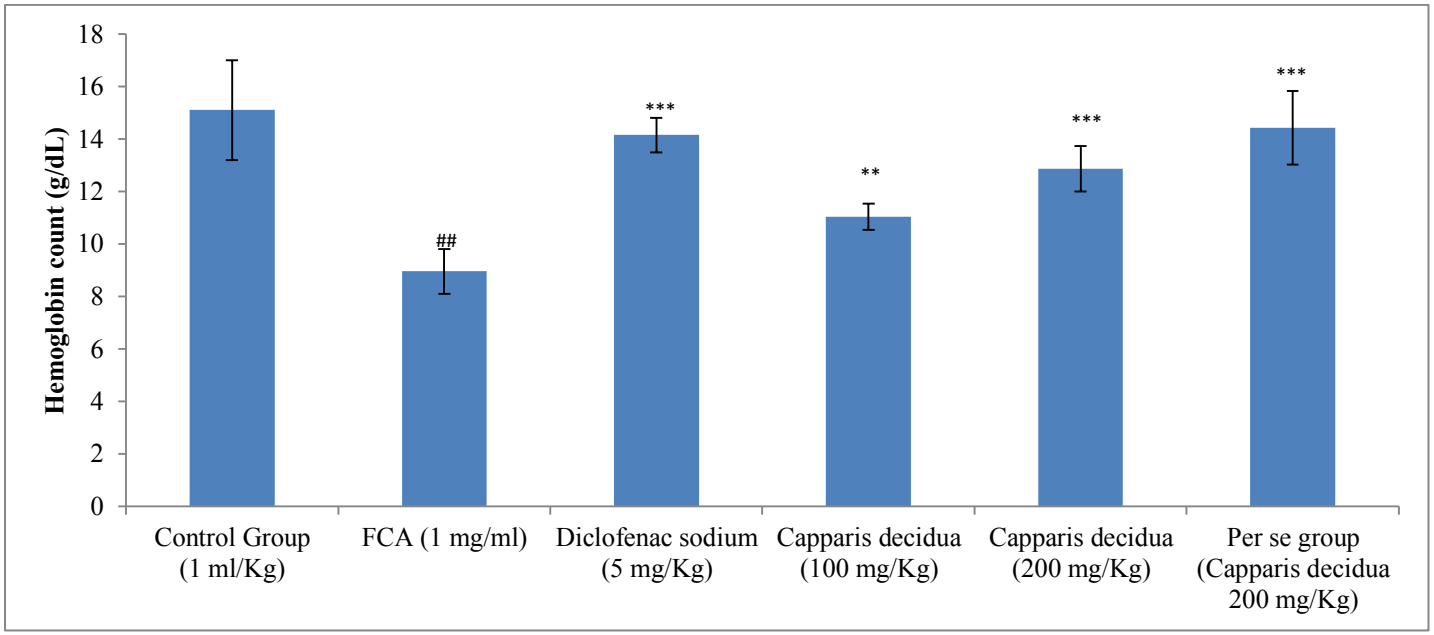


Figure 49: Effect of *Capparis decidua* (obtained from Jodhpur) on Hemoglobin count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

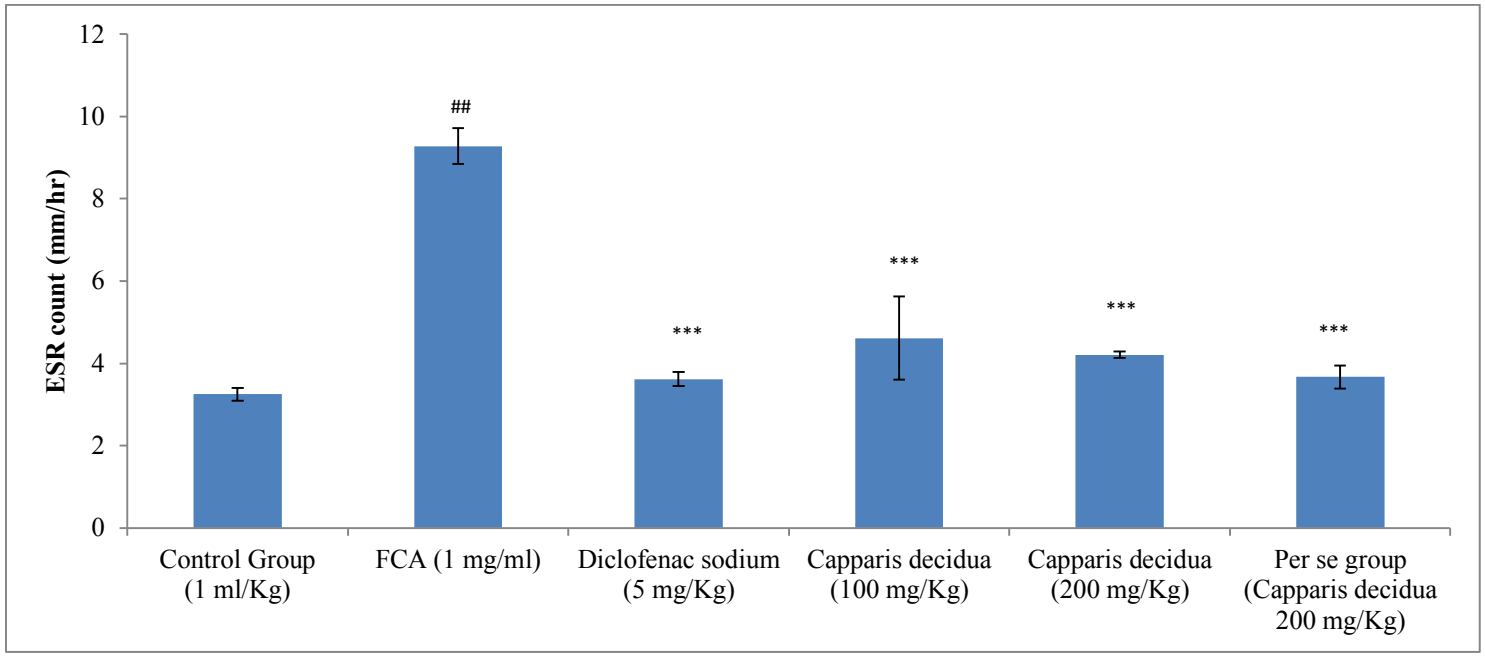


Figure 50: Effect of *Capparis decidua* (obtained from Jodhpur) on ESR count test in FCA-induced arthritic rats. Data are expressed as mean ± SD. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA treated.

5.22 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (OBTAINED FROM JODHPUR) ON BIOCHEMICAL PARAMETERS

As a result of FCA-induced arthritis, the serum levels of AST, ALT and ALP were increased significantly (P<0.001) and total protein level was decreased significantly (P<0.001) in FCA group. These enzyme levels were altered by treatment with *Capparis decidua* (100 and 200 mg/Kg), and Diclofenac (5 mg/Kg) group. The level of AST, ALT and ALP were significantly (P<0.001) decreased by treatment with *Capparis decidua* (100 and 200 mg/Kg), and Diclofenac 5 mg/Kg and the level of total protein was significantly (P<0.001) increased in *Capparis decidua* (100 mg/Kg; P<0.01, 200 mg/Kg; P<0.001) and Diclofenac group (P<0.001) as compared to FCA group **(Figures 51-54).**

Table 27: Effect of hydroalcoholic extract of *Capparis decidua* (obtained from Jodhpur) on AST, ALP, ALT, Total protein level in FCA induced arthritic model on rats.

Treatment	**AST/SGOT (IU/L)**	**ALP (IU/L)**	**ALT/SGPT (IU/L)**	**Total protein (g/dL)**
Control Group (1 ml/Kg)	81±3.28	72.66±3.55	52.66±2.58	7.01±0.3
FCA (1 mg/ml)	162±4.56$^{\#\#}$	174.16±9.66$^{\#\#}$	161.83±2.48$^{\#\#}$	4.41±0.37$^{\#\#}$
Diclofenac sodium (5 mg/kg)	92.16±3.65***	87.66±5.31***	63.66±6.4***	6.53±0.28***
***Capparis decidua* (100 mg/kg)**	101.16±2.04***	104.33±4.03***	112.16±8.37***	5.41±0.23***
***Capparis decidua* (200 mg/Kg)**	95.33±2.06***	98±8.07***	70.33±6.86***	5.95±0.13***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	81.33±6.5***	80.33±4.41***	57±7.74***	6.6±0.51***

Effect of *Capparis decidua* on biochemical parameters in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}$P < 0.001 as compared to control. ***P < 0.001 as compared to FCA

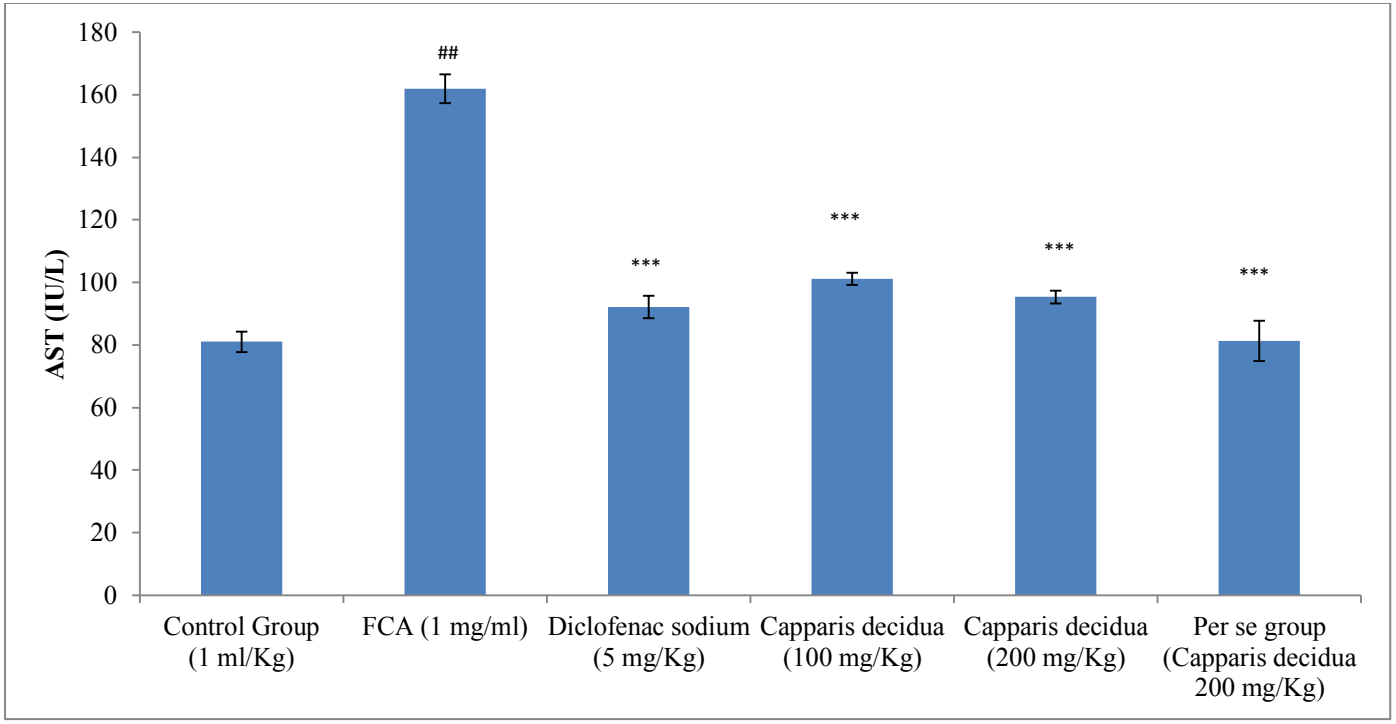


Figure 51: Effect of *Capparis decidua* (obtained from Jodhpur) on AST (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

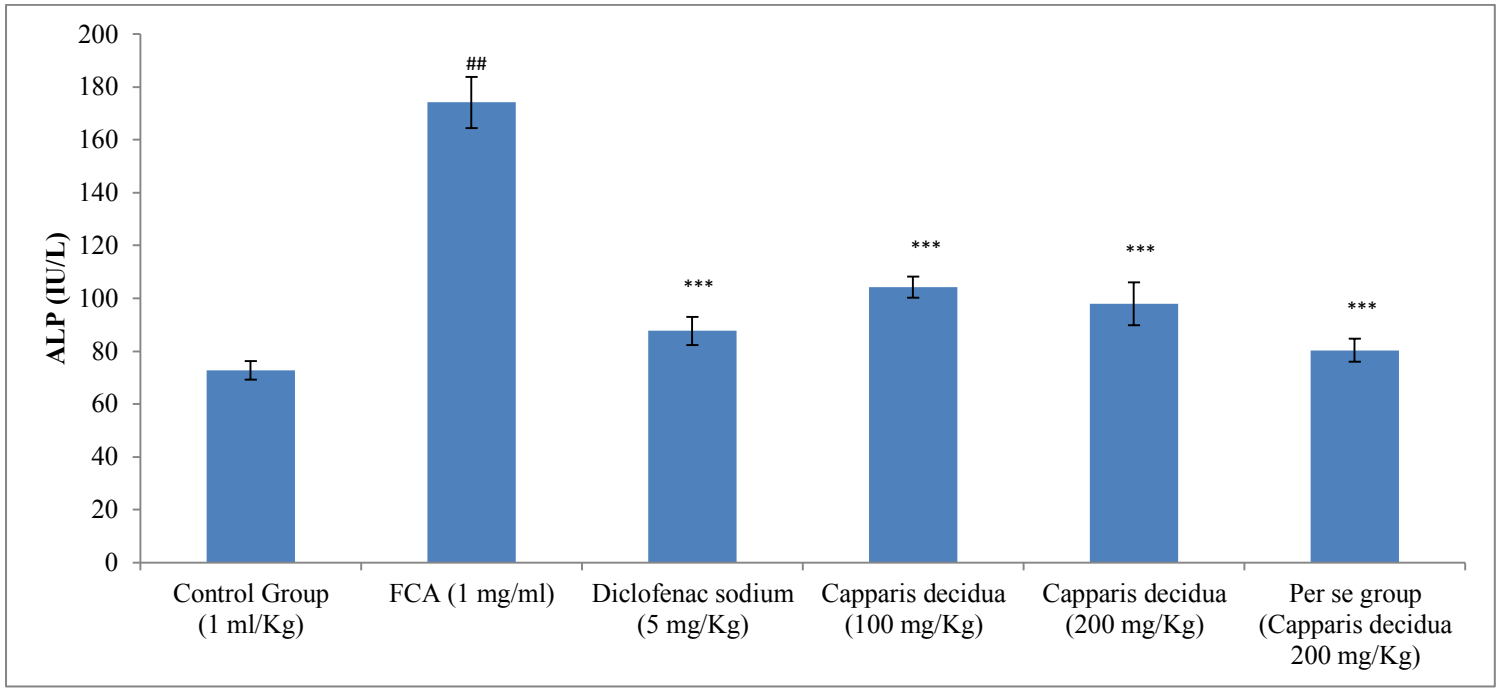


Figure 52: Effect of *Capparis decidua* (obtained from Jodhpur) on ALP (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

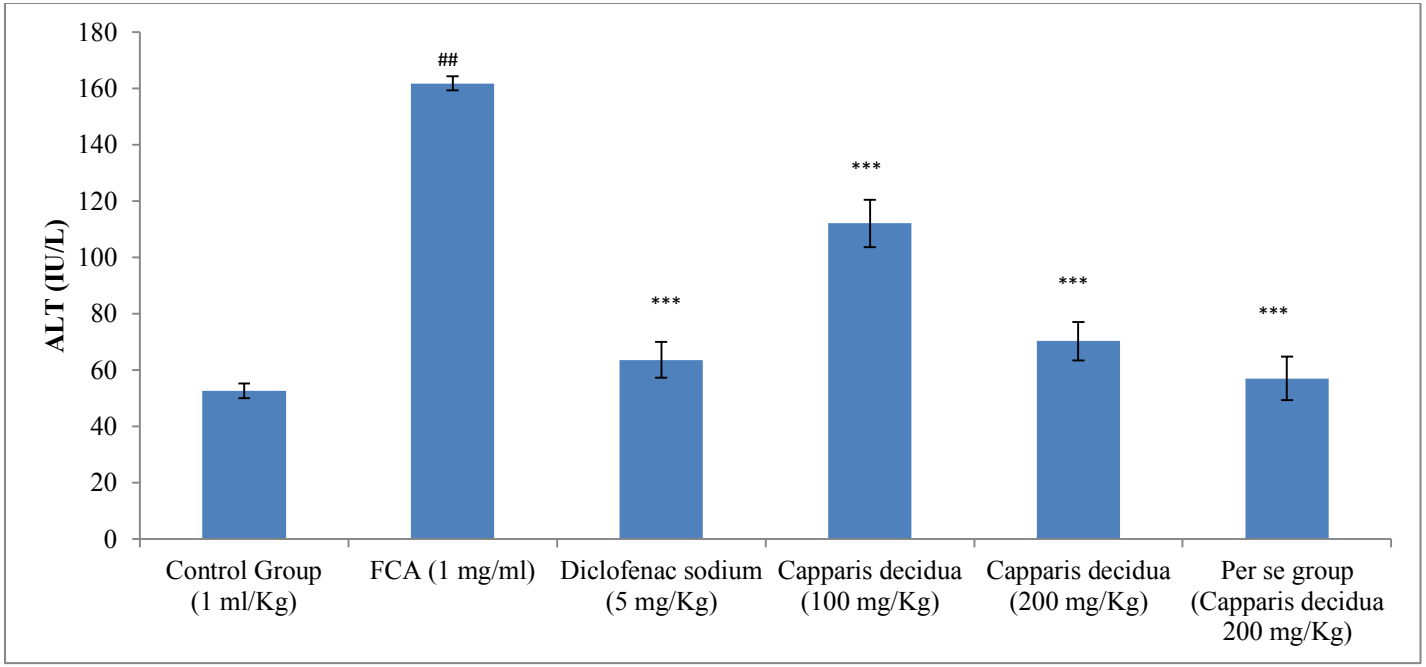


Figure 53: Effect of *Capparis decidua* (obtained from Jodhpur) on ALT (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.

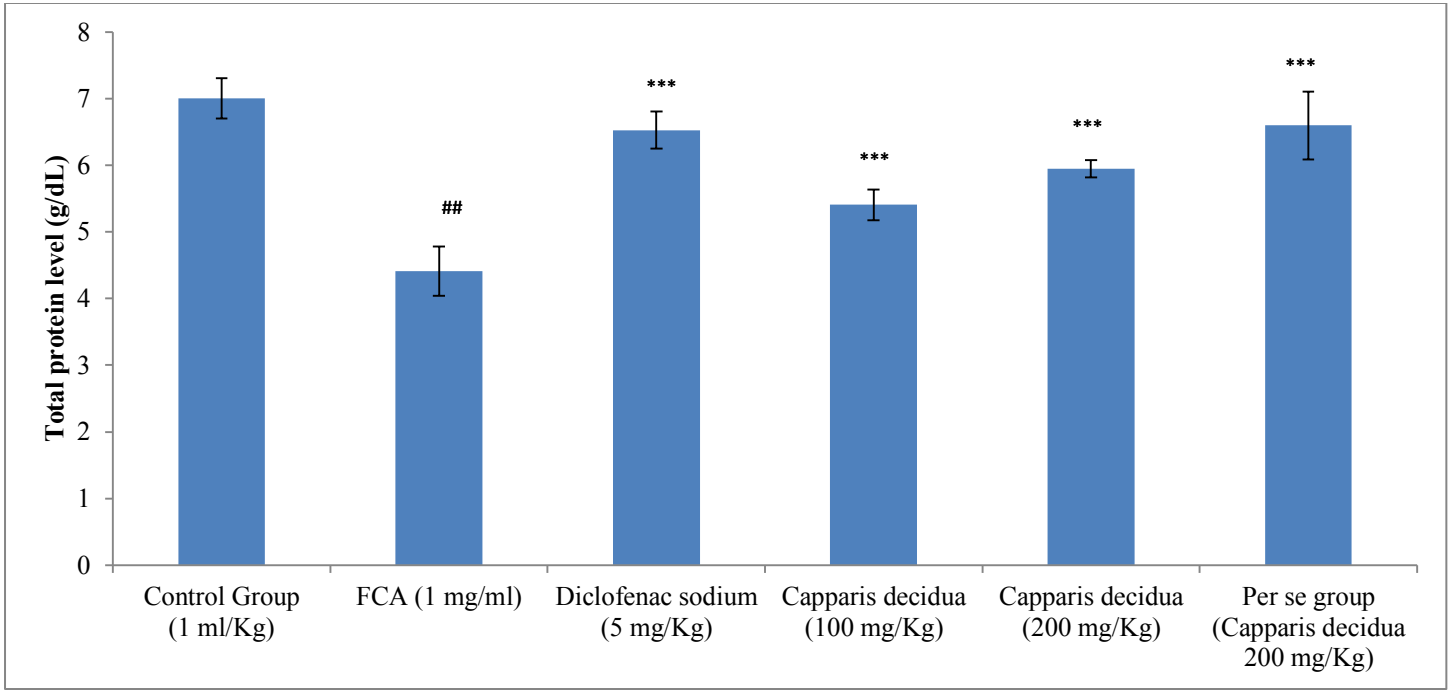
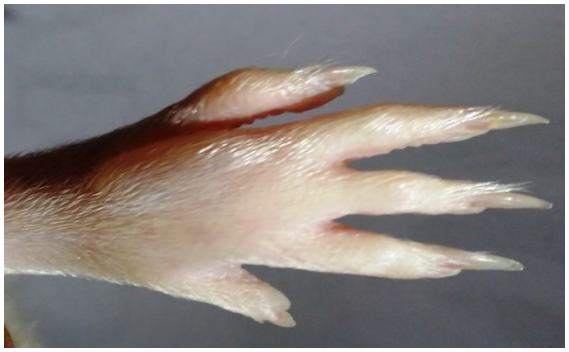
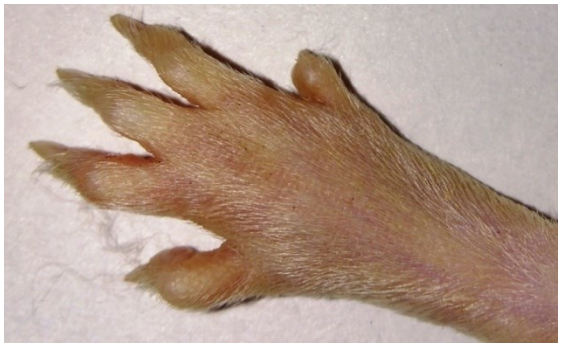


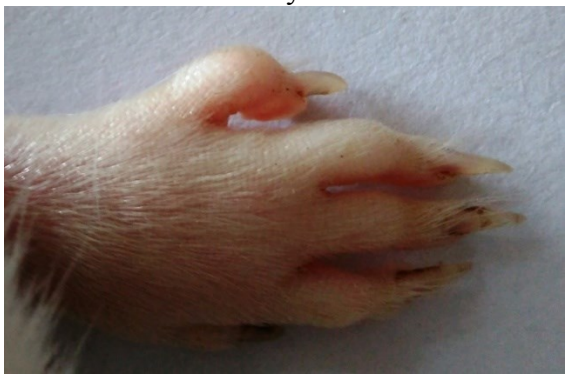
Figure 54: Effect of *Capparis decidua* (obtained from Jodhpur) on Total protein (g/dL) in FCA-induced arthritic rats. Data are expressed as mean ± S.D. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple tests for comparison.$^{\#\#}P < 0.001$ as compared to control. ***$P < 0.001$ as compared to FCA.



Day 0



Day 1



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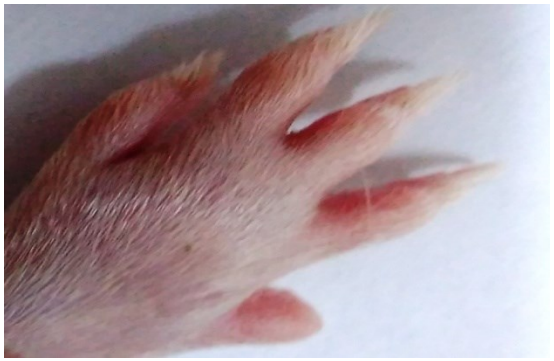
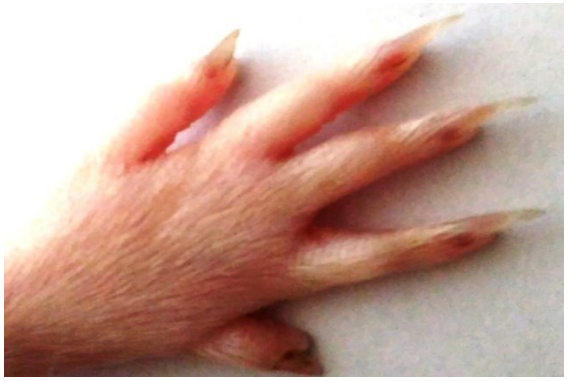
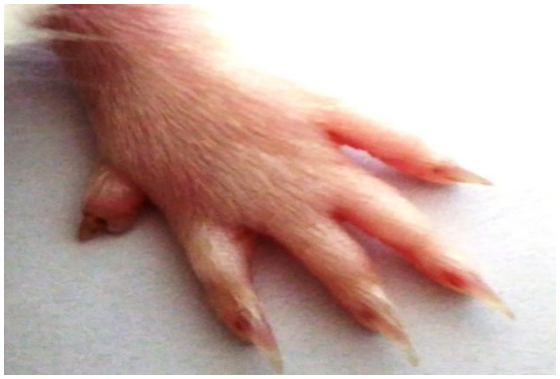


Day 10

Figure 55: Representation of paw swelling in FCA induced arthritic rats showing varying degree of swelling in rats on days 0, 4, 10, 14, 17, 21, 24, 28.



Day 14



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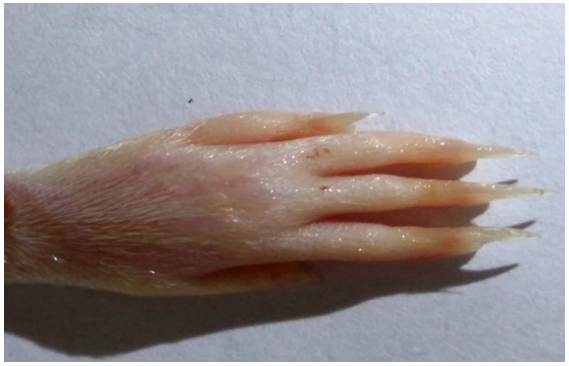
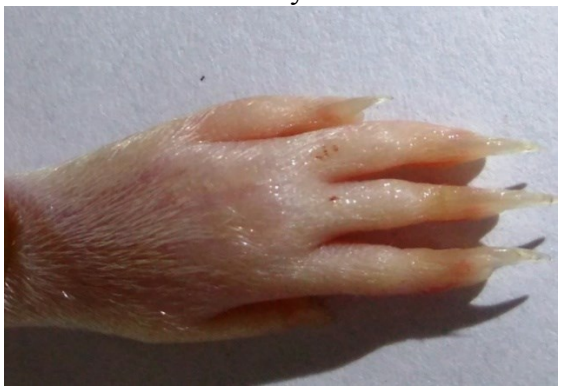


Day 24



Day 28

Figure 55: Representation of paw swelling in FCA induced arthritic rats showing varying degree of swelling in rats on days 0, 4, 10, 14, 17, 21, 24, 28.

Evaluation of aphrodisiac activity

5.23 EFFECT OF SEXUAL BEHAVIOR STUDY

The observations of sexual behavior are presented in **Table 28-33 and Figure 56-62.** Treatment with hydroalcoholic extract of *Capparis decidua* at different doses influenced the behavior of the treated animals in a dose-dependent manner. All the experimental groups significantly affected sexual behavior as compared with the control. *Capparis decidua* extract at the dose of 100 mg/Kg, significantly increased the Mounting Frequency (MF) P<0.05) on day 21 and 28, Intromission Frequency (IF) on day 21P<0.05) and day 28 (P<0.01), Ejaculatory Latency (EL) on day 21 (P<0.01) and day 28 (P<0.01) and caused significant reduction in the Mounting Latency (ML) (P<0.01) on day 28, Intromission Latency (IL) (P<0.01) on day 21 and 28, as compared to control group. The dose of 200 mg/Kg of the extract significantly increased the MF on day 14 P<0.05), day 21(P<0.01), day 28 (P<0.001); IF on day 14 P<0.05), day 21 and 28 (P<0.001); EL on day 7 ((P<0.01), day 14(P<0.01), day 21(P<0.001), day 28(P<0.001) and significantly decreased the ML on day 7(P<0.01), day 14(P<0.01), day 21(P<0.001), day 28(P<0.001); IL on day 7(P<0.01), day 14(P<0.05), day 21(P<0.001), day 28(P<0.001), in comparison with the control group. *Per se* group showed significant increase in MF on day 14P<0.05), day 21(P<0.01), day 28(P<0.01); IF on day 28 P<0.05); EL on day 14 (P<0.05), day 21(P<0.001), day 28(P<0.001) and significantly decreased the ML on day 7(P<0.05), day 21(P<0.001), day 28(P<0.001); IL on day 7(P<0.01), day 21(P<0.001), day 28(P<0.001) as compared to control group. Standard drug treated group gave highly significant increase in MF on day 7(P<0.01), day 14(P<0.001), day 21(P<0.001), day 28(P<0.001); IF on day 7 (P<0.05), day 14(P<0.001), day 21(P<0.001), day 28(P<0.001); EL on day 7, 14, 21 and 28 (P<0.001) and significant decrease in ML and IL on day 7, 14, 21 and 28 (P<0.001) as compared to control.

A dose-dependent increase in serum testosterone concentration were observed on the 21st and 28th day of the study in *Capparis decidua* extract (100 mg/Kg) (P<0.01), *Capparis decidua* extract (200 mg/Kg) (P<0.001), *per se* group (only *Capparis decidua* extract 200 mg/Kg) (P<0.001). While Sildenafil citrate group showed an increase in serum testosterone level on 14th, 21st and 28th day of the study as compared to control group

($P<0.001$) (Figure). After 7 days of treatment schedule i.e. 7 days after 28^{th} day the serum testosterone level was measured for all the groups. The *Capparis decidua* extract (200 mg/Kg), *per se* group, Sildenafil citrate gave significant results ($P<0.001$) while *Capparis decidua* extract (100 mg/Kg) showed slightly less significant result ($P<0.01$).

Table 28: Effect of hydroalcoholic extract of *Capparis decidua* on Ejaculation latency in male rats

Treatment	**Ejaculation latency**				
	day 0	**day 7**	**day 14**	**day 21**	**day 28**
Control Group (1 ml/Kg)	176.16±8.86	177.66±11.77	180.5±9.97	187.66±9.72	197.33±9.79
Sildenafil citrate (5 mg/Kg)	261.83±45.45	265.5±47.31***	270.33±50.75***	289.83±23.11***	299.16±22.08***
***Capparis decidua* (100 mg/Kg)**	209.16±18.86	212.5±19.07	221±19.17	235±19.23**	243.83±17.09**
***Capparis decidua* (200 mg/Kg)**	237.66±20.99	244±21.1**	253.16±21.18**	261.5±16.05***	274±17.14***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	220.33±17.9	230.83±19.48	239.33±20.31**	249.16±25.15***	259.16±25.96***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

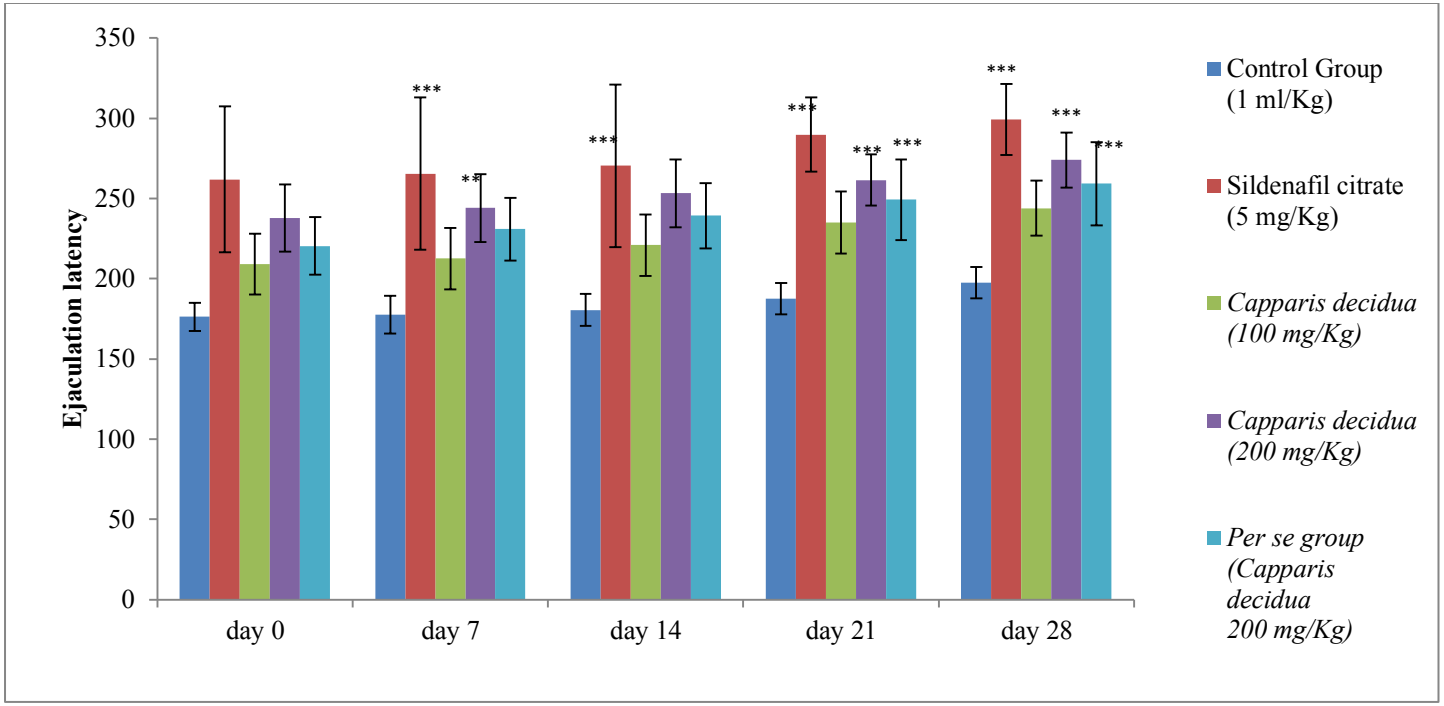


Figure 56: Effect of hydroalcoholic extract of *Capparis decidua* on Ejaculation latency in male rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

Table 29: Effect of hydroalcoholic extract of *Capparis decidua* on mounting latency in male rats

Treatment	Mounting latency				
	day 0	**day 7**	**day 14**	**day 21**	**day 28**
Control Group (1 ml/Kg)	135.96±7.16	130.16±6.71	116.25±5.02	110.45±5.38	93.36±3.36
Sildenafil citrate (5 mg/Kg)	109.14±9.91	97.28±5.94***	80.44±6.88***	75.67±3.46***	61.42±4***
***Capparis decidua* (100 mg/Kg)**	144.09±7.23	124.72±6.85	111.57±11.66	98.67±4.04	84.82±4.17**
***Capparis decidua* (200 mg/Kg)**	118.84±6.52	110.45±8.53**	99.17±7.87**	84.29±5.78***	70.63±3.61***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	120.16±7.55	113.5±10.09*	105.39±6.84	87.32±5.83***	77.14±2.94***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

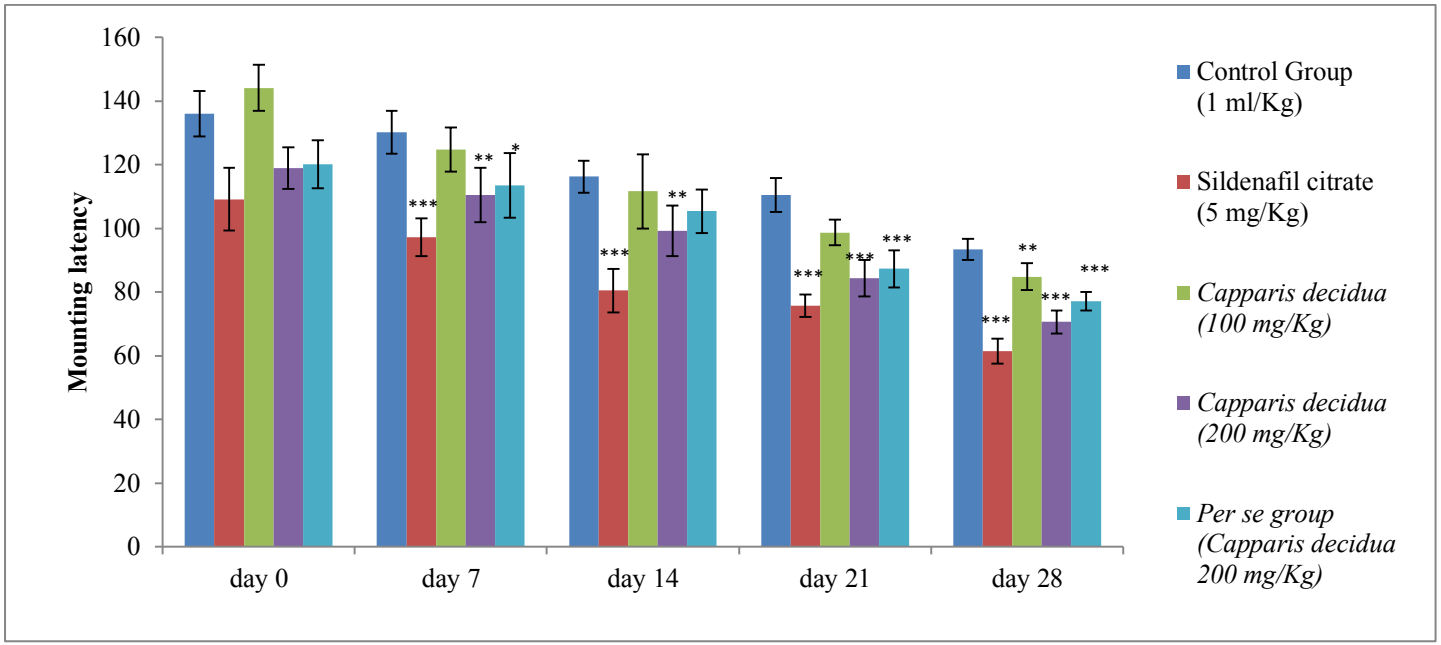


Figure 57: Effect of hydroalcoholic extract of *Capparis decidua* on mounting latency in male rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

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Table 30: Effect of hydroalcoholic extract of *Capparis decidua* on mounting frequency in male rats

Treatment	**Mounting frequency**				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	13.5±2.16	13.5±2.16	13±1.78	13.83±2.63	14.66±2.33
Sildenafil citrate (5 mg/Kg)	19.5±1.64	20±1.41**	20.5±1.64***	21.16±1.72***	22.5±1.87***
***Capparis decidua* (100 mg/Kg)**	14.83±2.63	15.16±1.83	15.83±2.13	18.16±1.16*	18.83±1.16*
***Capparis decidua* (200 mg/Kg)**	16.83±3.76	17.16±4.07	18±4.51*	19.33±3.44**	21±2.96***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	18±2.89	18±2.89	18.5±2.25*	19±1.89**	19.5±1.87**

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

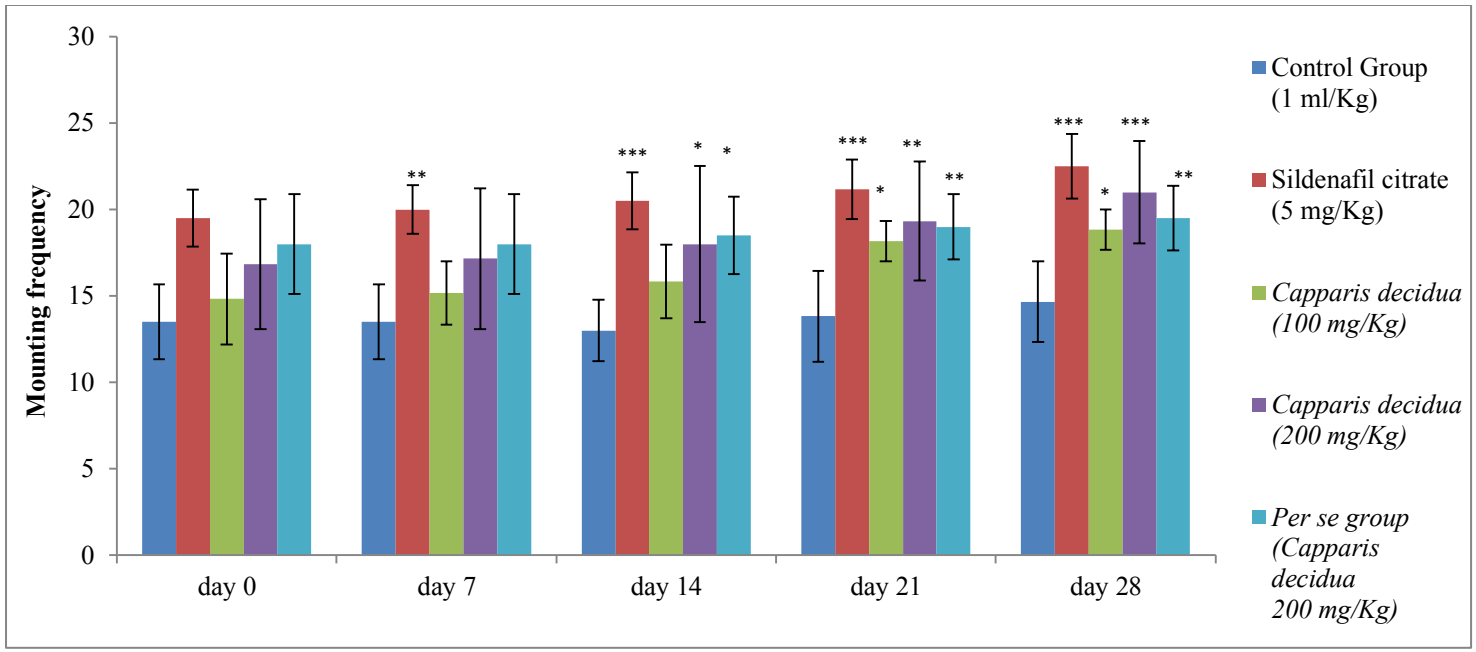


Figure 58: Effect of hydroalcoholic extract of *Capparis decidua* on mounting frequency in male rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

Table 31: Effect of hydroalcoholic extract of *Capparis decidua* on Intromission latency in male rats

Treatment	**Intromission latency (IL)**				
	day 0	**day 7**	**day 14**	**day 21**	**day 28**
Control Group (1 ml/Kg)	146.74±6.83	140.3±6.45	126.11±4.97	120.39±5.33	103.08±3.34
Sildenafil citrate (5 mg/Kg)	116.97±9.25	107.53±6.15***	87.7±5.86***	86.06±3.18***	71.68±4.18***
***Capparis decidua* (100 mg/Kg)**	154.17±7.27	133.33±6.67	122.84±11.22	108.94±4.76**	94.84±4.11**
***Capparis decidua* (200 mg/Kg)**	129.34±6.94	121.99±8.14**	109.09±7.96*	94.36±5.63***	80.64±3.54***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	129.74±6.49	123.39±10.21**	113.79±9	98.78±6.17***	87.14±2.89***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

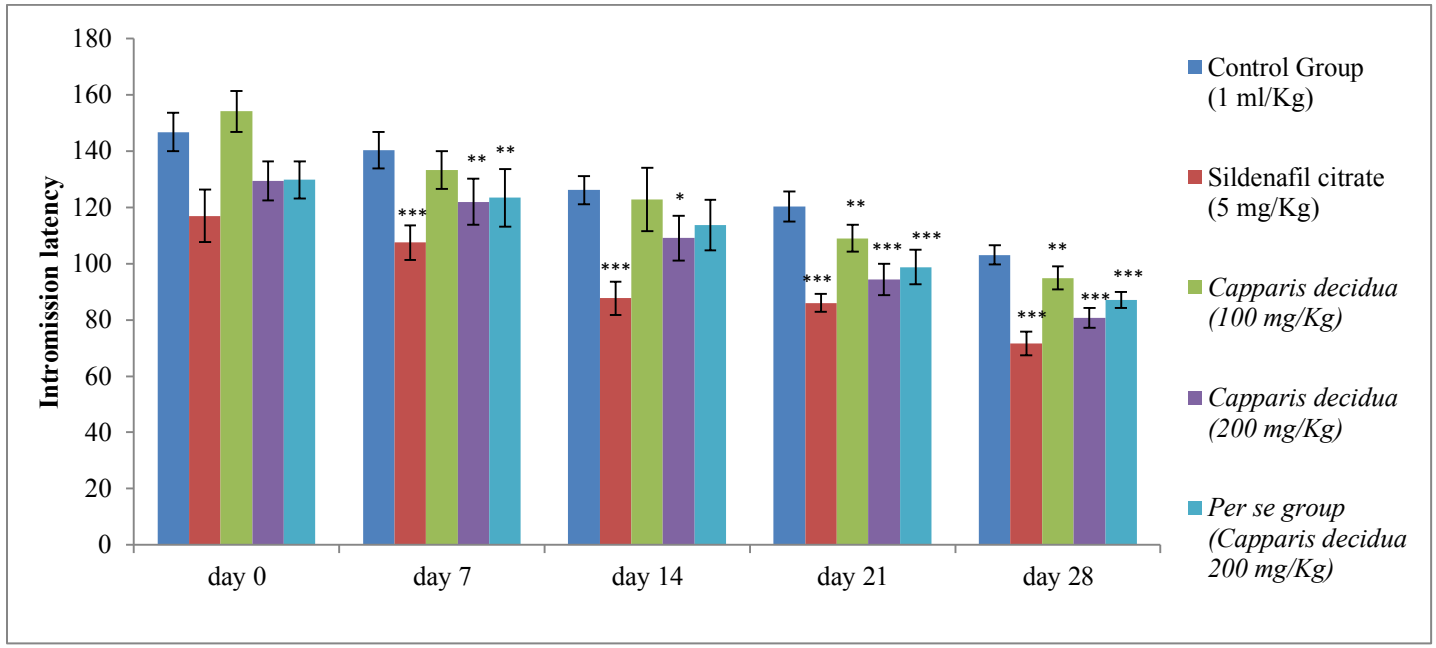


Figure 59: Effect of hydroalcoholic extract of *Capparis decidua* on Intromission latency in male rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

Table 32: Effect of hydroalcoholic extract of *Capparis decidua* on Intromission frequency in male rats

Treatment	**Intromission frequency (IF)**				
	day 0	**day 7**	**day 14**	**day 21**	**day 28**
Control Group (1 ml/Kg)	9.5±2.42	11.16±1.32	11.16±1.47	11.66±2.06	11.16±1.6
Sildenafil citrate (5 mg/Kg)	14.16±2.85	15.16±2.13*	17.66±2.06***	19.83±3.18***	21.66±3.93***
***Capparis decidua* (100 mg/Kg)**	10.83±1.47	11.16±1.72	13.83±3.54	16.16±1.72*	16.5±1.87**
***Capparis decidua* (200 mg/Kg)**	11.66±2.94	13.33±1.63	15.33±2.5*	17.5±1.64***	19.5±1.87***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	10.16±2.48	11.33±2.8	12.16±2.04	12.83±2.04	15.33±1.63*

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

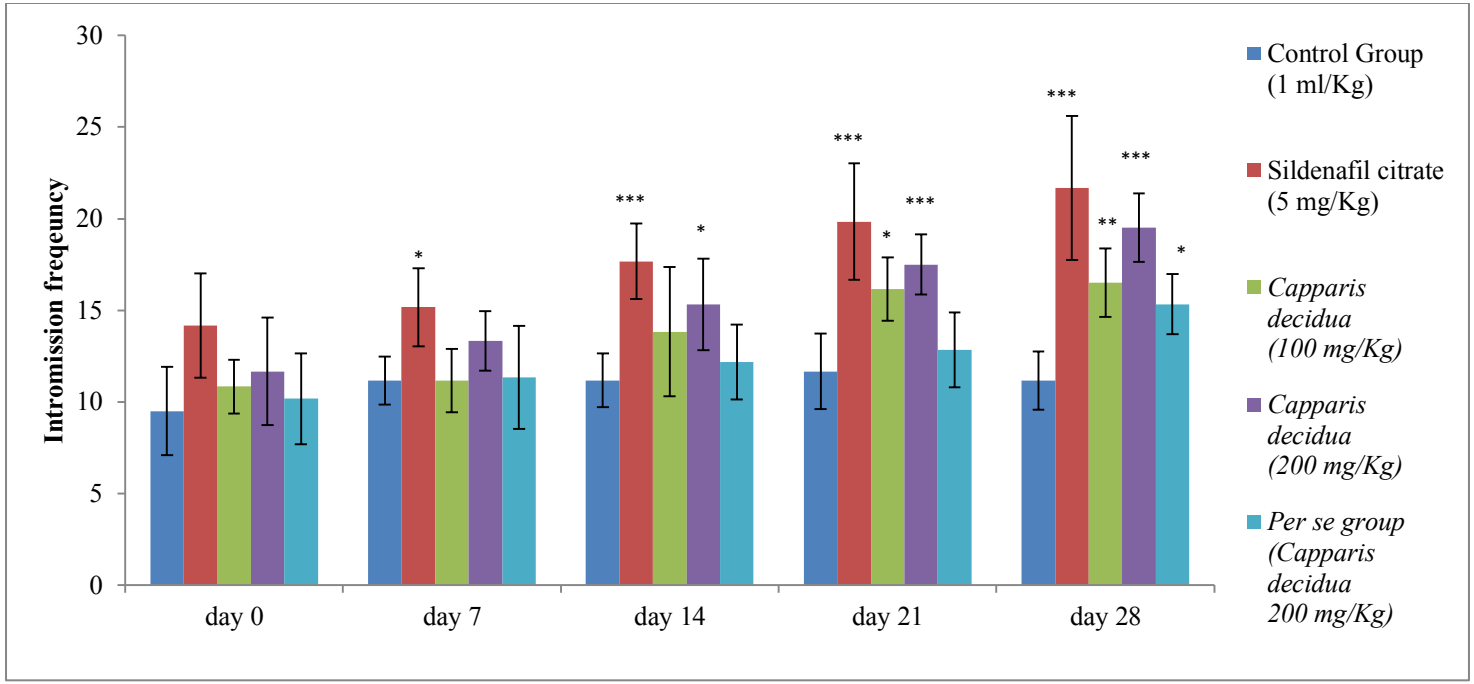


Figure 60: Effect of hydroalcoholic extract of *Capparis decidua* on Intromission frequency in male rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

Table 33: Effect of hydroalcoholic extract of *Capparis decidua* on serum testosterone level on male Wistar rats.

Treatment	Serum Testerone Level (ng/ml) on day						
	Before treatment	**day 0**	**day 7**	**day 14**	**day 21**	**day 28**	**7 days after treatment**
Control Group (1 ml/Kg)	1.17±0.04	1.17±0.04	1.16±0.04	1.16±0.04	1.18±0.04	1.2±0.03	1.2±0.03
Sildenafil citrate (5 mg/Kg)	0.95±0.08	0.94±0.07	1.22±0.18	2.22±0.49***	3.23±0.07***	3.73±0.27***	3.48±0.23***
***Capparis decidua* (100 mg/Kg)**	0.98±0.03	0.98±0.03	1.11±0.01	1.21±0.03	1.41±0.11	1.7±0.17**	1.65±0.12**
***Capparis decidua* (200 mg/Kg)**	0.99±0.02	0.99±0.02	1.12±0.01	1.32±0.09	1.61±0.2**	2.09±0.1***	2.01±0.1***
***Per se* group (*Capparis decidua* 200 mg/Kg)**	1.17±0.06	1.17±0.06	1.23±0.04	1.33±0.03	1.52±0.2*	1.92±0.31***	1.86±0.36***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

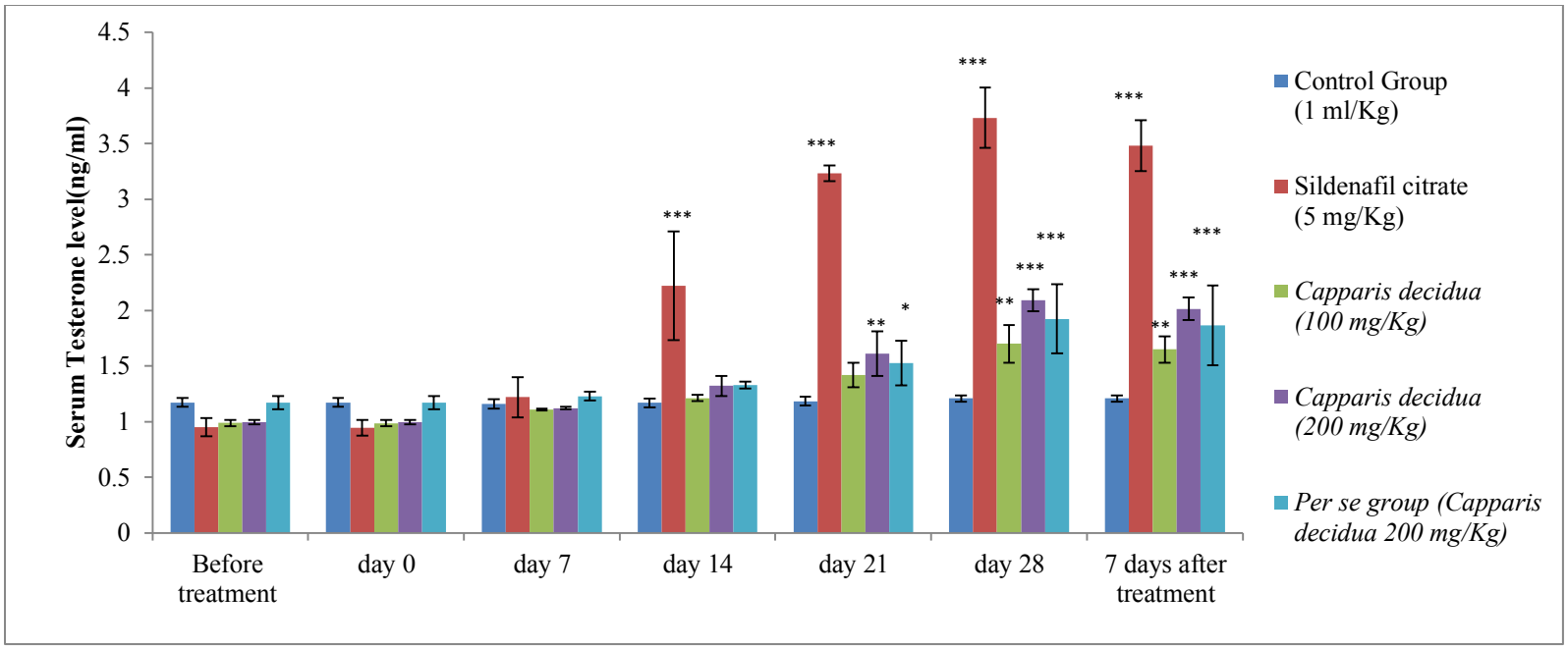


Figure 61: Effect of hydroalcoholic extract of *Capparis decidua* on serum testosterone level on male Wistar rats. Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P < 0.01; ***P < 0.001.

Figure 62: Representation of various coital positions of male and female Wistar rats during the sexual behavior studies in aphrodisiac activity.

Evaluation of wound healing activity

5.24 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CITRULLUS COLOCYNTHIS* IN EXCISION WOUND MODEL

The progress of the wound healing induced by *Citrullus colocynthis* fruit extract ointments (5% and 10% w:w) treated groups, normal control (distilled water) treated group and Povidone iodine (standard drug) treated group of animals are shown in Table 34. It is observed that the wound contracting ability of the extract ointment in different concentrations was significantly greater than that of the control group. The 5% & 10% (w:w) extract ointment treated groups showed significant ($P<0.001$) wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. Povidone iodine ointment treated group of animals ($P<0.001$). The wound closure time was lesser, as well as the percentage of wound contraction was much more with the *Citrullus colocynthis* extract 10% w:w ointment treated group (20 days for 100% contraction which was nearly similar to that of the Povidone iodine treated group that showed 100 % contraction on 18th day). *Citrullus colocynthis* 5% extract ointment treated group of animals showed significant wound contraction from the fourth day onwards and achieved 100% with the wound closure time of 20 days but wound area and percentage wound contraction was slightly less as compared to 10% ointment till 18th day. Povidone iodine ointment showed significant ($P<0.01$) decrease in wound area from 2nd day onwards and 100% wound contraction was obtained on 18th day after treatment began.

The present study shows that hydroalcoholic extract of *Citrullus colocynthis* fruit possesses a good wound healing activity. Topical administration of extracts reduced the epithelization time from 21.83(normal control group) to 18 days (*Citrullus colocynthis* 10% ointment) ($P<0.001$), 19 days (*Citrullus colocynthis* 5% ointment) ($P<0.01$) and reduced the scar area on complete epithelization from 54 (normal control group) to 38.16 mm2 (*Citrullus colocynthis* 10% ointment) ($P<0.001$) and 43 mm2 (*Citrullus colocynthis* 5% ointment) ($P<0.001$). Further theses are comparable to the standard drug Povidone iodine treatment group as it showed the epithelization time as 17.16 days ($P<0.001$) and scar area as 34.16 mm^2 ($P<0.001$). **Table 34-35 and Figure 63-67.**

5.25 EFFECT OF HYDROALCOHOLIC EXTRACT OF *CITRULLUS COLOCYNTHIS* IN INCISION WOUND MODEL

The measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 3. The tensile strength of the *Citrullus colocynthis* 10% ointment treated group showed significant tensile strength value of 420.5±9.58 g, while *Citrullus colocynthis* 5% ointment treated group gave value of 319.83±10.26 g as tensile strength and Povidone iodine ointment treated group gave value of tensile strength as 538.33±23.16 g. The 5% extract ointment treated group showed a lesser but significant increase in the tensile strength compared to the control group. Thus both concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound. **Table 36 and Figure 68-69.**

Table 34: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on wound area in excision wound healing model.

Treatment	Wound area(mm^2) mean±SD & (percentage wound contraction) on day										
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
Control group (1 ml/Kg)	512±1.41	495.8±3.76 (3.16)	488.33±1.63(4.62)	465.33±1.86(9.11)	401±4.33 (21.67)	368.5±4.76 (28.02)	307.66±5.08(39.91)	267.33±5.57(47.78)	203.33±4.17(60.28)	162.33±7.91	147.16±5.56
Povidone iodine ointment (5% topically)	518±0.89	490.33±2.16** (5.34)	402.16±2.04*** (22.36)	364.83±2.31***(29.56)	274.16±4.99*** (47.07)	186.66±7.55*** (63.96)	101.16±5.11*** (80.47)	69.83±3.6*** (86.51)	25.16±3.61*** (95.14)	0*** (100)	0*** (100)
Citrullus colocynthis ointment (5% topically)	521±1.54	495.33±2.16 (4.92)	412.83±1.47*** (20.76)	382.83±4.11***(26.52)	297.83±5.0*** (42.83)	215.33±4.71*** (58.66)	134.33±4.03*** (74.21)	91.8±3.12*** (82.37)	49.16±4.7*** (90.56)	12.66±2.8*** (97.57)	0*** (100)
Citrullus colocynthis ointment (10% topically)	522±1.26	492.33±1.03 (5.68)	405.83±1.96*** (22.25)	373.5±3.93*** (28.44)	277.83±6.04*** (46.77)	201±3.74*** (61.49)	122.66±2.33*** (76.50)	84±6.22*** (83.90)	38.5±2.73*** (92.62	6.5±1.37*** (98.75)	0*** (100)

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.

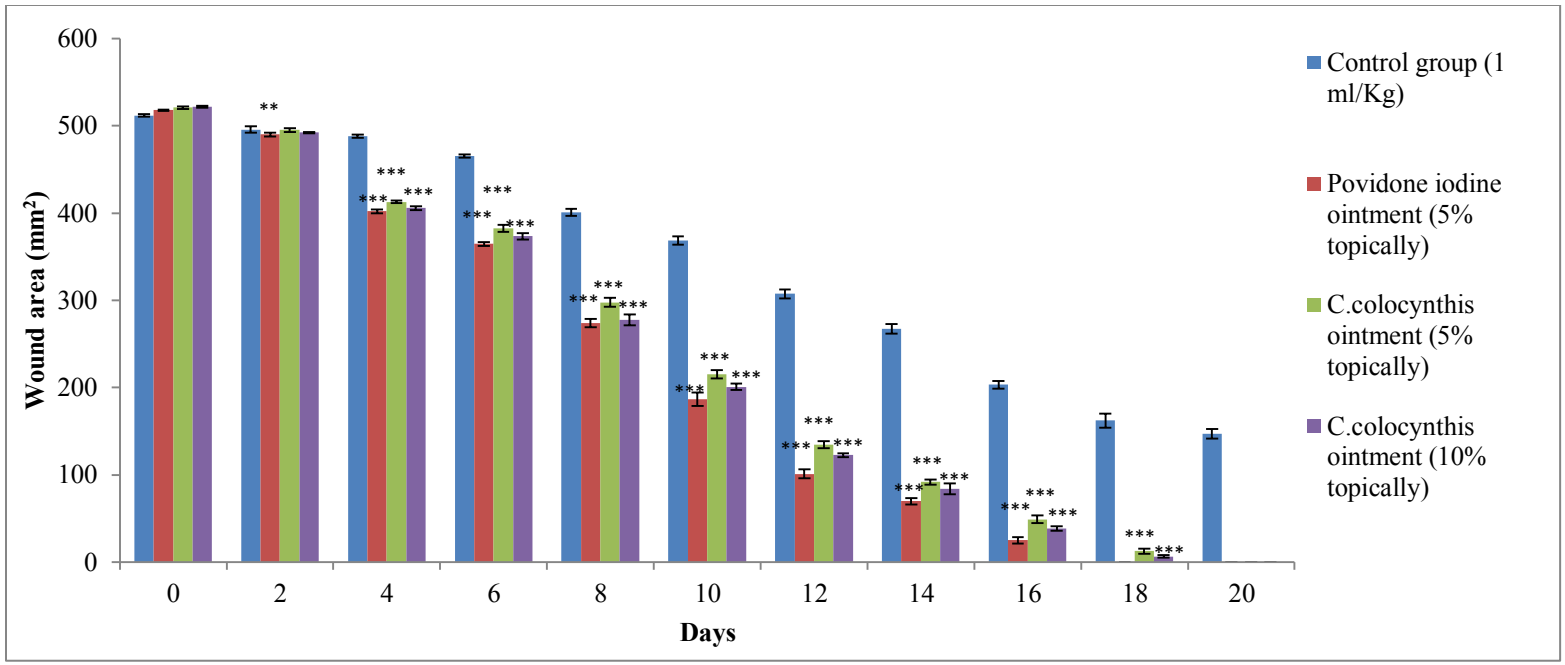


Figure 63: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on wound area in excision wound healing model. Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.

Table 35: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on epithelization time and scar area in excision wound healing model.

Treatment	Epithelization time (days)	Scar area (mm^2)
Control group (1 ml/Kg)	21.83±0.75	54±1.41
Povidone iodine ointment (5% topically)	17.16±1.16***	34.16±2.31***
Citrullus colocynthis ointment (5% topically)	19±1.78**	43±1.78***
Citrullus colocynthis ointment (10% topically)	18±0.89***	38.16±1.94***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: **P<0.01; ***P<0.001 as compared to control.

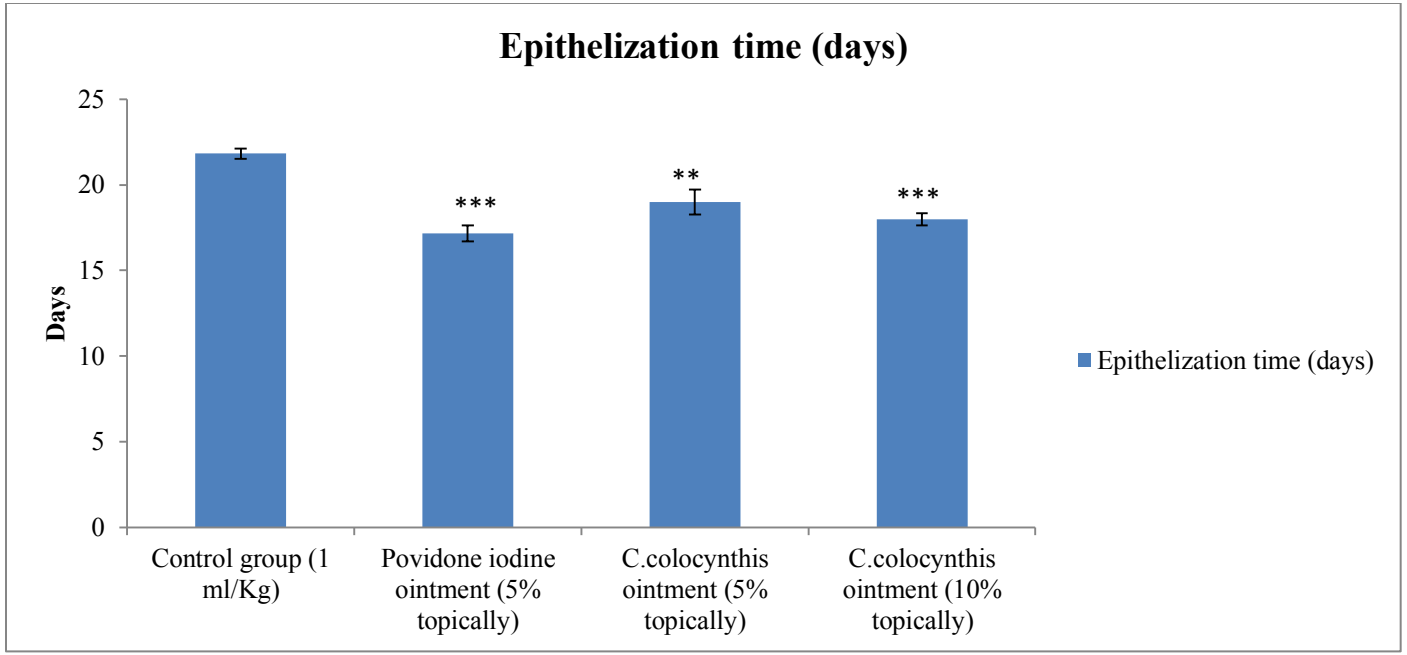


Figure 64: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on epithelization time in excision wound healing model. Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.

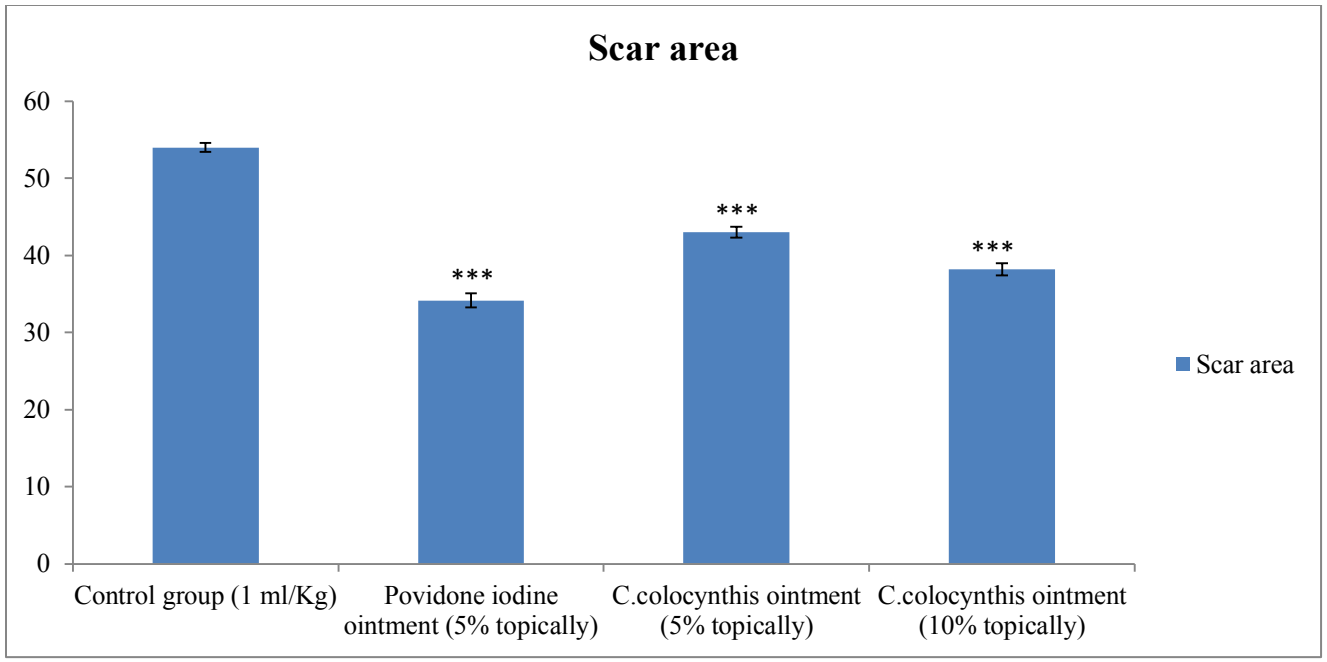


Figure 65: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on scar area in excision wound healing model. Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.

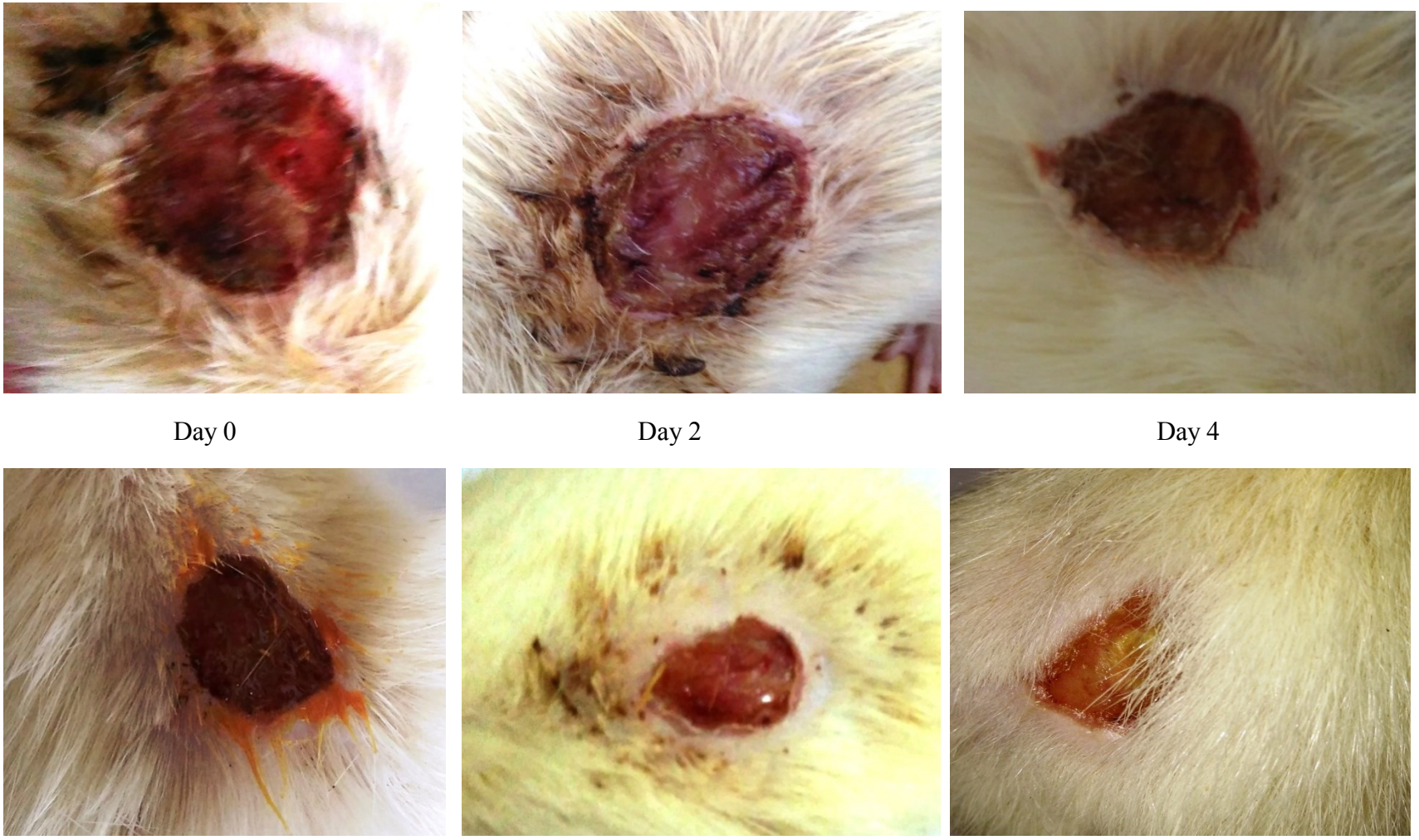
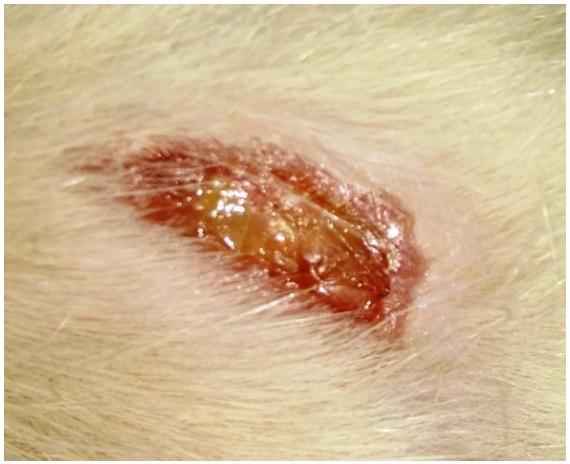
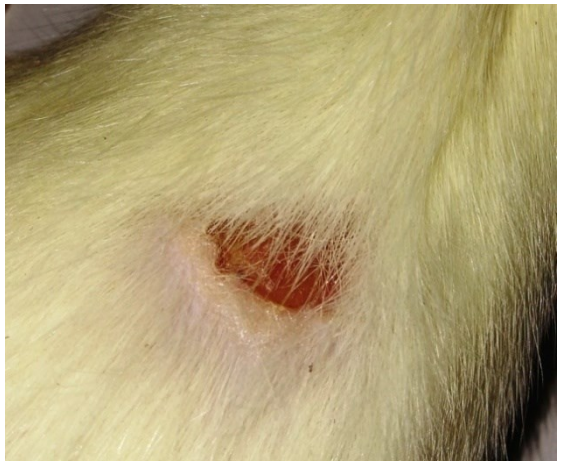


Figure 66: Representation of the wound healing on various days from 0 to 20 in excision wound healing model.



Day 12



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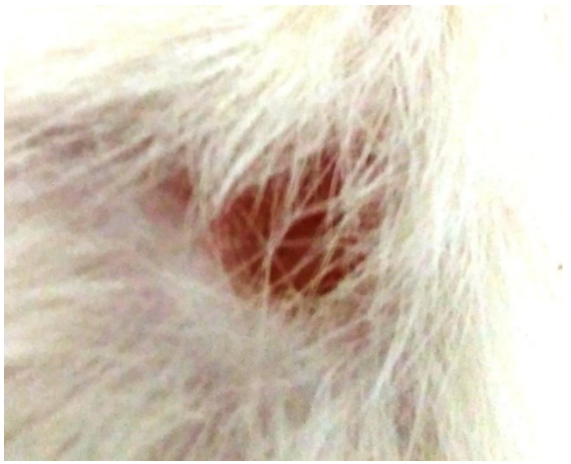

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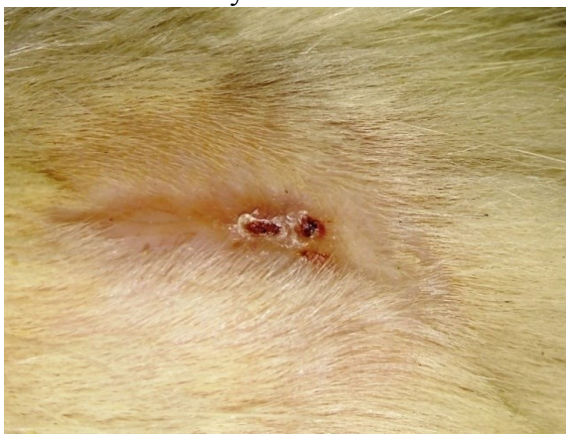
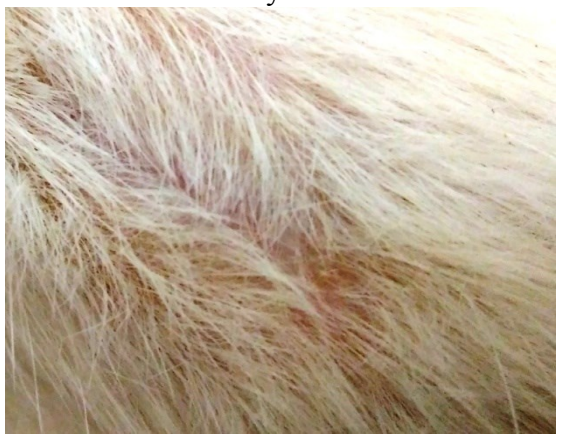


Day 18



Day 20

Figure 66: Representation of the wound healing on various days from 0 to 20 in excision wound healing model.

Table 36: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on tensile strength in incision wound healing model.

S.No.	Number of animals	Treatments	Tensile strength (g) mean±SD
1	6	Control group (1 ml/Kg)	244±14.29
2	6	Povidone iodine ointment (5% topically)	538.33±23.16***
3	6	*Citrullus colocynthis* ointment (5% topically)	319.83±10.26***
4	6	*Citrullus colocynthis* ointment (10% topically)	420.5±9.58***

Tabular values are expressed as mean ± SD, n = 6 (number of animals in each group); Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.

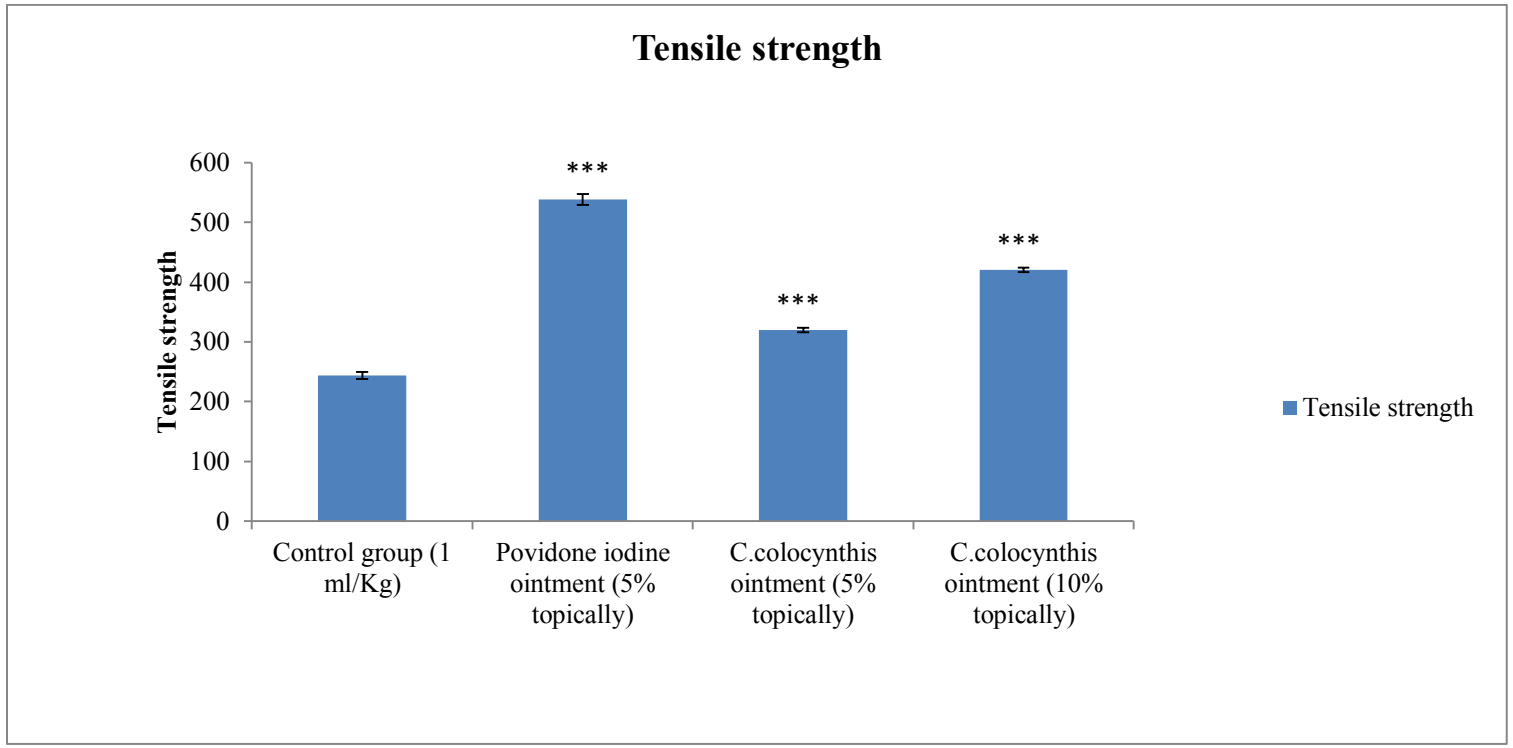
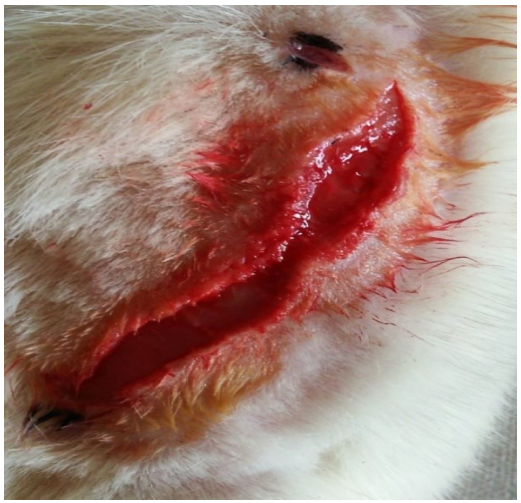
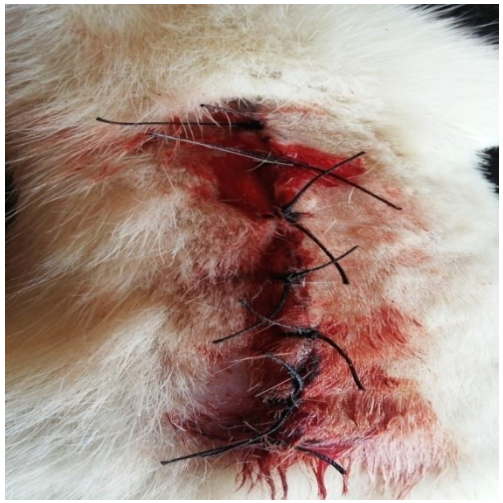


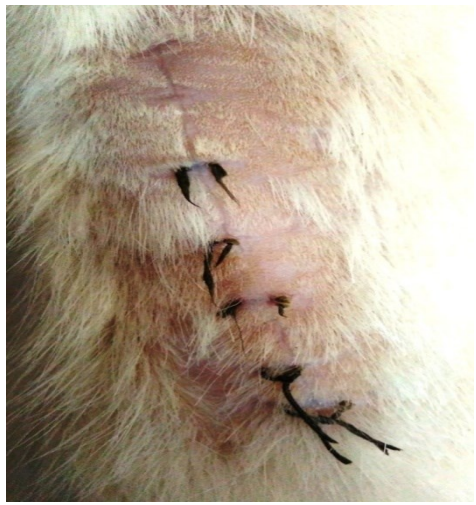
Figure 67: Effect of hydroalcoholic fruit extract of *Citrullus colocynthis* topically on tensile strength in incision wound healing model. Statistical evaluation of the experimental results was performed against the control using Dunnett's Multiple Comparison Test: $^{**}P<0.01$; $^{***}P<0.001$as compared to control.



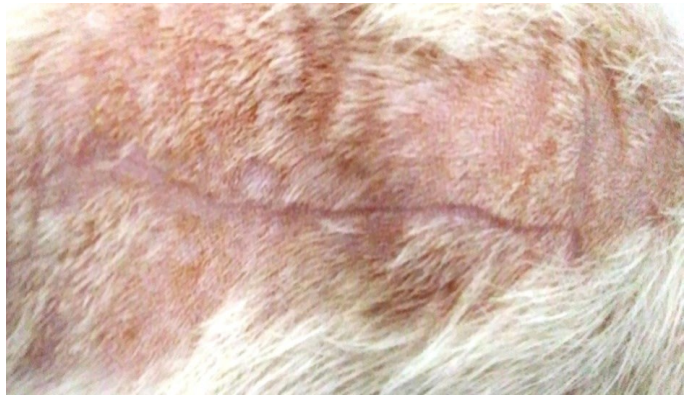
Day 0 (incision on paravertebral surface)



Day 0 (stitching)



Day 8 (dissolution of stitches)



Day 10 showing complete healing of incision

Figure 68: Representation of figures showing the wound healing in incision wound model.

Chapter 6

Discussions

Within the context of present study, anti-diarrheal and wound healing activity of hydroalcoholic extract of *Citrullus colocynthis* and anti-arthritic, aphrodisiac activity of the hydroalcoholic extract of *Capparis decidua* were investigated by using various animal models in order to assess the validity of the use of this plant.

Results of our investigation have established that fruits of *Citrullus colocynthis* possess the anti-diarrheal and wound healing potentials and root, stem and leaves of *Capparis decidua* have aphrodisiac and anti-arthritic potential. The activity could be attributed to the presence of phytochemical found in these plants as done in phytochemical screening.

In our experiment, the anti-diarrheal activities were evaluated in two *in-vivo* study models (COID and BSM). The hydroalcoholic extract of *Citrullus colocynthis* (fruits) inhibited the Castor oil induced diarrhea at doses- 100 mg/Kg and 50 mg/Kg. While *Per se* group (only 100 mg/Kg of *Citrullus colocynthis* extract administered without induction of diarrhea) also showed highly significant results which were used to evaluate the beneficial effects of *Citrullus colocynthis* extract in both the plants collected from Jaipur and Jodhpur. In COID model the hydroalcoholic extracts showed decrease in number of wet defecations in 6 h [100 mg/Kg ($P<0.001$), 50 mg/Kg ($P<0.01$), *Per se* ($P<0.001$)], weight of stool [100 mg/Kg ($P<0.001$), 50 mg/Kg ($P<0.05$), *Per se* ($P<0.001$)] and water content of feces [100 mg/Kg ($P<0.001$), 50 mg/ kg ($P<0.01$), *Per se* ($P<0.001$)]. The experimental groups of *Citrullus colocynthis* extract (CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/Kg) significantly diminished the severity of diarrhea with respect to decreasing in the rate of defecation and watery content of feces in Wistar rats. All the extracts showed significant anti-diarrheal activity demonstrating 52.48%, 21.29% and 71.32% reductions in diarrhea respectively in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/ kg as compared to that of Loperamide that demonstrated 72.16% reductions in diarrhea in *C. colocynthis*, obtained from Jaipur while in the studies conducted in plant obtained from Jodhpur 58.27%, 38.64% and 73.25% reductions in diarrhea respectively

in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/ kg as compared to that of Loperamide that demonstrated 76.37% reductions in diarrhea. While the percent inhibition of water content was found to be significant displaying 34.34%, 07.63% and 83.19% decrease in water content respectively in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/Kg as compared to that of the standard drug Loperamide that showed 100% reductions in water content in diarrhea in *Citrullus colocynthis* obtained from Jaipur while *Citrullus colocynthis* obtained from Jodhpur showed 35.01%, 7.46%, 83.86% decrease in water content respectively in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/Kg as compared to that of the standard drug Loperamide that showed 100% reductions in water content in diarrhea.

In the gastrointestinal motility test, all the doses of extract produced a significant decrease in intestinal motility. In gastrointestinal motility test with BSM, all the doses of hydro alcoholic extract of *Citrullus colocynthis* decreased intestinal transmit significantly ($P<0.001$). The normal control group showed 56.93% intestinal motility by the Barium Sulfate milk in gastrointestinal motility test. *Citrullus colocynthis* extracts showed intestinal motility as 41.8%, 47.08% and 43.41% respectively in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/Kg groups. CCE extracts also gave a significant inhibition of intestinal motility with values as 26.57% (CCE 100 mg/Kg), 17.3% (CCE 50 mg/Kg), and 23.74% (*Per se* 100 mg/Kg) while Loperamide (2 mg/Kg) had a value of 38.89% ($P<0.001$) of decrease in intestinal motility. While in *Citrullus colocynthis* obtained from Jodhpur normal control group showed 55.25% intestinal motility by the Barium Sulfate milk in gastrointestinal motility test. *Citrullus colocynthis* extracts showed intestinal motility as 42.49%, 48.77% and 42.83% respectively in CCE 100 mg/Kg, CCE 50 mg/Kg and *Per se* 100 mg/Kg groups. CCE extracts also gave a significant inhibition of intestinal motility with values as 23% (CCE 100 mg/Kg), 11.7% (CCE 50 mg/Kg), and 22.4% (*Per se* 100 mg/Kg) while Loperamide (2 mg/Kg) had a value of 33.24% ($P<0.001$) of decrease in intestinal motility.

Similar results were also ascertained in prior anti-diarrheal studies conducted which substantiates the medicinal use of *Citrullus colocynthis* in the treatment of diarrhea.

In prior anti-diarrheal studies, ethanolic extracts of *Cynodon dactylon* Pers. aerial parts (EECA) in Wistar rats demonstrates that EECA viably restrains the recurrence of wetting feces and defecation as well as inhibit the water content of total feces. In gastrointestinal motility test with BSM, the most astounding decrease of gastrointestinal motility is for Loperamide at a dose of 2 mg/Kg and inhibition of the distance traveled by BaSO4 milk is 39.6%. While the plant extracts decrease the distance of gastrointestinal motility of rats ranging from 58.57% (control group) to 47.12% and inhibition of distance traveled by barium sulfate milk is 19.55% at the dose of 1 g/kg of extract dose as compared to control.

In another anti-diarrheal investigation of methanol (MEHO), ethanol (EEHO) and water (AEHO) extracts of *H. odorata* leaves demonstrate critical ($p<0.001$) inhibition against castor oil-induced diarrhea. At the 400 mg/Kg dose, the extracts show significant anti-diarrheal activity ($P<0.001$) demonstrating 47.76 ± 2.36%, 58.21 ± 6.92% and 56.72 ± 5.48%, reductions in diarrhea respectively in AEHO, EEHO and MEHO comparable to that of the standard drug Loperamide with 59.70 ± 2.99% reduction in diarrhea. The normal control group demonstrates intestinal motility as 84.85 ± 2.88%. The 200 and 400 mg/Kg (p.o) of the extracts displays intestinal motility as 51.51 ± 0.97% to 62.05 ± 1.41%. Also, the extracts significantly inhibit intestinal motility as 22.82 ± 1.76% to 35.93 ± 1.21% at all the doses. Be that as it may, Loperamide (5 mg/Kg) shows a significant inhibition (43.6 ± 2.14%) in intestinal motility (DeSales et al, 2015).

Castor oil is obtained from the seeds of *Ricinus communis* (Family-Euphorbiaceae). Castor oil acts as a stimulant laxative which hydrolyzes to form ricinoleic acid, a local irritant and instigate changes in gastrointestinal mucosal fluid and electrolyte transport bringing about hypersecretory reaction and diarrhea. Experimental studies demonstrated that inflammatory response occurring due to ricinoleic acid causes generation of prostaglandins PGE2. Ricinoleic acid diminishes the active Na+ and K+ retention and declines Na+- K+- ATPase pump in small intestine and colon and henceforth hindering the mucosal c-AMP intervened dynamic secretion. Loperamide is an opioid derivative that functions through mu receptors on neurons in sub mucosal neural plexus of intestinal wall and moderates the intestinal motility, aside from it, likewise, indicates

antimuscarinic action in the gastrointestinal tract. So clearly Loperamide protected the Wistar rats through above mechanism (Hussain et al, 2014).

Standard chemical test carried out during the phytochemical screening of *Citrullus colocynthis* showed the presence of a number of bioactive constituents such as phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. The counter diarrheal action could be ascribed to these compounds. Previous literature survey and experimental studies also showed the presence of these compounds in the fruit of *Citrullus colocynthis* as per Kumar et al, (2009) and Umer et al, (2013). In earlier studies, anti-diarrheal activity is showed by the plants having alkaloids, tannins, saponins, steroids, terpenoids, and flavonoids. Hostile to the looseness of the bowels exercises of flavonoids have been credited to their capacity to hinder intestinal motility and hydro electrolytic discharges which are known to be modified in diarrheic conditions. Tannins denature proteins in the intestinal mucosa by shaping protein tannates which may lessen secretion (Yacob et al, 2016).

Studies on the on the useful part of tannins additionally uncovered that they can likewise lessen the peristaltic developments and intestinal discharges by decreasing the intracellular Ca2+ inward current or by the enactment of the calcium pumping system (which actuates the muscle unwinding) ascribed by calcium channel blocking and spasmolytic activities of tannins present in the plant extract. Sesquiterpenes, terpenes, diterpenes and various terpenoids derivatives are known for repressing release of prostaglandins and autacoids; in this way restrain the motility and secretion instigated by castor oil (Barsante et al, 2005).

In the present study, anti-arthritic effect of *Capparis decidua* was additionally affirmed by Freund's Complete Adjuvant arthritis in rats. The FCA model is an entrenched rat model to study the inflammation as per Ochaion et al, (2006). FCA comprises of inactivated and dried mycobacterium, which adequately fortifies cell intervened insusceptibility and eventually drives the immunoglobulin generation and further creation of prostaglandins. The Diclofenac, a non-steroidal anti-inflammatory drug was utilized for examination since it is usually recommended for the treatment of joint inflammation

and its activity is primarily through the hindrance of cyclooxygenase and prostaglandin creation according to Furst et al, (2001) and Issekutz et al, (1991). In the present investigation Diclofenac sodium kept the spread of adjuvant instigated joint pain which is predictable with past reports of different scientists as per Swierkot et al, (2006) and Mythilypriya et al, (2008). In the present examination, hydroalcoholic extract of *Capparis decidua* (100 and 200 mg/Kg) treatment showed anti-arthritic effect in all the arthritic parameters. It significantly decreased the inflammation compared to the FCA group as observed by decreased paw joint diameter and arthritic score. The present study revealed that paw joint diameter increases with ankle stiffness in FCA subjected rats. The analgesic effect of *Capparis decidua* (100 and 200 mg/Kg) in rats with FCA induced arthritis is also marked as evident by the increase in pain threshold. Muscle grip strength of FCA group rats markedly reduced and in *Capparis decidua* (100 and 200 mg/Kg) treated groups the fall of time in motor in-coordination test significantly increased suggesting the anti-arthritic activity of hydroalcoholic extract of *Capparis decidua*.

In the present study, the single intradermal injection with FCA (0.1 mL) significantly ($P<0.001$) elevated the serum ALP, AST and ALT level and decreased the total protein level. Evaluation of the serum levels of ALP, AST and ALT provides an excellent and simple tool to measure the anti-arthritic activity of the drug. The activities of aminotransferase and alkaline phosphatase rises significantly in arthritic rats, since these are good markers of liver and kidney disorders which is also considered a feature of adjuvant arthritis. Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as kinin in inflammatory process according to Glenn et al, (1965). The administration of *Capparis decidua* (100 and 200 mg/Kg) hydroalcoholic extract significantly ($P<0.001$) decreased the level of ALP, AST and ALT and increased the level of total protein that confirms the anti-arthritic activity of the extract.

In the present study, hydroalcoholic extract of *Capparis decidua* (100 and 200 mg/Kg) and *Per se* treatment showed an anti-arthritic effect in the inflammatory parameter like paw volume. *Capparis decidua* extract significantly ($P<0.001$ at 100 & 200mg/Kg) decreased the inflammation compared to the FCA treated the group as observed by

decreased paw volume. The present study revealed that paw volume rises with ankle bone hardness in FCA treated rats. The body weight of FCA treated group rats was prominently decreased compared with that of normal control rats. The results suggest that oral *Capparis decidua* extract (100 mg/Kg, 200 mg/Kg and *Per se*) reduced inflammatory body weight loss in arthritis induced rats. Thus, *Capparis decidua* gives protective action in terms of body weight. The decrease in body weight of FCA-induced arthritic rats in the present study is because of decreased intestinal absorption rate. Treatment with extract significant inhibited weight loss in arthritic rats. Thus, *Capparis decidua* extract may have the potential as a therapeutic agent used for symptomatic treatment of rheumatoid arthritis because of its anti-inflammatory action which delays progression of disease as per Wood et al, (1969). Increase in the WBC count in FCA treated group rats indicates the leukocytosis in the joint region by infiltration of neutrophil cells. This data may be affirmed by the earlier study conducted by Glenn et al, (1965) who reported neutrophilia and leukocytosis on day 28 post-induction.

In arthritis decreased level of hemoglobin (Hb) and red blood cells (RBCs) is caused because of the diminished reaction of the bone marrow erythropoietin and pulverization of untimely RBCs as per Patil et al, (2011). So also increment in the level of erythrocyte sedimentation rate (ESR) is credited to the quickened arrangement of endogenous proteins including plasma proteins, for example, fibrinogen, alpha and beta globulins. Henceforth these parameters are key biomarkers that are elevated during inflammation, stress and cell necrosis as per Maria et al, (1983). In our study, treatment with *Capparis decidua* extract in arthritic rats significantly increased the level of RBC and Hb while it decreased the level of ESR which can be credited to its mitigating potential. Ascend in a number of platelets in FCA treated group rats also indicated the inflammatory pathogenesis in the joint region while a decrease in the number of platelets in *Capparis decidua* extract treated groups suggest the protective role of this plant in arthritis.

The anti-arthritic effect of *Capparis decidua* hydroalcoholic extract set up in this investigation could be ascribed to the nearness of flavonoids, triterpenoids, saponins, tannins and steroids detected after phytochemical screening of the *Capparis decidua*. Triterpenoids are known to repress histamine discharge from mast cells and exert anti-

inflammatory effects. Non-specific anti-arthritic activity might be because of the consolidated impact of the distinctive phytoconstituents display.

In the present study, hydroalcoholic extract of *Capparis decidua* was tested in animal experimentation for its effect on sexual behavior, and Sildenafil citrate was used as the standard referent. Mating behavior test revealed that the extract of *Capparis decidua* significantly increased the Mounting Frequency (MF) and Intromission Frequency (IF) as compared to control but less than that of the standard drug. The (MF) and (IF) are considered as the exponents of sexual desire and potency. So, it indicates that the test drug possesses a sexual function improving effect. The extract of *Capparis decidua* significantly increased the EL as compared to control group animals, whereas a highly significant increase was observed with the standard drug Sildenafil citrate. The *Capparis decidua* extract was found to produce a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control while a highly significant decrease was found in ML of animals treated with Sildenafil citrate. This is also an evidence of the sexual function improving effect of the *Capparis decidua* extract. The significant increase in the Ejaculatory Latency (EL) indicates that the extract and standard drug prolonged the duration of coitus. These findings demonstrate that the extract produces a striking improvement of general sexual execution of rats in experimental groups. Moreover the proceptive behaviors were seen in the animals like darting, hopping and lordosis by female rats and precopulatory behavior in male rats as well which implicates the sexual arousal between opposite sex rats. Mount Frequency and Intromission Frequency are useful factors of sexual strength, sexual desire and potency. The number of mount (MF) reflects sexual motivation, and rise in the number of intromission (IF) shows the efficiency of erection.

Some of the medicinal plants are effective as aphrodisiac through mechanisms such as vasodilatation, generation of nitric oxide, elevation of androgens and gonadotropins as per Mills et al, (1996).

In the previous studies it is seen that Dehydroepiandrosterone (DHEA), a major circulating steroid in the plasma, and a common precursor for both androgens and

estrogens and its subsequent conversions to testosterone and its metabolites responsible for the effective masculine behavior in rats (Majewska et al, 1995).

The involvement of saponins in the biosynthesis of DHEA boosts the level of testosterone and therefore triggers the sexual desire in male rats (Gauthaman et al, 2008).

Steroidal nature of saponins makes it possible to act as intermediary in androgen synthesis where saponins binds to hormone receptor and undergoes conformational change to yield androgen production. Similarly the flavonoids due to its antioxidant property alter androgen levels and are responsible for the enhanced male sexual behavior. Alkaloids are reported to have ergogenic properties act on central nervous system by causing vasodilatation of the blood vessels through the production of endothelium dependent releasing factor i.e. nitric oxide and allowing erection or arouses steroidogenesis in the testes of the animals. Alkaloids also act on peripheral nervous system by relaxing Corpus cavernosum smooth muscle in the penis of the male rats (Yakubu et al, 2011).

In the previous studies ethanolic extract of rhizomes *Curculigo orchioides* evaluated for effect on sexual behavior in rats. 100 mg/Kg of extract change significantly the sexual behavior pattern assessed by parameters such as mating performance mount frequency and mount latency. The rhizome extract markedly affected sexual behavior of rats as seen in reduction of mount latency, an increase in mount frequency and enhanced attract ability towards female rats (Chauhan et al, 1968).

A significant ($P<0.001$) increase in testosterone level was found in the extract treated animals compared with control. It demonstrates that the extract has an impact at the endocrine level. Testosterone is the significant male gonadal hormone, and it is created by the interstitial Leydig cells in the testis. It is additionally the real factor for androgenicity. A specific concentration of androgens is required for the initiation and maintenance of spermatogenesis and for the start and support of spermatogenesis and for the incitement of development and the working of the prostate and original vesicles. The expansion in testosterone level may improve androgen-dependent parameters such as

mating behavior and the maintenance of spermatogenesis (Connel et al, 1968; Johnson 1971; Holt et al, 1973; Dorrington and Armstrong 1975)

During the phytochemical screening of extract of *Capparis decidua* there was occurrence of phenols, flavonoids, alkaloids, saponins and many other phytochemical. Thus, the resultant aphrodisiac activity of the test drug might be attributed to phytochemical like phenols, flavonoids, alkaloids, saponins.

In late decades more prominent comprehension of the ideas identified with healing have prepared the business to create and popularize more particular items that are powerful and sufficient for each type of wound as for the cost/advantage proportion. As of now, analysts are assessing changes in sub-atomic science concerning the amalgamation of substances involved in the healing phenomena. With so many resources to promote healing, in this study it was decided to evaluate hydroalcoholic extract of *Citrullus colocynthis* fruits in wound healing, as they positively affect the healing process. Wound healing process consists of different phases such as granulation, collagenization, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in this study two models were used to assess the effect of hydroalcoholic *Citrullus colocynthis* extracts on 5% and 10% ointment doses. The result of the present study showed that *Citrullus colocynthis* possesses a definite prohealing action. In excision wound healing model the hydroalcoholic extract of the fruits of the plant *Citrullus colocynthis* showed significant increase in percentage closure of excision wounds by enhanced epithelization. This enhanced epithelization may be due to the effect of *Citrullus colocynthis* extracts on enhanced collagen synthesis. Similarly, the breaking strength of the incision wounds was increased in hydroalcoholic extract treated groups in incision wound healing model. Deposition of newly synthesized collagens at the wound site increases the collagen concentration per unit area and hence the tissue tensile strength (Tsuchiya et al, 1996).

According to Scortichini and Pia (1991) studies have shown that phytochemical constituents like flavonoids and triterpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial properties, which appear to be

responsible for wound contraction and increased rate of epithelialization. The preliminary phytochemical analysis of the *Citrullus colocynthis* fruit extract showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. Any one of the observed phytochemical constituents present in *Citrullus colocynthis* may be responsible for the wound healing activity.

So the present study proposes the wound healing role of *Citrullus colocynthis* extract ointment at both the dose of 5% and 10%.

On the basis of rainfall intensities the Rajasthan state has been divided into arid (low rainfall 10-20 cm), semi-arid (slightly improved rainfall 20-40 cm) and sub-humid regions (better rainfall 40-60 cm). Jodhpur district lies in the arid zone receiving low rainfall while Jaipur district lies in the semiarid zone receiving improved rainfall than Jodhpur. *Capparis decidua* and *Citrullus colocynthis* plants grows well in dry and less rainfall conditions. Also these plants are regarded as climate change indicators in dry regions. *Capparis decidua* and *Citrullus colocynthis* are fully laden with the fruits and flowers in drought and high temperature regions like in Jodhpur (arid zone) which do suggest the higher proportion of phytoconstituents in various parts of this plant. While in good rainfall regions like Jaipur plants like *Capparis decidua* and *Citrullus colocynthis* bears only average fruits and flowers and low proportion of phytoconstituents. Hence *Capparis decidua* and *Citrullus colocynthis* hydroalcoholic extracts obtained from the Jodhpur district showed much better results as compared to that obtained from Jaipur district.

Hence *Capparis decidua* showed better anti-arthritic activity, aphrodisiac activity and *Citrullus colocynthis* showed anti-diarrheal, wound healing activities in plants obtained from Jodhpur region as compared to that obtained from Jaipur region.

Chapter 7
Summary and Conclusion

Capparis decidua Linn. (Family: Capparidaceae) is commonly known as 'Kair. The plant is xerophytic and is growing commonly throughout the dry areas of tropical and subtropical regions. According to Ayurveda literature, the plant possesses anti-inflammatory, anti-rheumatic, hepatoprotective, analgesic, diaphoretic, astringent, laxative, anthelmintic and antibacterial properties. It is reported to be used in traditional systems of medicine in the treatment of various diseases, like rheumatism, gout, boils and swellings, jaundice, biliousness, dropsy, diabetes, cough, asthma, cardiac troubles, urinary purulent discharges, piles and ulcers.

Citrullus colocynthis (L.) Schrad. is a Cucurbitaceae family plant. The fruit is intense and globular with a smooth surface. It is hard and has a skin around it and contains 200–300 seeds/gourd. Seeds are small, ovoid, compressed, smooth and brownish when ripe. *Citrullus colocynthis* has a wide range of therapeutic and nutritional uses. Traditionally this plant is used in the treatment of diseases like cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases.

The fruits of *Citrullus colocynthis* were evaluated for preliminary phytochemical investigations, various ash and extractive values, anti-diarrheal and wound healing activities.

The root, stem and leaves of *Capparis decidua* was evaluated for preliminary phytochemical investigations, various ash and extractive values, anti-arthritic and aphrodisiac activities.

Preliminary phytochemical screening indicated that the fruits of *Citrullus colocynthis* contained phenolic compounds, flavonoids, alkaloids, terpenoids, saponins, steroids, tannins, cardiac glycosides, carbohydrates.

Preliminary phytochemical screening indicated that the root, stem and leaves of *Capparis decidua* contained phenolic compounds, flavonoids, alkaloids, terpenoids, saponins, steroids, tannins, cardiac glycosides, carbohydrates.

Loss on drying of *Capparis decidua* was found to be 40.16%, Ash value was found as Total ash (7.14%), Acid insoluble ash (0.62%), Water soluble ash (6.01%), Extractive values of *Capparis decidua* was found as Water soluble extractive (18%), Alcohol Soluble extractive (8.51%).

Loss on drying of *Citrullus colocynthis* was found to be 35.24%, Ash value was found as Total ash (76.31%), Acid insoluble ash (0.54%), Water soluble ash (7.06%), Extractive values of *Citrullus colocynthis* was found as Water soluble extractive (17.09%), Alcohol Soluble extractive (9.32%).

The anti-diarrheal activity was evaluated in hydroalcoholic extract of Citrulus colocynthis fruits using Castor oil induced diarrhea (COID) model and Barium sulfate milk (BSM) model. The results indicated that both the doses of *Citrullus colocynthis* extract (100 mg/Kg and 50 mg/Kg) showed protection against COID model and BSM model. The hydroalcoholic extract of *Citrullus colocynthis* at 100 mg/Kg showed highly significant results such as prolonged the latency time, reduced the defecation frequency, number of wet defecations, the weight of stool and water content of feces when compared with the disease control group ($P<0.001$) but at 50 mg/Kg the response obtained was less significant ($P<0.01$) as compared to 100 mg/Kg. In BSM model the percentages of inhibition of gastrointestinal motility at 100 mg/Kg, 50 mg/Kg groups compared to control group was 26.57%, 17.30% respectively.

During the comparative analysis of *Citrullus colocynthis* plant obtained from Jaipur and Jodhpur in COID model and BSM model the results were significant in terms of values. While both the plants proved to be effective in treating the diarrhea ailment in wistar rats the values of various parameters seen in *Citrullus colocynthis* obtained from Jodhpur district showed slightly better results in terms of values.

The anti-arthritic activity was evaluated in hydroalcoholic extract of *Capparis decidua* root, stem and leaves using FCA induced arthritis model. Hydroalcoholic extract of *Capparis decidua* (100 and 200 mg/Kg) treatment showed anti-arthritic effect in all the arthritic parameters. It significantly decreased the inflammation compared to the FCA group as observed by decreased paw joint diameter and arthritic score. The analgesic effect of *Capparis decidua* (100 and 200 mg/Kg) in rats with FCA induced arthritis is also marked as evident by the increase in pain threshold. The fall of time in motor in-coordination test in *Capparis decidua* (100 and 200 mg/Kg) treated groups significantly increased. The administration of *Capparis decidua* (100 and 200 mg/Kg) hydroalcoholic extract significantly ($P<0.001$) decreased the level of ALP, AST and ALT and increased the level of total protein. *Capparis decidua* extract significantly ($P<0.001$ at 100 & 200mg/Kg) decreased the inflammation compared to the FCA treated the group as observed by decreased paw volume. In our study, treatment with *Capparis decidua* extract in arthritic rats significantly increased the level of RBC and Hb while it decreased the level of ESR which can be credited to its mitigating potential. A reduction in the number of platelets in *Capparis decidua* extract treated groups suggests the protective role of this plant in arthritis. Among the different doses of *Capparis decidua* extract the more significant results were seen at 200 mg/Kg ($P<0.001$) as compared to 100 mg/Kg ($P<0.01$) dose.

While comparative analysis of *Capparis decidua* plant obtained from Jaipur and Jodhpur districts in FCA induced arthritis, the results were significant. Both the plants proved to be effective in treating the arthritis disease in Wistar rats but the values of various parameters seen in *Capparis decidua* obtained from Jodhpur district showed slightly better results in terms of values.

The aphrodisiac activity was evaluated in hydroalcoholic extract of *Capparis decidua* root, stem and leaves using sexual behavior study. Mating behavior test revealed that the extract of *Capparis decidua* at 200 mg/Kg ($P<0.001$) and 100 mg/Kg ($P<0.01$) significantly increased the Mounting Frequency (MF) and Intromission Frequency (IF). The extract of *Capparis decidua* at 200 mg/Kg ($P<0.001$) and 100 mg/Kg ($P<0.01$) significantly increased the Ejaculatory Latency (EL) as compared to control group

animals. The *Capparis decidua* extract at 200 mg/Kg ($P<0.001$) and 100 mg/Kg ($P<0.01$) was found to produce a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) A significant ($P<0.001$) increase in testosterone level was found at 200 mg/Kg ($P<0.001$) and 100 mg/Kg ($P<0.01$) *Capparis decidua* extract. Between both the doses of 200 mg/Kg and 100 mg/Kg the results at 200 mg/Kg were much better as compared to 100 mg/Kg.

The wound healing activity was evaluated in hydroalcoholic extract of *Citrullus colocynthis* fruits using excision and incision wound model. In excision wound model the 5% & 10% extract ointment treated groups showed significant ($P<0.001$) wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. povidone iodine ointment treated group of animals ($P<0.001$). The wound closure time was lesser, as well as the percentage of wound contraction was much more with the *Citrullus colocynthis* extract 10% ointment treated group ($P<0.001$). *Citrullus colocynthis* 5% extract ointment treated group of animals showed significant wound contraction from the fourth day onwards and achieved 100% with the wound closure time of 20 days but wound area and percentage wound contraction was slightly less as compared to 10% ointment till 18th day ($P<0.001$). Topical administration of extracts reduced the epithelization time from 21.83(normal control group) to 18 days (*Citrullus colocynthis* 10% ointment) ($P<0.001$), 19 days (*Citrullus colocynthis* 5% ointment) ($P<0.01$) and reduced the scar area on complete epithelization from 54 (normal control group) to 38.16 mm^2 (*Citrullus colocynthis* 10% ointment) ($P<0.001$) and 43 mm^2 (*Citrullus colocynthis* 5% ointment) ($P<0.001$). In the incision model the tensile strength of the *Citrullus colocynthis*10% ointment treated group showed significant tensile strength value of 420.5±9.58g ($P<0.001$), while *Citrullus colocynthis* 5% ointment treated group gave value of 319.83±10.26 g ($P<0.001$) as tensile strength.

In the light of the above, the study demonstrated significant anti-diarrheal, and wound healing activities of *Citrullus colocynthis* as well as anti-arthritic and aphrodisiac activities of *Capparis decidua*, thereby providing a scientific base to the traditional claims of the therapeutic uses of this plant in multiple range of diseases. However, further studies are needed to elucidate the actual mode of action behind these activities.

During the phytochemical studies of *Capparis decidua* plant numerous phytoconstituents were found and previous studies have already isolated compounds of this plant. Majority of isolated compounds were alkaloids, phenolics and flavonoids but earlier studies indicated that the highest percentage of phenolic compounds in *Capparis decidua*. So here we can attribute phenolic compounds responsible for the anti-arthritic and aphrodisiac activities in the present study. Similarly the previous studies of *Citrullus colocynthis* have isolated numerous phytoconstituents belonging to the category of glycosides, flavonoids, saponins, tannins but earlier studies indicated the highest percentage of cucurbitacins in *Citrullus colocynthis* so that we can attribute the role of cucurbitacins for the anti-diarrheal and wound healing potential in the present study.

Important points of experimental investigations:

- The phytochemical screening showed the presence of phenolics, flavonoids, alkaloids, terpenoids, saponins, steroids, tannins, cardiac glycosides, carbohydrates in the *Capparis decidua* and *Citrullus colocynthis* plants.
- The anti-diarrheal study of *Citrullus colocynthis* in COID model at 100 mg/Kg gave highly significant results ($P<0.001$) by prolonging the latency time, reducing the defecation frequency, decreasing number of wet defecations, weight of stool and water content of feces while in BSM model 100 mg/Kg gave highly significant results ($P<0.001$) by inhibiting the gastrointestinal motility.
- In comparative study of *Citrullus colocynthis* in COID model and BSM models, plant obtained from Jodhpur district showed better significance level($P<0.001$) and values in various parameters as compared to Jaipur district.
- The anti-arthritic activity of *Capparis decidua* in FCA model at 200 mg/Kg gave significant results ($P<0.001$) for the arthritic score, nociceptive threshold, fall of time, body weight changes, paw joint diameter, hematological parameters and biochemical parameters.
- In comparative study of *Capparis decidua* plant in FCA induced arthritis model, plant obtained from Jodhpur district showed better significance level($P<0.001$) and values in various parameters as compared to Jaipur district.

- The aphrodisiac activity of *Capparis decidua* in sexual behavior study at 200 mg/Kg gave significantly ($P<0.001$) increased the Mounting Frequency (MF), Intromission Frequency (IF) and Ejaculatory Latency (EL) and decreased Mounting Latency (ML) and Intromission Latency (IL).
- The aphrodisiac activity of *Capparis decidua* at 200 mg/Kg showed significant ($P<0.001$) increased in serum testosterone levels.
- The wound healing activity in excision wound model showed that 10% *Citrullus colocynthis* extract ointment gave significant ($P<0.001$) values for epithelization time and reduction in scar area while in incision wound model 10% extract gave significant ($P<0.001$) result of tensile strength.

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DETAILS OF PATENTS

1. Patent on “A Pharmaceutical Composition using Plant Extract for Skin Healing Applications”; Ref. No. 201811007080.
2. Patent on “Herbal skin care formulation and a process for the preparation thereof.” Ref. No. 201811014445.
3. Patent on “A synergistic herbal topical composition and process of preparation thereof” (Filed).

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1	201811007080	TEMP/E-1/7450/2018-DEL	1600	5088	FORM 1	A Pharmaceutical Composition using Plant Extract for Skin Healing Applications

TransactionID	Payment Mode	Challan Identification Number	Amount Paid	Head of A/C No
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Application Details

APPLICATION NUMBER	201811007080
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	26/02/2018
APPLICANT NAME	1 . PRAMOD KUMAR SHARMA 2 . PRASHANT KUMAR DHAKAD
TITLE OF INVENTION	A PHARMACEUTICAL COMPOSITION USING PLANT EXTRACT FOR SKIN HEALING APPLICATIONS
FIELD OF INVENTION	PHARMACEUTICALS
E-MAIL (As Per Record)	pujakr@gmail.com
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	NA
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	06/04/2018

Application Status

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1	201811014445	TEMP/E-1/15098/2018-DEL	1600	9824	FORM 1	Herbal skin care formulation and a process for the preparation thereof

TransactionID	Payment Mode	Challan Identification Number	Amount Paid	Head of A/C No
N-0000366612	Online Bank Transfer	02806341604201850947	1600.00	1475001020000001

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Application Details

APPLICATION NUMBER	201811014445
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	16/04/2018
APPLICANT NAME	1 . PRAMOD KUMAR SHARMA 2 . PRASHANT KUMAR DHAKAD
TITLE OF INVENTION	HERBAL SKIN CARE FORMULATION AND A PROCESS FOR THE PREPARATION THEREOF
FIELD OF INVENTION	PHARMACEUTICALS
E-MAIL (As Per Record)	pujakr@gmail.com
ADDITIONAL-EMAIL (As Per Record)	pujakr@gmail.com
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PRIORITY DATE	NA
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Application Status

APPLICATION STATUS	Application Published

DETAILS OF PUBLICATIONS

1. A Review on Ethnobiological & Medicinal Potential of Capparaceae Family Plant: *Capparis decidua (Forssk.) Edgew.* Advances in Pharmacology and Pharmacy 4(3): 27-39, 2016.Doi: 10.13189/app.2016.040302

2. Phytochemical Investigation and Anti-Diarrheal Activity of Hydroalcoholic Extract of Fruits of *Citrullus colocynthis (L.) Schrad.* (Cucurbitaceae). J Mol Genet Med 11: 305 Doi:10.4172/1747-0862.1000305 **(Impact Factor 1.73)**

3. Evaluation of Anti-arthritic Activity of Hydroalcoholic Extract of *Capparis decidua (Forssk.) Edgew. on Freund's Complete Adjuvant-induced Arthritis in Rats.* Immunology and Infectious Diseases 6(1): 6-15, 2018. Doi: 10.13189/iid.2018.060102

4. Effect of hydroalcoholic extract of *Capparis decidua (forssk.) Edgew on serum testosterone and spermatogenesis in rats. Eur. Chem. Bull., 2017, 6(12), 554-557.* Doi: 10.17628/ecb.2017.6.554-557

5. Antiarthritic Potential of *Capparis decidua (Forssk.) Edgew on FCA Induced Arthritis in Wistar Rats. J Stem Cell Res. 2017; 2(1): 1-6.*

6. Effect of Hydroalcoholic Extract of *Capparis decidua* (Forssk.) Edgew on Sexual Behavior of Male Rats. Advances in Pharmacology and Pharmacy 6(2): 50-56, 2018. Doi: 10.13189/app.2018.060203

7. A Review on Phytochemical Studies and Biological Potential of *Citrullus colocynthis (L.) Schrad. (Cucurbitaceae).* Bioengineering and Bioscience 5(4): 55-64, 2017. Doi: 10.13189/bb.2017.050401

8. Phytochemical analysis and pharmacological spectrum of *Citrullus colocynthis (L.) Schrad. (Cucurbitaceae).* Edorium J Biomed Sci 2017;2:9–16. Doi:10.5348/B04-2017-3-ED-2.

Advances in Pharmacology and Pharmacy 4(3): 27-39, 2016
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A Review on Ethnobiological & Medicinal Potential of Capparaceae Family Plant: *Capparis decidua* (Forssk.) Edgew

Prashant Kumar Dhakad[1,*], Pramod Kumar Sharma[1], Sokindra Kumar[2]

[1]Department of Pharmacy, Galgotias University, India
[2]R.V. Northland Institute (RVNI), Chithera, Dadri, Greater Noida-II, India

Abstract Rajasthan state is rich in flora of xerophytic plants. *Capparis decidua* (Forssk.) Edgew. is a wild bushy plant found in hot arid regions. This plant has wide range of distribution in many parts of India. Ethnobiologically, this plant is useful as its various parts like immature flower buds, semi-mature fruits, young shoots with small leaves are pickled for use as a condiment and traditional people use this plant in treating ailments like digestive diseases, anodyne, sudorific, constipation, gout, cough, flu, dropsy, palsy, asthma, and intestinal worms, lumbago, odontalgia. *Capparis decidua* (Forssk.) Edgew. contains chemical compounds like alkaloids, flavanoids, terpenoids, phenolic compounds, steroids, vitamins, quarternary ammonium compounds and many more phytoconstituents that are responsible for its medicinal value. Different parts of this plant like seed, root, stem, flowers, fruits and leaves have medicinal importance and has shown numerous pharmacological activities like antimicrobial, antibacterial, antifungal, anti-inflammatory, antioxidant, hepatoprotective, anthelmintic, antidiabetic, antisebum, antihyperlipidemic, antisclerotic, antitermite, antiplaque, analgesic, sedative and anticonvulsant. Economic importance of this plant has tended for harvesting, yield and marketing specifically in Thar Desert. Future potential of this crop is very promising as it is a drought and heat tolerant plant which makes it a good weather forecasting species; also it provides people with food (pickle & vegetable), fodder, wood and fuel. The presence of numerous phytoconstituents makes it a medicinally important crop for treating deadly diseases. This review covers taxonomy, distribution, phytochemicals, and nutritional value, and commercial value, traditional and pharmacological aspects of *Capparis decidua* (Forssk.) Edgew.

Keywords *Capparis decidua* (Forssk.) Edgew., Xerophytic, Phytoconstituents, Pharmacological Activities

1. Introduction

The wild plants have been utilized by native community in a medicinal way to cure cuts, wounds, burns and other disease ailments having dietary or pathogenic origin. Rajasthan state is having extremely dry climatic conditions and local community is coupled with poverty and natural disaster like drought which is challenging for them to cope up with food and medicine. But the flora of this state comprises of drought resistant plants having photochemical and mineral ingredients to carry out nearly all biological reactions of body. [1-4]. As per the reports, it is estimated that about 70–80% of world population, especially in developing countries, depends on plant medicine to prevent and cure diseases. In addition, it has been reported that about 25% of the synthesized drugs are being derived from medicinal plants [4, 5]. *Capparis decidua* (Forssk.)Edgew.(Kair) is a multipurpose perennial woody plant, of caper family (Capparaceae), found chiefly in hot arid region of different parts of world. The caper family includes 650 species of plants found in 30 genera located principally in tropical and warm temperate regions. Nearly 26 of these species are reported to occur in India [6]. Because of its xerophytic adaptive nature this plants grows well under the harsh climatic conditions of arid regions. *Capparis decidua* (Forssk.) Edgew. is salt-tolerant and grows along saline hard planes in Thar Desert. Mature plants develop extensive root systems that penetrate deeply into the soil. Leaf stipules form into spines to reduce transpiration. It also protects birds and animals from scorching heat during summers. [7]

1.1. Taxonomical Classification [8]

Kingdom: Plantae
Division: Phanerogamae
Subdivision: Angiospermae
Class: Dicotyledonae

Subclass: Polypetalae
Order: Thalamiflorae
Suborder: Parietales
Family: Capparaceae
Genus: Capparis
Species: deciduas

1.2. Monograph

Arabic: Hanbag, Kiabara, Margh, Sodab, Tundub
Deccan: Karyal
English: Caper berry, Caper bush, Caper plant
Hindi: Kabra, Kachra, Karer, Karil, Karu, Kurrel, Pinju, Teent, Tent, Tenti
Sanskrit: Apatra, Chakrak, Granthil, Gudhpatra, Kantaki
Rajasthani: Kair, Kareal, Kerro, Taint
Scientific Name: Capparis deciduas
Family: Capparaceae
Duration: Perennial

Growth Habit: Grows abundantly in dry, arid and exposed habitat like wastelands, ditches, drying ponds, cultivated lands, road sides and surrounding plains of hills as it is tolerant to prolonged drought due to its excellent adaptation to arid conditions.

Nativity: Dry places in Sind, Baluchistan, Western Rajputana, Deccan Peninsula, Egypt, Socotra, Arabia, Tropical Africa, Central India, Punjab, Gujarat, Tinnevelly and Pakistan.

2. Botanical Description

Capparis decidua (Forssk.) Edgew.(Kair) is a spiny, much dense and slender branched, green twiggy looking shrub or small tree growing gregariously bearing dense spherical crowns. The stem bark is smooth, green when young and turns yellow or whitish grey as it matures [9]. Leaves are deciduous, glabrous, small caducous, succulent that appear for maximum of one month, on new shoots. New leaves sprout from January to November [10], being sessile with very short petioles, pointed and small (2–12 mm in length and 1–3 mm in width). Fruits are globose, borne on a long stalk, green when immature and red or pink when ripened. Ripe fruits contain a sweet yellow pulp with many seeds. Flowering occurs on young shoots of the current year. Narrow leaves and stipular spines on shoots help reduction in loss of water due to transpiration in extremely drought conditions. It has well developed tap root systems which uptake water within ground at a depth of up to 4m [11].

3. Distribution in Hot Arid Regions of India

The natural habitat of *Capparis decidua* (Forssk.) Edgew. is on the lowerside of plains all over the hot and dry regions, semi- stabilized dune peripheries [12]. *Capparis decidua* (Forssk.) Edgew flourishes well on shallow hard soils and rocky outcrops but not on shifting sand dunes or water logged areas [13]. It grows abundantly in sandy, saline and gravy soils of pH 6.5-8.5. It can tolerate temperatures as high as 50ºC and as low as 0 ºC. It has also been found to thrive well on saline sodic soils [14]. This species shows wide genetic variability in plant size, morphology, fruit production and dimorphism in seeds. [15] (Figure 1)

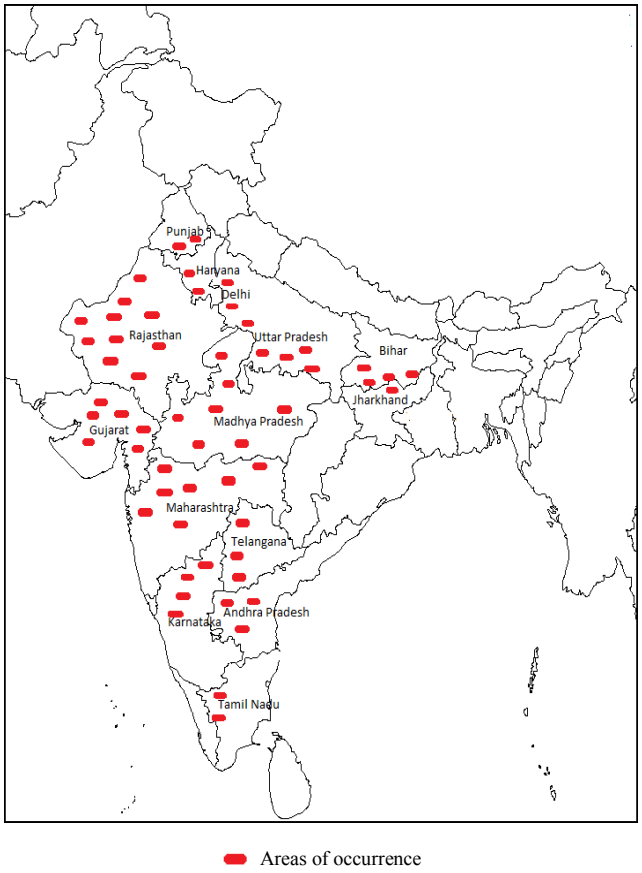


Figure 1. Map of India showing growth of *Capparis decidua* (Forssk.) Edgew.

4. Commercial Use

Capparis decidua (Forssk.) Edgew has been cultivated since past for several purposes. Different parts of caper plant are utilized in the form of drugs, foods and cosmetics [16, 17]. The commercially significant parts of *Capparis decidua* (Forssk.) Edgew are the immature flower buds, which are used as food in the form of curry, pickled in vinegar or preserved in granular salt and as condiment mostly by the people living around the desert. Semi-mature fruits are called as caperberries and young shoots with small leaves pickled for use as a condiment. The flavor of caper can be considered as similar to mustard and black pepper. The strong aroma of caper comes from mustard oil: methyl isothiocyanate (released from glucocapparin molecules) arising from crushed plant tissues. Mature and semi-mature

fruits are also eaten as a cooked vegetable after removal of bitterness and pungency by keeping in salt solution. Ashes obtained after incineration of caper roots may be utilized as a source of salt. In cultivating land this plant is grown to form hedges for protection [18]. *Capparis decidua* (Forssk.) Edgew. seeds are rich in oil, fibers and proteins. Seed oil contains prominent amount of oleic and linoleic acids. Hence, seeds can be used in various forms for food and feed [19]. Capparis is also used for landscaping, control of erosion and animal feeding [16, 17]. Capparis wood is moderately hard and heavy, resistant to termite attack [20] and is utilized for making tool handles, boat knees, burning fuel etc. [21]. In Rajput territory and Sudan, this plant is fed to camels and goats [22].

5. Harvesting, Yield and Marketing Fruits

Plants grown through seed start bearing fruits at 6 to 7 years of age, and vegetative propagated plants start fruiting after four years. The harvesting of the fruits is done manually. The thorns of the plant poses great difficulty for hand picking the fruits and natives collect the fruits by beating the crown with wooden sticks. The fruits are sold fresh in the local markets. The fresh fruits show astringency due to tannins and phenolic compounds. The astringency is removable by immersing fruits in a 5% solution of common salt, or in butter milk, for 4-5 days in an earthen pot. After the removal of astringency, the green fruits can be made into a vegetable or they can be preserved by sun drying for use in the off season. The dried fruits, with 5-7% moisture, can be preserved for 2-3 years in airtight containers and can be marketed later. Fruits can also be exported to foreign, urban and national markets. [23]

6. Traditional & Therapeutic Uses

Capparis species have been utilized in medicine since ancient times. These plants were used first time about 2000 years BC by Sumerians [24, 25]. The roots, flowers, and fruits of these plants with potential medicinal benefits, have been in use since that time against infectious diseases without any side effects [26]. Sharma and Kumar (2008)[27] suggested that the biological effects of *Capparis decidua* (Forssk.) Edgew may be ascertain to presence of antimicrobial bioactive compounds, like phenolics, flavonoids, polyamine alkaloids, glucosinolates, and vitamins that decrease the growth of microorganism, and are negligibly harmful for their hosts. The medicinal use of *Capparis decidua* (Forssk.) Edgew is also mentioned in ancient books. By Kavirajas, the plant is regarded as acrid, laxative, counterirritant and stimulant. They often prescribe it in heart diseases, colic pains, scurvy and phthisis [22]. The plant act therapeutically in flatulence, anorexia, respiratory disorders, skin diseases, in general weakness and also act as anthelmintic and diuretic [28,29]. Infusion of *Capparis decidua* (Forssk.) Edgew is used externally for eruptions, boils, joint diseases and internally in cough and as an antidote in case of poisoning. Juice of fresh plant is used to kill worms in ear. It acts as a good substitute of senega [22, 30]. Crushed bark is applied as poultice for treatment of wounds [31]. Roots are acts as sudorific, thermogenic, expectorant, carminative, digestive, stimulant, antibacterial, aphrodisiac, anodyne, anthelmintic and useful in arthritis, dyspepsia, constipation, lumbago, odontalgia, amenorrhoea and dysmenorrhoea [32]. Root bark is known to be astringent, alterative, acrid, diaphoretic, alexeteric. Powder or infusion of root bark is used in gout, rheumatism, cough, dropsy, palsy, asthma, intestinal worms and intermittent fever. The root powder is applied externally on malignant ulcer [33]. Coal paste obtained after burning the wood is applied to muscular injuries [34]. Fresh leaves and young shoots, when chewed, relieves toothache immediately [35]. The local people of India and Pakistan consider caper fruits having anti-diabetic, eye smoothing, and laxative properties so, they use caper fruits in pickles and curry [22, 28, 31, 36]. Hakeems in India, suggest using Caper fruit powder mixed with sugar ameliorate rheumatism and diarrhea in livestock animals. Plants of genus Capparis contain spermidine, glucosinolate, alkaloids, phenols, glycosides, and flavonoids, which have various pharmacological properties [37-41] and anti-inflammatory activities [42]. Polyamine alkaloid called as spermidine, reported in caper species, delays aging in yeast, flies, worms, and human immune cells through the induction of autophagy [43]. Pichiah et al. (2011) [44] suggested that spermidine is used for treating type 2 diabetes. Isocodonocarpine, isolated firstly from *Capparis decidua* (Forssk.) Edgew, found useful against inflammation and asthma [36]. β-Sitosterol showed a significant anti-inflammatory activity, similar to indomethacin, in carageenan-induced rat paw edema. β-sitosterol showed to inhibit ear inflammation induced by multiple applications of tetradecanoylphorbol-13-acetate in mice. It also inhibited adjuvant-induced rat paw edema by inhibition of cyclooxygenase and 5-lipoxygenase pathways [45]. Phenols, flavonoids, and indoles are reported as bioactive constituents with anti-inflammatory effects in many other plants [46, 47]. Compounds extracted from Capparis species have also shown to be useful for controlling the metabolism of lipids. Alcoholic extracts of bark, flowers, and roots of *Capparis decidua* (Forssk.) Edgew reduced cholesterol, triglycerides, LDL (low-density lipoproteins), and VLDL (very low-density lipoproteins) levels [48], whereas, *Capparis decidua* (Forssk.) Edgew fruit extract showed beneficial effect on blood sugar levels, glycated hemoglobin levels, and lipid profiles in diabetic and normal male rats. Rahmani et al. (2013) [49] study concluded that consumption of *Capparis decidua* (Forssk.) Edgew fruits might decrease levels of sugar in blood and improve lipid

profile. Various uses of *Capparis decidua* (Forssk.) Edgew. are shown in Table 1.

7. Nutritional Value

Plants with adequate amount of protein, fiber, and essential minerals are valued for livestock and human nutrition [57-60]. Capparis species contains minerals in floral buds and fruits, which are used as vegetable and is pickled. The buds and fruit are also rich in protein, carbohydrates, lipids, and vitamins [55]. *Capparis decidua* (Forssk.) Edgew. is reported to have higher potassium content than several other nutritious trees. Kumar et al. (2013)[61] reported the presence of P(219.05 mg 100 g−1), Mg (49.16 mg 100 g−1), Fe (4.64 mg 100 g−1),Zn (0.31 mg 100 g−1), Cu (1.94 mg 100 g−1), Na (160.64 mg 100 g−1),proline (11.76 mg 100 g−1), Ca (3.24%), crude protein (14.94%), total carbohydrates (73.48%), soluble carbohydrates (18.03%), starch(15.28%), crude fiber (10.94%), neutral detergent fiber (30.48%),hemicelluloses (11.45%), cellulose (8.91%), lignin (7.62%), crude fat(5.38%) and total ash (5.97%) in *Capparis decidua* (Forssk.) Edgew. fruits. Arginine is involved in rapid regeneration of adenosine triphosphate, cell proliferation, vasodilatation, neurotransmission, calcium release, and imparting immunity [62]. *Capparis decidua* (Forssk.) Edgew. berries acts as a good source of arginine. The *Capparis decidua* (Forssk.) Edgew. seed oil contains oleic acid, linoleic acid and palmitic acid in increasing order. So *Capparis decidua* (Forssk.) Edgew seed oil is a healthy source of fatty acids and thus, can be used in diet. The mature fruits, young shoots with small leaves and immature fruits are pickled in vinegar or granular salt. The presence of reducing sugar, fats, vitamin C, antioxidants, alkaloids, and carotene makes it a dietary supplement [63]. *Capparis decidua* (Forssk.) Edgew. bud and ripened fruits extract are also used in the food processing industry as a flavor agent. [64]

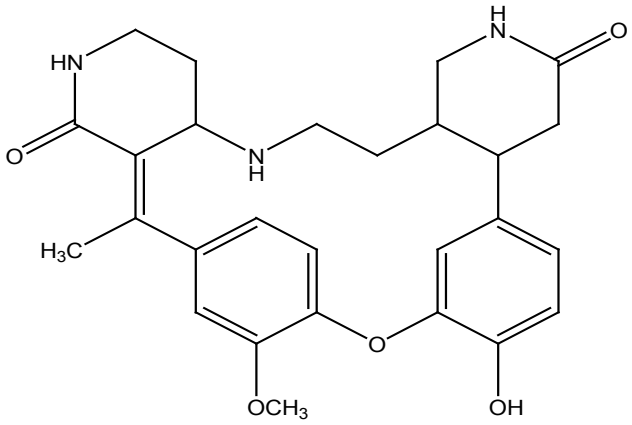
8. Chemical Constituents

Isocodonocarpine (A), Spermidine alkaloid (B), Capparisinine (C), Capparidisine (D), Capparine, and capparinine have been isolated from Caper roots [48, 56]. Codonocarpine (E), capparisine (F), cadabacine-26-O-d-glucoside, and capparipine-26-O-d-gluc oside have also been isolated from dry root bark of *Capparis decidua* (Forssk.) Edgew. plant [7, 48, 56, 65, 66,67]. N-acetylated spermidine alkaloids- 15-N-acetyl capparisine (G), and 14-N-acetyl isocodonocarpine (H) obtained from the root bark of *Capparis decidua* (Forssk.) Edgew.. It can be considered that roots of Capparis species are rich in spermidine alkaloid compounds [54] and can be used as a natural source for isolation of these polyamine alkaloids for formation of phytomedicines. Spermidine and spermine polyamines exhibit antioxidant and anti-allergenic activities, and suppression on glycation process. Spermidine is a class of multifunctional polyamines, found in some animals and microorganisms. Spermidine and spermine polyamines are essential in the proliferation, growth, and development of mammalian cells. These polyamines exhibit antioxidant and anti-allergenic activities, and suppression on glycation process [68, 69]. Polyamines prevent arteriosclerosis and promote healthy hair growth attributed to their anti-inflammatory properties and cell proliferative properties [70-73]. β-Sitosterol (I), is a principal phytosterol present in several plant including *Capparis decidua* (Forssk.) Edgew has partial antimicrobial effect through inhibition of cyclooxygenase and 5-lipoxygenasepathways. Flavonoids are known to be the most abundant plant compounds in human diet. Flavonoids are commonly found in cell vacuoles of the outer coloring parts of the flowers, fruits, and leaves [74] and show anti-stress effects in plants. Seemingly, the concentrations of phenolics and flavonoids vary depending on the extraction methods, genetic factors, and climatic/growing conditions of different sites [75, 76]. Baghiani et al. (2012) [77] reported that ethyl acetate extracts of *Capparis decidua* (Forssk.) Edgew. leaves showed higher amounts of phenolic compounds and flavonoids, followed by the chloroform extracts of roots. Mann et al. (2013) [78] also investigated that the content of different compounds in extracts of *Capparis decidua* (Forssk.) Edgew, alters depending on the solvent used. *Capparis decidua* (Forssk.) Edgew. fruits also contain carotene, ascorbic acid, phytic acid and oxalic acid.

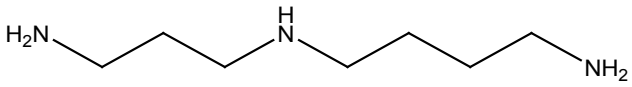
According to previous studies, water extract from roots of Capparis species exhibited better purgative effect as compared to alcoholic extracts indicating that different extracts can exhibit different pharmacological potential [79, 80]. Oil extract from leaves of *Capparis decidua* (Forssk.) Edgew. contains phenyl propanoid, terpenoids, isothiocyanate, and n-alkalenes [81]. Recently, isothiocyanates have shown as anti-cancer agents [82, 83]. In another investigation, the oil extract of *Capparis decidua* (Forssk.) Edgew. showed presence of thymol, isopropyl isothiocyanate, butyl isothiocyanate, and 2-hexenol. Gupta and Ali [84] explored oxygenated heterocyclic constituents from the alcoholic extract of root-bark of *Capparis decidua* (Forssk.) Edgew. Quaternary ammonium compounds and alkaloids were isolated from *Capparis decidua* (Forssk.) Edgew leaves [85, 86]. (Figure 2) (Table 2)

Table 1. Representation of traditional and therapeutic uses of various plant parts of *Capparis decidua* (Forssk.) Edgew. in treating different body ailments.

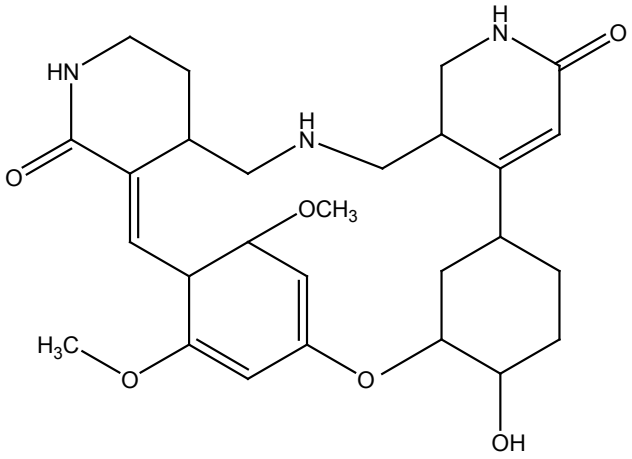
Plant parts	Ailments	References
Roots and root barks	Digestive diseases, stimulant, anodyne, sudorific,constipation, Gout, cough, flu, dropsy, palsy, asthma, and intestinal worms, lumbago, odontalgia, and amenorrhoea, intermittemt fever, arthritis, thermogeic, expectorant, carminative, aphrodisiac, anthelmintic, dyspepsia, astringent, diaphoretic, alexeteric	[32,50,51]
Leaves and young shoots	Toothache, swellings, and blisters, Hypercholesterolemia	[28,52]
Stem barks	Toothache, cough, asthma, intermittent fever, rheumatism, inflammation, kidney infection, and treatment of wounds as poultice	[31,53-55]
Fruits and flowers	Diabetes, respiratory diseases, skin, anthelmintic, diuretic, cardiac and biliousness diseases, anti-diabetic and eyesight smoothing properties, laxative potential, atherosclerosis, and plaque	[22,27,28,31,36,50,56]



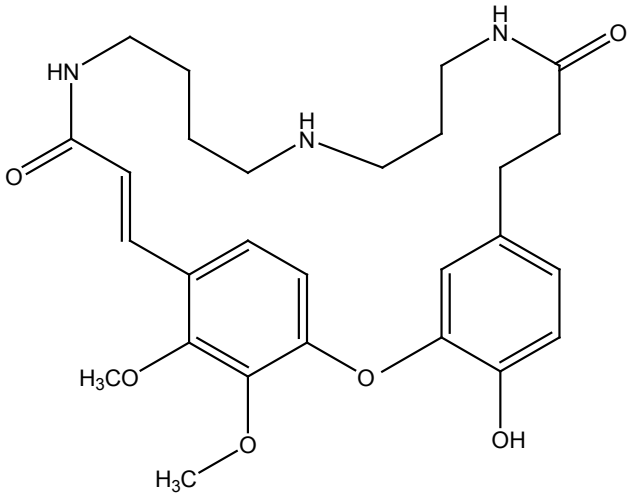
(A) Isocodonocarpine



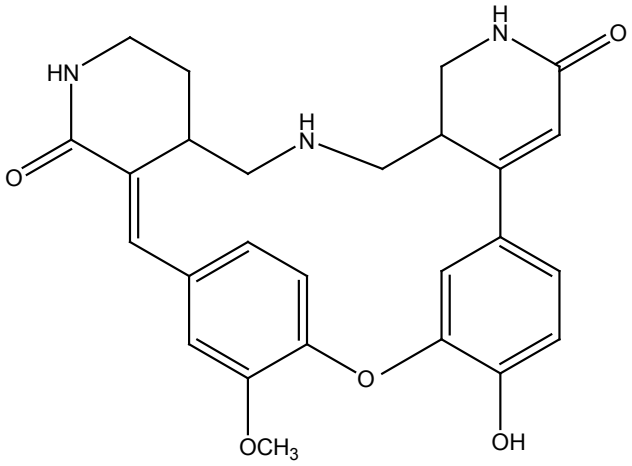
(B) Spermidine alkaloid



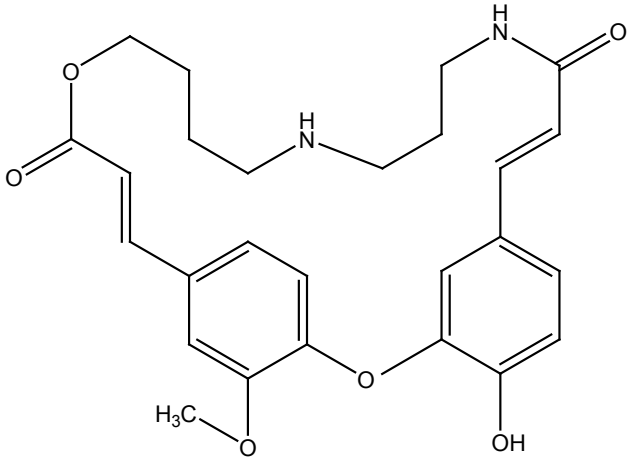
(C) Capparisinine



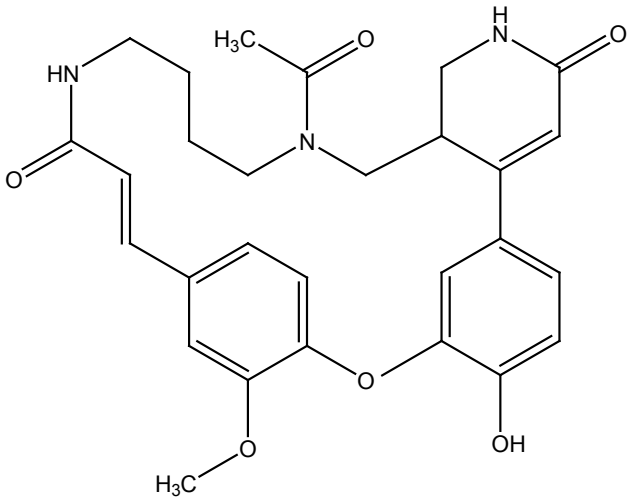
(D) Capparidisine



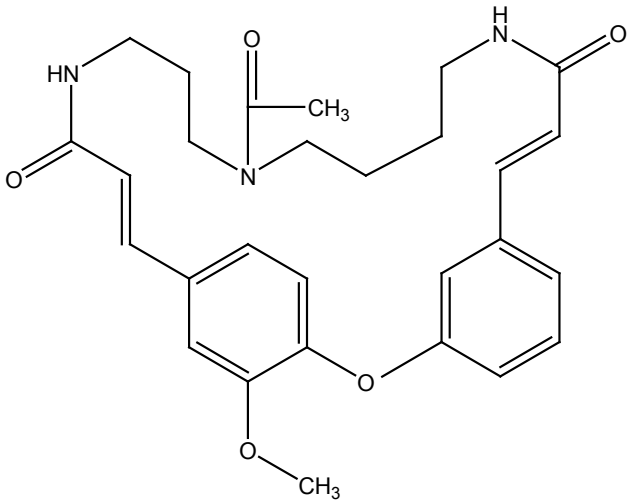
(E) Codonocarpine



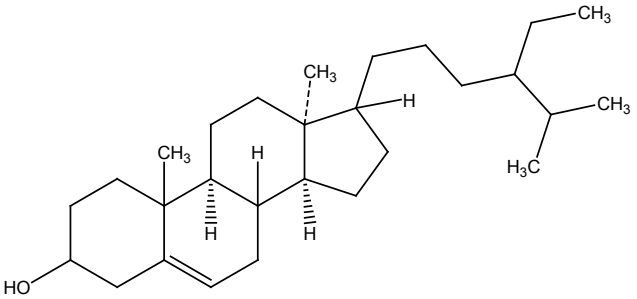
(F) Capparisine



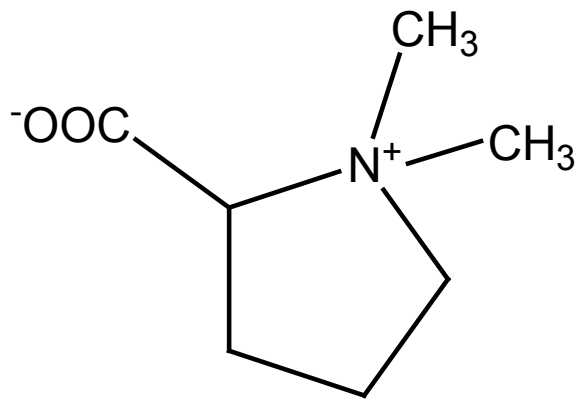
(G)15-N-acetyl capparisine



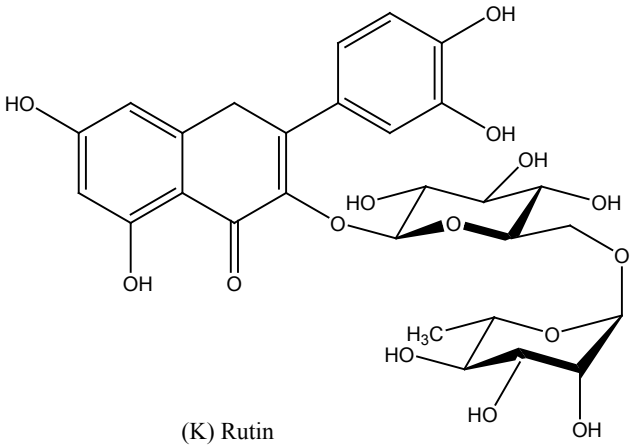
(H) 14-N-acetylcodonocarpine



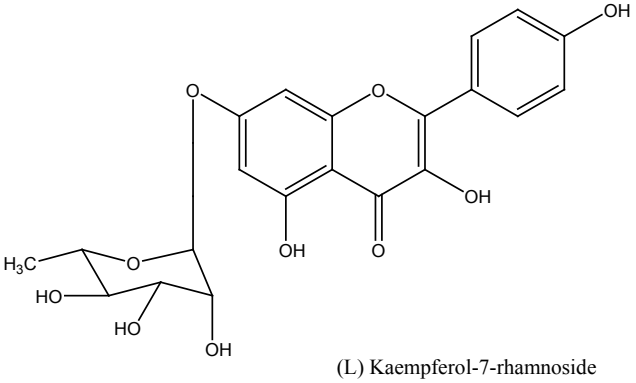
(I) β-sitosterol



(J) l-Stachydrine



(K) Rutin



(L) Kaempferol-7-rhamnoside

Figure 2. Structure of various chemical constituents of *Capparis decidua* (Forssk.) Edgew.(A to L)

Table 2. Representation of chemical constituents found in various plant parts of *Capparis decidua* (Forssk.) Edgew.

Plant Parts	Chemical constituents	References
Fruits	n-Triacontane, n-Pentacosane, β-Carotene, Carbohydrates, Proteins, Glucosinolates, n-Triacontanol,Tetrahydropyran-2-one,2-Carboxy-1-dimethylpyrrolidine	[56, 67]
Flower	Nonacosane, n-Triacontane, n-Pentacosane, n-Triacontanol	[48, 67]
Flower buds	n-Triacontane, n-Pentacosane, Quercitin, Isodulcite, Nonacosane	[22, 48, 67]
Shoots	Thymol, Isopropyl isothiocyanate, Butyl isothiocyanate, 2-Hexenol	[48]
Leaves	Phenyl propanoid, Terpenoids, Isothiocyanate, n-Alkalenes. Thomnocitrine, Kaempferol, Quercetin, Isorhamnetin, rhamnocitrin, rhamnetin, rhamnazin, quaternary ammonium compounds, alkaloids	[81, 83, 85-88]
Roots	Sitosterol, Spermidine alkaloid, Isocodonocarpine, Capparine, Capparinine, Cappariline, Codonocarpine	[7, 13, 56, 48]
Root barks	Cadabacine-26-O-β-d-glucoside, Isocodonocarpine, Capparine, *Capparis*ine, Codonocarpine, *Capparis*pine-26-O--d-glucoside Capparinine, N-acetylated spermidine 14-N-acetyl isocodonocarpine, 15-N-acetyl*capparis*ine, Rutin(K), I-Stachydrine(J), β–Sitosterol, Terpenoids, *Capparis*esterpenolide	[7, 48, 54, 65, 66]
Seeds & Seed oil	Glucocapparin, n-Pentacosane, n-Triacontanol, β–Sitosterol, Capric acid, Monoterpenes, Sesquiterpenes, Tocopherols (Vitamin E)	[25, 27, 67, 89]

Table 3. Representation of pharmacological activities shown by various plant parts of *Capparis decidua* (Forssk.) Edgew. and phytoconstituents responsible for pharmacological activity

Plant parts	Pharmacological activity	Phytoconstituents	References
Capparis decidua (Forssk.) Edgew. stems and flowers	Antibacterial, antifungal, antiparasital activity	Quarternary ammonium compounds, Glucosinolate	[90, 91]
Capparis decidua (Forssk.) Edgew. root, stem, fruit	Antimicrobial activity, antifungal activity	Phenolic and flavanoid compounds	[27, 92]
Capparis decidua (Forssk.) Edgew. seeds	Antimicrobial activity	Isothiocyanate aglycon	[13, 93, 94]
Capparis decidua (Forssk.) Edgew. leaves	Antioxidant activity	Phenolic compounds, Polyphenols, Tocopherols, Carotenoids	[95-97]
Capparis decidua (Forssk.) Edgew. leaves	Antiplaque activity	Volatile oil-Thymol	[56, 98]
Capparis decidua (Forssk.) Edgew. stem	Hepatoprotective activity	Flavonoids, Cyanogenic glycosides, Triterpenes, Vit. C	[99]
Capparis decidua (Forssk.) Edgew.. root bark	Anthelmintic activity	Spermidine alkaloids, Tannins	[54, 100, 101]
Capparis decidua (Forssk.) Edgew. powdered fruit	Antidiabetic activity	Alkaloids	[36, 102]
Capparis decidua (Forssk.) Edgew.. Fruit and shoot	Antisclerotic activity	Vitamins, Alkaloids, Phenolic compounds	[52, 103-106]
Capparis decidua (Forssk.) Edgew. fruit, flower, bark	Antihyperlipidemic activity	Saponins, Tannins	[52]
Capparis decidua (Forssk.) Edgew. plant	Antisebum activity	β-sitosterol, Essential fatty acids,Thioglu-cosides	[107]
Capparis decidua (Forssk.) Edgew. flowers, stem	Sedative and anticonvulsant activity	Alkaloids	[108]
Capparis decidua (Forssk.) Edgew. stem	Analgesic and antinociceptive activity	Tannins, Diterpenes, Triterpenes, steroids	[109]
Capparis decidua (Forssk.) Edgew. stem, root, root bark	Antiinflammatory activity	Isocodonocarpine, β-sitosterol	[36, 45]
Capparis decidua (Forssk.) Edgew. stem, flower	Anti-termite activity	Heneicosylhexadecanoate, triacontanol, 2-carboxy-1, 1-dimethylpyrrolidine, 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one	[110]

9. Pharmacological Activities

Pharmacological activities of various parts of *Capparis decidua* (Forssk.) Edgew. have been shown in Table 3.

10. Conclusions

Capparis decidua (Forssk.) Edgew. has been used as an ethnic medicine in different parts of the world. Several researchers keenly participated and explored this plant for identifying, isolating, and extracting potential medicinal constituents which proved various pharmacological activities. Many of pharmacological activities have already been mentioned in text above but still a lot of focus needs to be addressed regarding this plant by scientists, chemists, pharmacists, microbiologists, and nutritionists. Despite the multitude of pharmacological activities shown by this plant some activities like antirheumatic, aphrodisiac which are traditionally well known but has not been authenticated by any researcher yet. Moreover clinical studies have not been conducted so far to conform to the results of preclinical studies.

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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Research Article Open Access

Phytochemical Investigation and Anti-Diarrheal Activity of Hydroalcoholic Extract of Fruits of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae)

Dhakad PK*

Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India

Abstract

Background: Cucurbitaceae family is one of the best genetically assorted accumulations of restorative plants in the plant kingdom. Previous studies have suggested that *Citrullus colocynthis (L.)* Schrad. plant parts (root, stem, leaf, fruits, and seeds) have been utilized in the traditional system of medicine. Pharmacological activities reported for this plant include antioxidant activity, antimicrobial activity, anti-diabetic activity, anti-hyperlipidemic activity. The antidiarrheal activity of hydroalcoholic extract of fruits of this plant is reported for the first time in the present study.

Objective: To evaluate the anti-diarrheal activity of *Citrullus colocynthis (L.) Schrad.* (Cucurbitaceae) in experimentally induced diarrhea in Wistar rats.

Materials and methods: Hydroalcoholic extract of fruits of *Citrullus colocynthis* was examined for its acute toxicity on rats, in order to establish the safe doses. Castor oil induced diarrhea model and gastrointestinal motility test using barium sulfate milk were done to assess the antidiarrheal activity of plant extracts. Extract of *Citrullus colocynthis* at the dose of 50 mg/kg, 100 mg/kg and per se group (100 mg/kg) were used in Wistar rats of either sex. Loperamide (2 mg/kg) was taken as a standard drug in both the models.

Results: Phytochemical analysis showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. The acute toxicity studies revealed that extract is relatively safe when given orally; no death was recorded at a dose of 2000 mg/kg. The dose of 100 mg/kg (P<0.001) and 50 mg/kg (P<0.01) of plant fruit extract significantly reduced defecation frequency in 6 h and also increased the latency time which showed similar effects as produced in loperamide treated group. Both doses of fruit extract and loperamide reduced the gastrointestinal motility in Wistar rats significantly (P<0.001).

Conclusion: The hydroalcoholic extract of fruits of *Citrullus colocynthis* showed significant antidiarrheal activity and supports its use as a complementary and alternative medicine for treatment of diarrhea.

Keywords: *Citrullus colocynthis;* Hydroalcoholic extract; Antidiarrheal activity; Acute toxicity; Cucurbitaceae; Phytochemical analysis

Introduction

Enough experimental and epidemiological researchers have indicated that diarrhea is one of the common gastrointestinal disorders that make a passage to mortality and morbidity in children especially in developing countries including India. Diarrhea is an intestinal disorder involving abnormal fluid content and defecation frequency resulting in increased motility in the colon [1,2]. Different multidrug-resistant pathogenic microbes' have driven treatment of this sickness more troublesome. The subclass of secretory looseness of the bowels is normally prompted by bacterial pathogens like *Vibrio cholerae*, enterotoxin *E. coli*. It is portrayed by the dynamic discharge of chloride or potentially bicarbonate into the digestive tract and consequently by lost liquid [3]. Clinically diarrhea shows increased liquidity of stool, along with increased stool frequency and weight. Despite the understanding causes, treatment and counteractive action of diarrheal illnesses, an expected 4.6 million individuals, with 2.5 million kids, bite the dust from looseness of the bowels consistently, especially in developing countries [4]. Regardless of the variety of etiologies of the runs, literary works express that there are four noteworthy pathophysiologies that lead to diarrhea. These incorporate increased electrolytes secretion (secretory diarrhea), increased luminal osmolarity (osmotic diarrhea), deranged intestinal motility and decreased electrolytes absorption causing a decreased transit time. Conversely, purgatives and diarrhea-causing agents improve gastrointestinal motility. For example, castor oil; which is utilized as an inducer of looseness of the bowels in this investigation, is known for its purgative effects due to active principle, retinoic acid [5].

Although WHO launched Diarrhea Disease Control Program in 1983 to eradicate diarrhea in developing countries included measures like use of traditional remedies, but more than 85% of plants still wait for scientific results in terms of their pharmacological activities [6]. Herbal medicines indicate potential uses in future in light of the fact that the greater part of the plants, their activities, and pharmacological actions have not been investigated totally [7]. The more noteworthy efficiency of plant-origin medicines is because of the antioxidative role which prevents oxidation and provides protection to living beings from harm caused by excessive generation of ROS, lipid peroxidation, DNA strand breaking and protein damage. Prevention of cell oxidative damage, therefore, limits the events of the vast majority of the illnesses. It is the world's third most elevated executioner illness to undernourished, children [8]. It is explicitly stated that diarrhea mars the intestinal antioxidant defense system which will make it confused and cause other oxidative stress disorders and in this way,

***Corresponding author:** Dr. Prashant Kumar Dhakad, Department of Pharmacy, School of Medical and Allied Sciences, Plot No. 2, Sector 17 A, Yamuna Expressway, Galgotias University, Greater Noida, Uttar Pradesh, India, Tel: 8510860363; E-mail: dhakadprashant654@gmail.com

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antioxidants may take up an essential application in the treatment of diarrhea [9]. The revelation of viable anti-microbial agents, vaccines have diminished the overwhelming effect of irresistible maladies and enhanced personal satisfaction. However, the efficacy of many antimicrobials is being undermined by the development of microbial resistance to existing chemotherapeutic agents [10]. Despite many efforts taken by the government and international organizations as well as advancement in medicines current rate of diarrheal patients is still high at large. It is necessary to identify and evaluate the indigenous plants for the treatment of diarrhea as allopathic medicines have both adverse effects and toxicities.

Citrullus colocynthis (L.) Schrad. Citrullus colocynthis Schrad (family Cucurbitaceae), also known as colocynth or bitter apple or Indian wild gourd (Hindi-Indrayan) is a desert plant wildly distributed in hot arid areas of the world, including Pakistan, India and Saudi Arabia [11]. It is a perennial herbaceous crawling plant, possessing rough and angular stems. Leaves are rough, 5 to 10 cm long, 3 to 7-lobed and fruits are almost globular, 4 to 10 cm in breadth about the extent of a little orange. Customarily, the poultice of colocynth is utilized to battle rheumatic agony. The leaves have been utilized for the agonizing feminine cycle and in the treatment of asthma. The fruit pulp is laxative, utilized as diuretic, cathartic and furthermore utilized against gonorrhea [12]. The watery pulp extract of *Citrullus colocynthis* fruits is used for the treatment of kidney, liver-related diseases. The fruits and leaves of this plant contain cucurbitacins A, B, C and D and α elaterin and probably other constituents. Phytochemical investigations of its bitter principles 'cucurbitacins' were numerous [13]. Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc Cucurbitacins are reported to be the main constituent of fruits of this plant [14]. The various antidiarrheal therapies and their mechanisms of action include proabsorptive action by ORS/RS-ORS (Oral rehydration solution/ Resistant starch-based oral rehydration solution) responsible for increase in glucose/SCFA (Short-chain fatty acids) absorption, antisecretory action through following ways – (a) CFTR (Cystic fibrosis transmembrane conductance regulator) and CaCC (Ca^{+2} activated chloride channels), (b) decrease in bile salt in lumen (c) decreasing PG (Prostaglandins) synthesis which results in reduction of intestinal secretions. Antimotility action has also been seen by various compounds like loperamide act by increasing μ opioid receptor hence decreasing the motility, hyoscyamine reduces the acetylcholine (Ach) action on muscles thereby decreasing muscle contractions, Alosetron works by inhibiting serotonergic (5 HT_3) receptor action and decreases the intestinal motility as well as secretion, Racecadotril increases encephalin endogenously which also decreases gut secretions. Clonidine acts on adrenergic receptors and activates the α2 adrenoceptor which results in increasing the absorption. Anti-inflammatory action is seen by corticosteroids through immunosuppression by decreasing prostaglandins and increasing IL-10 (Interleukins), while Anti-TNFα causes a decrease in blood and tissue TNFα (Tumor necrosis factor) [15].

Currently, there is no scientific evidence in the literature on the effect of *Citrullus colocynthis* on diarrhea. Hence present investigation was undertaken to evaluate the effect of dried fruit extract of *C. colocynthis* on the experimentally induced diarrhea in Wistar rats. Acute toxicity study and phytochemical investigations of dried fruit extract were also done.

Materials and Methods

Plant materials

Citrullus colocynthis plant wildly grows in Jaipur region and the fruits of this plant were collected fresh from Smriti Van, Jaipur, Rajasthan, India in the month of August 2016. They were taxonomically identified and authenticated by Dr. Manju Sharma, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (R.No: RUBL 211645) has been deposited at the Herbarium of Department of Botany, University of Rajasthan, Jaipur, India.

Preparation of crude extract

Citrullus colocynthis fruits were washed with tap water followed by distilled water and then cut and dried under the shade. The dried fruits were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. The dried and powdered plant material was Soxhlet extracted with water and alcohol. The extraction was carried out for 24 hr at room temperature with mild shaking. The extract was filtered and concentrated at 48°C and weight of residue was recorded. The percentage yield of hydro-alcoholic extract was found to be 38.5%. The collected extract was stored in a sterile container for further use.

Experimental animals

Healthy Albino Wistar rats of both sexes weighing 150-250 g were obtained from Central Animal Facility AIIMS New Delhi. The experimental protocol was approved by Institutional Animal Ethics Committee CPCSEA No. - 1149/PO/ERe/07/CPCSEA. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30% to 70%) with 12:12 light: dark cycle. The animals were given standard pellet diet and water ad libitum. All the experimental procedures involving animals were conducted in accordance with Institutional Animal Ethics Committee (IAEA) (OECD guideline no.420) and approved by IAEA.

Chemicals

Castor oil (Jayant Agro-Organics, Mumbai Maharashtra), Loperamide (Arene Life Sciences, Andhra Pradesh), Barium Sulfate (Oasis Fine Chem, Vadodara) and distilled water were used in this study.

Phytochemical analysis

The extract was analyzed for the presence of pharmacologically active constituents such as phenols, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and carbohydrates [16].

Test for phenolic compounds

50 mg of *C. colocynthis* extract was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. The appearance of bluish black color indicated the presence of phenolic compounds.

Test for flavonoids

Few drops of dilute sodium hydroxide solution were added into the *C. colocynthis* extract (0.5 ml) to give intense yellow color which disappears after addition of dilute hydrochloride acid showed the presence of flavonoids.

Test for terpenoids

The extract (0.5 mg) of *C. colocynthis* was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Formation of the reddish-brown ring at the interface indicated the presence of terpenoids.

Test for saponins

C. colocynthis extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min using hands. The formation of two cm layer of foam layer indicated the presence of saponins.

Test for alkaloids

About 50 mg of *C. colocynthis* extract was shaken with few ml of dilute hydrochloric acid and filtered. Few drops of Wagner's reagent were added at the side of the test tube. The appearance of reddish-brown precipitate indicated the presence of alkaloids.

Test for cardiac glycosides

C. colocynthis extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface is a feature of cardenolide deoxy sugar and appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

Test for steroids

C. colocynthis extract (1 gm) was dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The color of the upper layer changed to red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [17].

Test of carbohydrates

Molisch test: To 2–3 ml of the aqueous. *C. colocynthis* extract added two drops of alpha-naphthol solution in alcohol, shaken and added conc. H_2SO_4 from the sides of the test tube. Violet ring was formed [18].

Monosaccharide Barfoed's test: Equal volumes of Barfoed's reagent and the *C. colocynthis* extract were mixed to form a solution. Heated for 1–2 min in a boiling water bath and cooled. Red color indicated the presence of monosaccharides [18].

Test for tannins

C. colocynthis extract (1 gm) dissolved in water in a test tube and diluted with chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully to the side of the test tube to the solution. A green color was formed which showed the presence of tannins [19].

Acute toxicity studies

Lorke method [20] was used in this study. Twenty-five Wistar rats of both sexes were randomly grouped into five with five rats in each group and were fed orally with graded doses 100, 500, 1 000, 1 500 and 2 000 mg/kg of a hydroalcoholic extract of *Citrullus colocynthis* by gastric gavage. The animals were allowed free access to feed and water. They were observed over a 48 h period for acutely toxic signs and death.

Experimental Methods

Castor oil-induced diarrhea (COID) in rats

The antidiarrheal activity of *C. colocynthis* extract was estimated as per the method of Awouters et al. [21]. Thirty Wistar rats were allowed to fast for 18 h. Animals were divided randomly into five groups of six animals each (n=6) as a control group, standard group, and test groups. At first, *C. colocynthis* extract and the standard drug will be provided orally and after 1 h castor oil (2 ml/rat) will be supplied for inducing diarrhea. Only distilled water (2 ml/rat) will be supplied for the control group and standard drug loperamide (2 mg/kg) will be provided for the positive control group. Treated Groups III, IV received *C. colocynthis* extract at the dose of 50 mg/kg and 100 mg/kg respectively. Group V i.e., per se group will receive only plant extract at the dose of 100 mg/kg. Separate cages will be used for each rat and sheets of paper will be placed below the cage for the collection of fecal matters. The presence of stool with fluid material that stained the paper will be placed beneath the cages indicated diarrhea. During the observation period of 6 h, parameters such as latency time, defecation frequency, no. of wet defecations, the weight of stool and water content of feces were recorded. The total score of disease control group was considered as 100%. The water content of feces was expressed in terms of percentages using the formula:

$$W_c(\%) = \frac{F_W - D_W \times 100}{F_W}$$

Where W_c = Water content of feces; F_w = Fresh weight (g); D_w = Dry weight (g).

Gastrointestinal motility test with barium sulfate milk (BSM) model for diarrhea

This experiment was carried out by the method developed by Chatterjee [22]. By random selection Wistar rats (overnight fasted for 18 h) was divided into five groups of six rats each. Group, I recognized as normal control was administered distilled water of 2 ml/rat orally. Commercially available reference antidiarrhoeal drug loperamide at the dose of 2 mg/kg was provided orally for Group II marked as a positive control group. The hydroalcoholic extract of *C. colocynthis* was orally treated at a dose of 50 mg/kg and 100 mg/kg for groups III, and IV respectively assigned as treated groups and group V as Per se group where an only hydroalcoholic extract of *C. colocynthis* with 100 mg/kg was administered. After 30 min, 2 ml of 10% barium sulfate solution was administered in all groups. Rats were sacrificed after 30 min of extract and drug administration. The distance traversed by barium sulfate milk was measured and expressed as a percentage of the total length of the small intestine (from the pylorus to the ileocecal junction). The percentage of inhibition compared with the control group was determined by using the following equation:

$$Inhibition\ (\%) = \frac{extract\ -\ control\ \times\ 100}{control}$$

Statistical Analysis

The data were represented as a mean ± standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software.

Results

Preliminary qualitative phytochemical screening and acute toxicity studies

Preliminary qualitative phytochemical analysis of hydroalcoholic fruit extract of *C. colocynthis* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. Acute toxicity studies did not show any signs of death at administered graded doses of 100, 500, 1000, 1500 and 2000

mg/kg of a hydroalcoholic extract of *C. colocynthis* by gastric gavage. The LD_{50} value for oral administration of the plant extract was found to be greater than 2000 mg/kg body weight. Based on the results of acute toxicity studies the doses of 50 and 100 mg/kg of plant extract were selected for administration.

Effect of *C. colocynthis* extract on Castor oil induced diarrhea (COID) model

The results about the antidiarrheal effect of loperamide and hydroalcoholic extract of *C. colocynthis* in COID on Wistar rats are shown in Table 1. The results indicated that both the doses of *C. colocynthis* extract (100 mg/kg and 50 mg/kg) showed protection against COID model. The hydroalcoholic extract of *C. colocynthis* at 100 mg/kg showed highly significant results such as prolonged the latency time, reduced the defecation frequency, number of wet defecations, the weight of stool and water content of feces when compared with the negative control group ($P<0.001$). In addition, this dose of plant extract had shown results comparable to loperamide while at a dose of 50 mg/kg the results were same as the significant value of P<0.01. Per se group showed a highly significant result ($P<0.001$) on all the parameters discussed above as compared to negative control group and it contributes to the beneficial effects of *C. colocynthis* extract in the treatment of diarrhea.

Effect of *C. colocynthis* extract on Barium sulfate milk (BSM) model

The results of the gastrointestinal motility test with BSM of hydroalcoholic extract of *C. colocynthis* and loperamide on Wistar rats have been shown in Table 2. The treatment with standard drug loperamide and with all the doses of hydroalcoholic extract of *C. colocynthis* significantly inhibited the gastrointestinal motility of rats. The percentages of inhibition of 100 mg/kg, 50 mg/kg, and per se groups compared to control group was 26.57%, 17.30%, 23.74% respectively. While standard group exhibited 38.89% inhibition.

Discussion

In our experiment, the anti-diarrheal activities were evaluated in two *in-vivo* study models (COID and BSM). The hydroalcoholic extract of *C. colocynthis* (fruits) inhibited the Castor oil induced diarrhea at doses- 100 mg/kg and 50 mg/kg. While per se group (only 100 mg/kg of *C. colocynthis* extract administered without induction of diarrhea) also showed highly significant results which were used to evaluate the beneficial effects of *Citrullus colocynthis* extract. In COID model the hydroalcoholic extracts showed decrease in number of wet defecations in 6 h [100 mg/kg ($P<0.001$), 50 mg/kg ($P<0.01$), per se ($P<0.001$)], weight of stool [100 mg/kg ($P<0.001$), 50 mg/kg ($P<0.05$), per se ($P<0.001$)] and water content of feces [100 mg/kg ($P<0.001$), 50 mg/kg ($P<0.01$), per se ($P<0.001$)] (Table 1). The experimental groups of *C. colocynthis* extract (CCE 100 mg/kg, CCE 50 mg/kg and per se 100 mg/kg) significantly diminished the severity of diarrhea with respect to decreasing in the rate of defecation and watery content of feces in Wistar rats. All the extracts showed significant anti-diarrheal activity demonstrating 52.48%, 21.29% and 71.32% reductions in diarrhea respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg as compared to that of loperamide that demonstrated 72.16% reductions in diarrhea. While the percent inhibition of water content was found to be significant displaying 34.34%, 07.63% and 83.19% decrease in water content respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg as compared to that of the standard drug loperamide that showed 100% reductions in water content in diarrhea. In the gastrointestinal motility test, all the doses of extract produced a significant decrease in intestinal motility. In gastrointestinal motility test with BSM, all the doses of hydro alcoholic extract of *C. colocynthis* decreased intestinal transmit significantly ($P<0.001$). The normal control group showed 56.93% intestinal motility by the Barium Sulfate milk in gastrointestinal motility test. *C. colocynthis* extracts showed intestinal motility as 41.8%, 47.08% and 43.41% respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg groups. CCE extracts also gave a significant inhibition of intestinal motility with values as 26.57% (CCE 100 mg/kg), 17.3% (CCE 50 mg/kg), and 23.74% (Per se 100 mg/kg) while loperamide (2 mg/kg) had a value of 38.89% (P<0.001) of decrease in intestinal motility (Table 2).

Similar results were also ascertained in prior anti-diarrhoeal studies conducted which substantiates the medicinal use of *Citrullus colocynthis* in the treatment of diarrhea.

Groups	Dose (mg/kg)	Latency Time (min)	Defecation Frequency in 6 hrs.	% Inhibition of defecation	No. of wet defecation within 6 hrs.	% Inhibition of defecation	Wt. of stool (gm)	Wt. of wet stool (gm)	Water content of feces (%)	% Inhibition of water content
Control	2 ml/rat	107.33 ± 2.24	20.33 ± 0.88		12.66 ± 0.88		0.63 ± 0.04	0.57 ± 0.03	87.60 ± 1.14	
Loperamide	2 mg/kg	304.16 ± 1.74[c]	5.66 ± 0.66[c]	72.16	2.66 ± 0.33[c]	78.98	0.13 ± 0.02[c]	0.10 ± 0.02	00 ± 000	100[c]
CCE	100 mg/kg	233.66 ± 1.94[c]	9.66 ± 0.88[c]	52.48	5 ± .73[c]	60.5	0.32 ± 0.01[c]	0.23 ± 0.03	57.51 ± 1.25	34.34[c]
	50 mg/kg	117.83 ± 0.98[b]	16 ± 0.44[b]	21.29	9.5 ± 0.42[b]	24.96	0.51 ± 0.02[a]	0.41 ± 0.01	80.91 ± 0.63	07.63[b]
Per se	100 mg/kg of CCE	200.66 ± 2.51[c]	5.83 ± 0.30[c]	71.32	2 ± .36[c]	84.2	0.27 ± 0.009[c]	0.06 ± 0.006	14.72 ± 1.50	83.19[c]

Note: Values are expressed as mean ± SEM (n=6); [a,b,c] p<0.001 refers to significant difference compared to control with positive control and extract.

Table 1: Effects of a hydroalcoholic extract of *C. colocynthis* on castor oil induced diarrhea model in wistar rats.

Group	Dose (mg/kg)	Length of GIT (cm)	Distance passed by $BaSO_4$ (cm)	$BaSO_4$ Transverse (%)	Inhibition (%)
Control	2 ml/rat	119.24 ± 0.94	67.89 ± 0.31	56.93	--
Loperamide	2 mg/kg	112.16 ± .60	39.03 ± 0.37	34.79[c]	38.89
CCE	100 mg/kg	99.95 ± 0.42	41.78 ± 0.27	41.8[c]	26.57
	50 mg/kg	106.74 ± 0.63	50.26 ± 0.35	47.08[c]	17.3
Per se	100 mg/kg of CCE	103.10 ± 0.44	44.76 ± 0.50	43.41[c]	23.74

Note: Values are expressed as mean ± SEM (n=6); [c] P<0.001 refers to significant difference compared to control with positive control and extract.

Table 2: Effects of a hydroalcoholic extract of *C. colocynthis* on gastrointestinal motility with barium sulphate milk model in rats.

In prior anti-diarrheal studies, ethanolic extracts of Cynodon dactylon Pers. aerial parts (EECA) in Wistar rats demonstrates that EECA viably restrains the recurrence of wetting feces and defecation as well as inhibit the water content of total feces. In gastrointestinal motility test with BSM, the most astounding decrease of gastrointestinal motility is for loperamide at a dose of 2 mg/kg and inhibition of the distance traveled by BaSO4 milk is 39.6%. While the plant extracts decrease the distance of gastrointestinal motility of rats ranging from 58.57% (control group) to 47.12% and inhibition of distance traveled by barium sulfate milk is 19.55% at the dose of 1 g/kg of extract dose as compared to control [23].

In another antidiarrheal investigation of methanol (MEHO), ethanol (EEHO) and water (AEHO) extracts of H. odorata leaves demonstrate critical ($p<0.001$) inhibition against castor oil-induced diarrhea. At the 400 mg/kg dose, the extracts show significant anti-diarrheal activity ($P<0.001$) demonstrating 47.76 ± 2.36%, 58.21 ± 6.92% and 56.72 ± 5.48%, reductions in diarrhea respectively in AEHO, EEHO and MEHO comparable to that of the standard drug loperamide with 59.70 ± 2.99% reduction in diarrhea. The normal control group demonstrates intestinal motility as 84.85 ± 2.88%. The 200 and 400 mg/kg (p.o) of the extracts displays intestinal motility as 51.51 ± 0.97% to 62.05 ± 1.41%. Also, the extracts significantly inhibit intestinal motility as 22.82 ± 1.76% to 35.93 ± 1.21% at all the doses. Be that as it may, Loperamide (5 mg/kg) shows a significant inhibition (43.6 ± 2.14%) in intestinal motility [8].

Castor oil is obtained from the seeds of Ricinus communis (Family-Euphorbiaceae). Castor oil acts as a stimulant laxative which hydrolyzes to form ricinoleic acid, a local irritant and instigate changes in gastrointestinal mucosal fluid and electrolyte transport bringing about hypersecretory reaction and diarrhea. Experimental studies demonstrated that inflammatory response occurring due to ricinoleic acid causes generation of prostaglandins PGE_2. Ricinoleic acid diminishes the active Na^+ and K^+ retention and declines Na^+- K^{+-} ATPase pump in small intestine and colon and henceforth hindering the mucosal c-AMP intervened dynamic secretion. Loperamide is an opioid derivative that functions through mu receptors on neurons in submucosal neural plexus of intestinal wall and moderates the intestinal motility, aside from it, likewise, indicates antimuscarinic action in the gastrointestinal tract. So clearly loperamide protected the Wistar rats through above mechanism [24].

Standard chemical test carried out during the phytochemical screening of *C. colocynthis* showed the presence of a number of bioactive constituents such as phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. The counter diarrheal action could be ascribed to these compounds. Previous literature survey and experimental studies also showed the presence of these compounds in the fruit of *C. colocynthis* [25-27]. In earlier studies, anti-diarrhoeal activity is showed by the plants having alkaloids, tannins, saponins, steroids, terpenoids, and flavonoids. Hostile to the looseness of the bowels exercises of flavonoids have been credited to their capacity to hinder intestinal motility and hydroelectrolytic discharges which are known to be modified in diarrhoeic conditions. Tannins denature proteins in the intestinal mucosa by shaping protein tannates which may lessen secretion [28]. Studies on the on the useful part of tannins additionally uncovered that they can likewise lessen the peristaltic developments and intestinal discharges by decreasing the intracellular Ca^{2+} inward current or by the enactment of the calcium pumping system (which actuates the muscle unwinding) ascribed by calcium channel blocking and spasmolytic activities of tannins present in the plant extract. Sesquiterpenes, terpenes, diterpenes and various terpenoid derivatives are known for repressing release of prostaglandins and autocoids, in this way restrain the motility and secretion instigated by castor oil [29].

Conclusion

The present results validate the use of a hydroalcoholic extract of *Citrullus colocynthis* as an anti-diarrheal agent as it showed significant inhibition of defecation-related parameters in castor oil induced diarrhea model and gastrointestinal motility and secretion in barium sulfate milk model. Phytochemical screening of *Citrullus colocynthis (L.)* fruits rationalizes the presence of active phytoconstituents in this plant. We suppose that the anti-diarrheal effect of *Citrullus colocynthis* is related to its bioactive components such as polyphenols, flavonoids, alkaloids, saponins, terpenoids. Therefore, further investigations are ongoing to isolate and identify specific constituents which will explicate the mechanism of action for the anti-diarrheal potential of *Citrullus colocynthis*. Hence the evaluation of the phytoconstituents and anti-diarrheal activity of hydroalcoholic extract of fruits of *Citrullus colocynthis (L.) Schrad.* (Cucurbitaceae) in experimentally induced diarrhea in Wistar rats affirm fruits of this plant for its effective anti-diarrheal use.

Conflicts of Interest

We declare that we have no conflict of interest.

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Evaluation of Anti-arthritic Activity of Hydroalcoholic Extract of *Capparis decidua* (Forssk.) Edgew. on Freund's Complete Adjuvant-induced Arthritis in Rats

Prashant Kumar Dhakad[1,*], Pramod Kumar Sharma[1], Sokindra Kumar[2]

[1]Department of Pharmacy, Galgotias University, India
[2]R.V. Northland Institute (RVNI), India

Abstract *Capparis decidua* has been traditionally used in the Ayurveda to treat rheumatoid arthritis and it is reported to have anti-inflammatory and analgesic activity. Considering its anti-inflammatory activity the present research work has been designed to assess the anti-arthritic activity in Wistar rats. The anti-arthritic activity of hydroalcoholic extract of *C. decidua* root, stem and leaves was evaluated using Freund's complete adjuvants (FCA) induced arthritic models in Wistar rats. Oral administration of *C. decidua* extract at the dose of 100mg/kg, 200mg/kg, per se group (only 200mg/kg of *C. decidua*) was subjected to Wistar rats for 28 days. Standard drug Diclofenac sodium at the dose of 5mg/kg and FCA at 1mg/ml was used in the study. The normal control group was administered only distilled water at 1ml/kg without induction of arthritis. The arthritic investigation was carried out on basis of parameters including changes in body weight, paw volume, hematological studies like ESR count, RBC count, WBC count, Hemoglobin count, platelet count. At the end of study period, animals were sacrificed and histological parameters were evaluated. Phytochemical analysis of *C. decidua* extract was done to assess the various constituents present in *C. decidua*. The results of *C. decidua* extract administration significantly ($P<0.001$) attenuated the body weight, paw volume, hematological alteration induced by the FCA in dose-dependent manner. The tarsal joint was extracted for histopathological studies. The overall results indicate that *C. decidua* extract (100mg/kg and 200mg/kg) showed a potent protective effect against FCA induced arthritic rats which could be attributed to phytoconstituents present in *C. decidua* and its effect is comparable to the standard drug diclofenac sodium.

Keywords *Capparis decidua*, Hydroalcoholic Extract, Freund's Complete Adjuvant, Rheumatoid Arthritis

1. Introduction

Joint inflammation and related issue, including rheumatoid arthritis (RA), are normal illnesses influencing a great many individuals. RA is characterized by articular wounds having an inflammatory propagation of synovial cells, achieving an almost entire functional defect. It influences around 1% of the all-inclusive community. RA is a kind of chronic inflammatory immune system infection. In spite of the fact that a number of medications utilized as a part of the treatment of RA have been developed over the previous couple of decades, there is as yet a requirement for more effective drugs with lower side effects [1]. This autoimmune disorder is characterized by pain, synovial membrane inflammation and confined joint development because of tissue harms. In RA, bone disfigurements and inability of joint capacity occurs due to dynamic disintegration of articular ligament in synovial joint via generation and invasion of auto-antibodies in it. The main pathological changes of RA incorporate hyperplasia of synovial membrane, penetration of fiery cell, and neovascularization, which eventually prompt ligament disintegration and articular destruction. Degradation of cartilage is a more mind-boggling occasion including the local arrival of proinflammatory substance, for example, prostaglandins, leukotrienes, elastase, and proteases including metalloproteases and lysosomal compounds that intervene aggravation in joints and in the synovial liquid in RA[2]. Women are three times more prone to get RA than men. The fundamental classes of medications used to treat rheumatoid joint inflammation are analgesics, disease-modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and immunosuppressive drugs. In any case, these medications deliver some undesirable symptoms, for example gastrointestinal ulcergenicity and renal morbidity. Thus, these days restorative herb in the treatment and counteractive action of illnesses is drawing attention by

researchers around the world [3]. *Capparis decidua* Edgew., belonging to the family Capparidaceae, is a glabrous, highly branched, spiny, spiked, relatively leafless bush or little tree developing fiercely in dry, open badlands all through the parched and semi-dry zones of India and diverse parts of the world. It is commonly called as Kair, or Karil [4]. Ripened fruits of this plant has sharp hot taste; astringent to the entrails, decimates foul breath, biliousness and urinary purulent releases; it is useful in cardiovascular inconveniences. This plant is being utilized as a laxative, emmenagogue, alexipharmic, and aphrodisiac. It improves appetite and is good for rheumatism, cough, lumbago, hiccough, and asthma [5]. *Capparis decidua* (Forssk.) Edgew. contains constituents like flavanoids, phenolic compounds, steroids, alkaloids, vitamins, quarternary ammonium compounds, terpenoids and many more phytoconstituents that are responsible for its medicinal value [6]. Enough phytochemical work has been done so far on *C. decidua*, which has been reported to contain indoles, β-sitosterols, oxygenated heterocyclic compounds, aliphatic constituents, isocodonocarpine, tannins, diterpene alcohol, β-carotene, and sufficient quantities of alkaloids [7]. The stem bark of *C. decidua* showed the presence of n-triacontanol, n-pentacosane, and β-sitosterol, 1-stachydrine. Root bark contains Capparis diterpene, Capparideciduasterol, capparisterol, capparisditerpenyl ester. Previous studies conducted on various parts of this plant showed numerous pharmacological activities like antibacterial, antifungal, antiparasital [8-9], antimicrobial, anti-inflammatory [10], antioxidant, antiplaque [11-12], anthelmintic [13], antidiabetic [14], hepatoprotective [15], antisebum [16], antisclerotic [17], antihyperlipidemic [18], anti termite [19], analgesic, sedative, and anticonvulsant [20].

2. Material and Methods

2.1. Procurement and Authentication of Plant Material

The complete plant of *Capparis decidua* was collected fresh from Jaipur, Rajasthan, India. The plant was taxonomically identified and authenticated by Prof. Kailash Agrawal, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited at the herbarium of the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India (R. No.-RUBL 211645).

2.2. Preparation of Plant Extract

C.decidua root, stem and leaves were washed with tap water followed by distilled water and then cut and dried under the shade. The dried plant parts were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. The dried and powdered plant material was Soxhlet extracted with water and ethyl alcohol (99.9%) in the ratio of 30:70. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered and concentrated at 48˚C by keeping on a water bath and weight of residue was recorded. The percentage yield of hydroalcoholic extract was found to be 42.8%. The collected extract was stored in a sterile container for further use.

2.3. Drugs and Chemicals

Freund's complete adjuvant (FCA) was obtained from Sigma-Aldrich Ltd. (USA). Diclofenac sodium was procured as gift sample from Afton Pharma, Gujarat, India. All other chemicals and reagents used for study were of analytical grade procured from approved organization.

2.4. Acute Toxicity Studies

The acute toxicity of the extract was studied in adult male Wistar rats. They were divided into five groups each consisting of five rats. The suspension of the extract was administered orally at four different doses of 500, 1000, 2000 and 4000 mg/kg, respectively, to different groups of rats separately. Control animals received 10 ml/kg of distilled water orally. The animals were observed continuously for the initial 4h for behavioral changes and mortality and intermittently for the next 6h and then again at 24h and 48h after dosing. The behavior parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration.

2.5. Animals

Female Wistar rats of body weight 150–200g were used for the study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

2.6. Freund's Complete Adjuvant-Induced Arthritis

Arthritis was be induced to all the groups of animals except normal control group by single intra-dermal injection of 0.1mL of Freund's Complete Adjuvant (FCA) containing 1mg.mL^{-1}Mycobacterium tuberculosis H37Ra suspension in sterile paraffin oil into a foot pad of the left hind paw of female rats. The rats were anesthetized with ether inhalation prior to and during adjuvant injection, as the very viscous nature of the adjuvant exerts difficulty while injecting.

Treatment with hydroalcoholic extract of *C. decidua*, Diclofenac sodium and normal control (Distilled water)

was started on the 14th day after arthritis induction and continued for 28 days. The paw volume of all the animal groups was measured by plethysmograph at 1, 4, 10, 14, 17, 21, 24 and 28 after the injection of Freund's complete adjuvant. [21]

The animals were divided into six groups consisting of six animals per group

Group I: Normal control group (distilled water 1ml/Kg p.o) (non-arthritic), (n=6)

Group II: FCA injected arthritic control; (n=6)

Group III: Arthritic animals treated with Diclofenac Sodium (5mg/kg/day), (n=6)

Group IV: Arthritic animals treated with hydroalcoholic extracts of *C. decidua* (100mg/kg body weight/day p.o), (n=6)

Group V: Arthritic animals treated with hydroalcoholic extracts of *C. decidua* (200mg/kg body weight/day p.o), (n=6)

Group VI: Per se group (normal group where only plant extract with 200mg/kg will be administered p.o)

Anti-arthritic effect of hydroalcoholic extract of *C. decidua* was evaluated on body weights changes and paw volume on day 1, 4, 10, 14, 17, 21, 24 and day 28. On day 28 the animals were anesthetized with ether and the blood was withdrawn by tail vein for the estimation of various hematological parameters followed by histopathological analysis of ankle joint of rats.

Measurement of Bodyweight - Body weight was recorded on day 0 just before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28. [22]

Measurement of paw volume - Paw volume was measured using a Plethysmometer (UGOBasile, Italy) on day 0 before FCA injections and thereafter on day1, 4, 8, 12, 16, 20, 24, and day 28 [19]. The change in paw volume was calculated as the difference between the final and initial paw volume. [23]

Haematological parameters - On day 28, haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and platelets (PLT) were determined by usual standardized laboratory method. [24]

Histopathological analysis of ankle joints - On day 28, ankle joints were separated from the hind paw and immersed in 10% buffered formalin for 24h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5µ thickness. The sections were stained with haematoxylin-eosin and evaluated under light microscope with 10 times magnifications for the presence of inflammatory cells, hyperplasia of synovium, pannus formation and destruction of joint space [25].

2.7. Statistical Analysis

The data were represented as a mean ± standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software.

3. Results

The oral administration of hydroalcoholic extract of *C. decidua* did not provoke any gross behavioral changes or manifestations of toxic symptoms such as increased or decreased motor activity, loss of right reflex, ataxia, clonic convulsions, muscle relaxation spasticity, tremors, tonic extensions, lacrimation, salivation, weight loss, watery diarrhea, writhing and urination over a period of 48h. The hydroalcoholic extract of *C. decidua* was found to be non-lethal even at the maximum single dose of 4.0g/kg. The dose of hydroalcoholic extract of *C. decidua* was selected on this basis and as per the earlier studies conducted by Goyal et al 2009 [7] where 100mg/kg and 200mg/kg of *C. decidua* showed significant results ($P<0.05$) without any toxic effects at these doses.

The rats in the FCA treated group lost body weight as compared with the *C. decidua* extract treated and diclofenac treated groups. The body weight of *C. decidua* at 100mg/kg, 200mg/kg and per se significantly ($P<0.001$) increased from day 17th onwards till day 28th as compared to FCA treated group rats. While Diclofenac treated groups also showed the significant result ($P<0.001$) from day 17th onwards till day 28th as compared to FCA treated group rats. The effect produced by *C. decidua* extract at 100 mg/kg and 200mg/kg produced a similar result as seen in a diclofenac-treated group on days 17, 21, 24 and 28**.** (Figure 1)

There was significant ($P < 0.001$) increase in paw volume of all the rats treated with FCA compared to control groups' rats. Hydroalcoholic extract of *C. decidua* (100 and 200 mg/kg) significantly ($P < 0.001$) lowered the paw volume from day 14 onwards as compared to FCA control group. Per se group also showed significant ($P<0.001$) reduction in paw volume from day 4 onwards till 28th day. *C. decidua* extract at 100 mg/kg was less effective initially ($P< 0.05$) till 14th day but thereafter showed more significant result ($P < 0.001$). Diclofenac 5mg/kg showed significant ($P < 0.001$) reduction in paw volume from day 4 onwards. (Figure 2)

The significant increase in levels of platelets ($P< 0.001$), ESR count ($P < 0.001$) and WBC ($P < 0.001$) and significant decrease in levels of RBC ($P < 0.001$) and Hb ($P < 0.001$) were observed in FCA group as compared to normal control group indicating a stimulation of immune response towards FCA in arthritic rats. Treatment with hydroalcoholic extract of *C. decidua* (100 & 200 mg/kg), per se group significantly ($P < 0.001$) inhibited the stimulation of immune response towards FCA by decreasing blood WBC, ESR, and increasing Hb and RBC compared to FCA treated group. Diclofenac sodium treated

rats also showed significant result ($P < 0.001$) by reducing the WBC, ESR count and platelet and increasing hemoglobin and RBC levels. (Figure 3-7)

As shown in Figure 4, the histopathological evaluation of the ankle joint in FCA treated group exhibited dense neutrophil cell infiltration causing edematous synovium, destructive lesions in articular cartilage, vascularity formation into the joint space, synovial hyperplasia, pannus formation and cartilage erosion. Treatment with extract of *C. decidua* reduced infiltration of inflammatory cells, joint space narrowing, pannus formation, synovial hyperplasia and cartilage erosion in a dose-dependent manner as evidenced from the histopathology sections of *C. decidua* treated rats. (Figure 8.1-8.6)

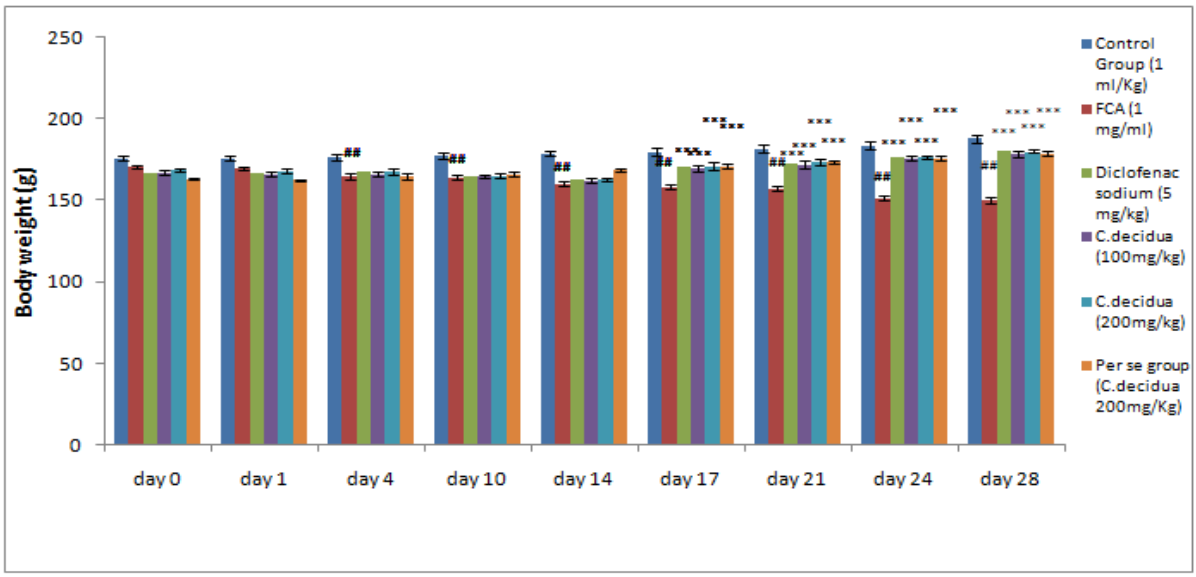

Figure 1. Effect of *Capparis decidua* on body weight (g) in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison tests. ##P < 0.001 as compared to control. ***P < 0.001 as compared to FCA treated

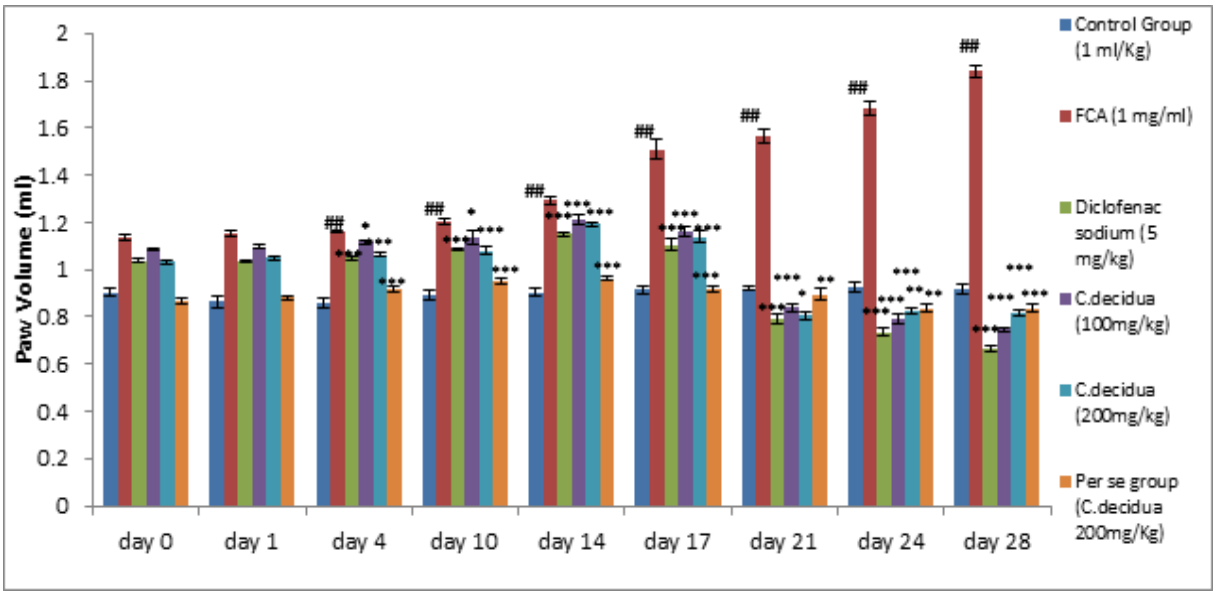

Figure 2. Effect of *Capparis decidua* on Paw volume test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzedby one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P < 0.001 as compared to control. ***P < 0.001 as compared to FCA. **P < 0.01 as compared to FCA.*P < 0.05 as compared to FCA.

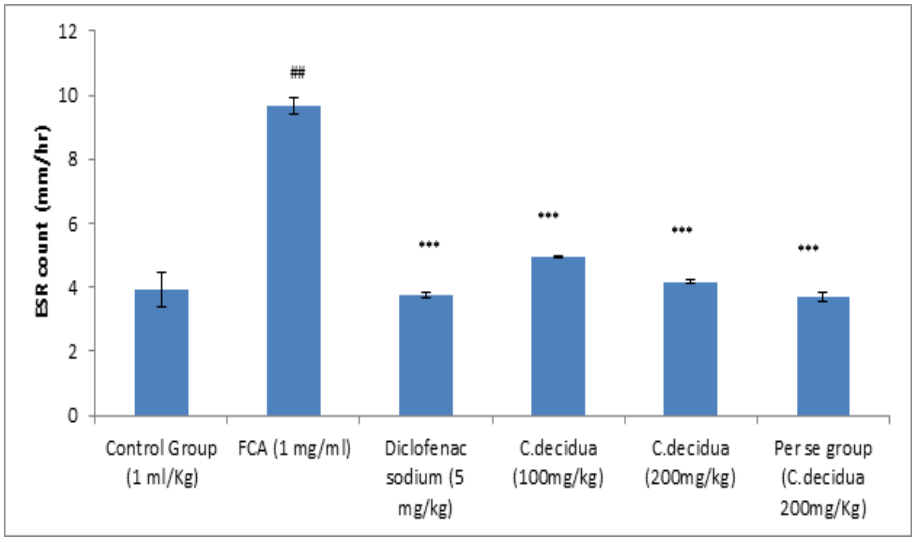

Figure 3. Effect of *Capparis decidua* on ESR count mm/hr test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison. ##P < 0.001 as compared to control. ***P < 0.001 as compared to FCA

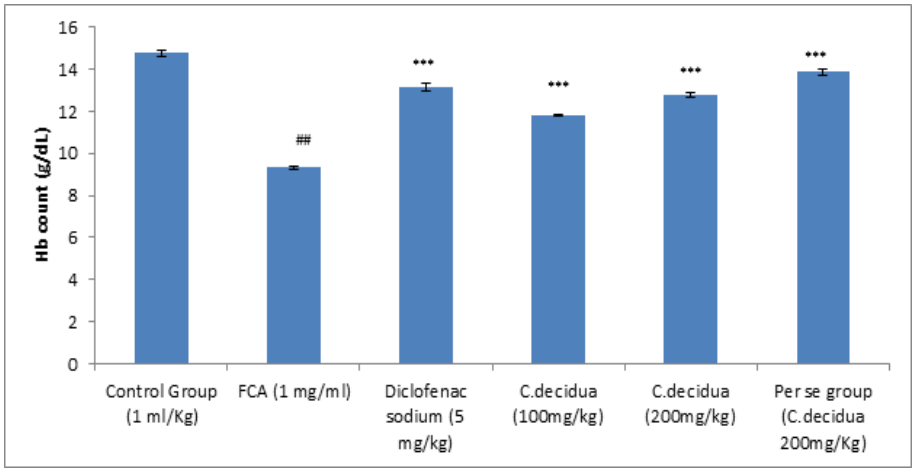

Figure 4. Effect of *Capparis decidua* on Hemoglobin count g/dL count test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzedby one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison. ##P < 0.001 as compared to control. ***P < 0.001 as compared to FCA.

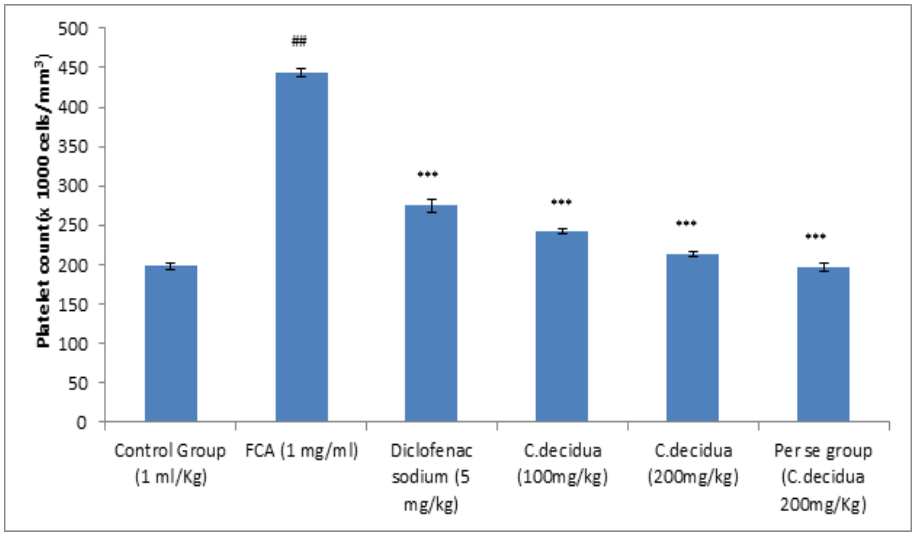

Figure 5. Effect of *Capparis decidua* on Platelet count (x 1000 cells/mm^3) test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzedby one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison. ##P < 0.001 as compared to control. ***P < 0.001 as compared to FCA.

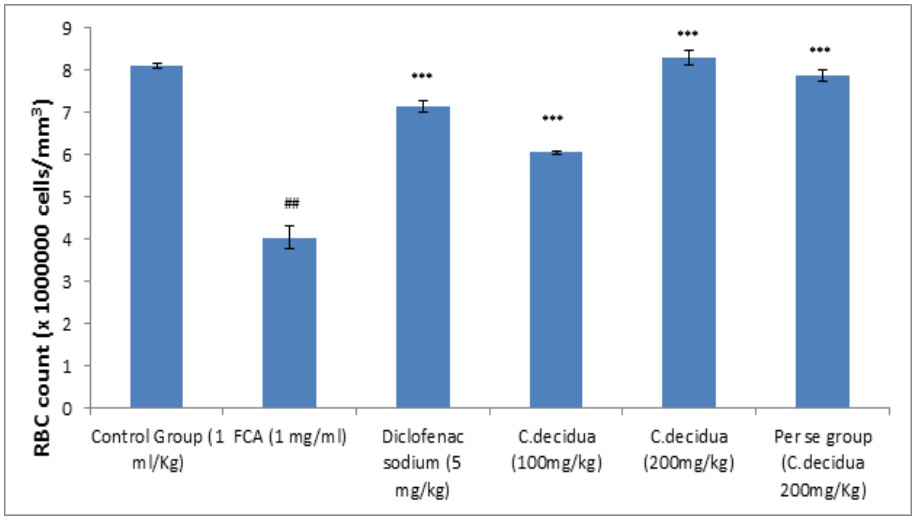

Figure 6. Effect of *Capparis decidua* on RBC count test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzedby one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison. $^{\#\#}P < 0.001$ as compared to control. ***P < 0.001 as compared to FCA treated.

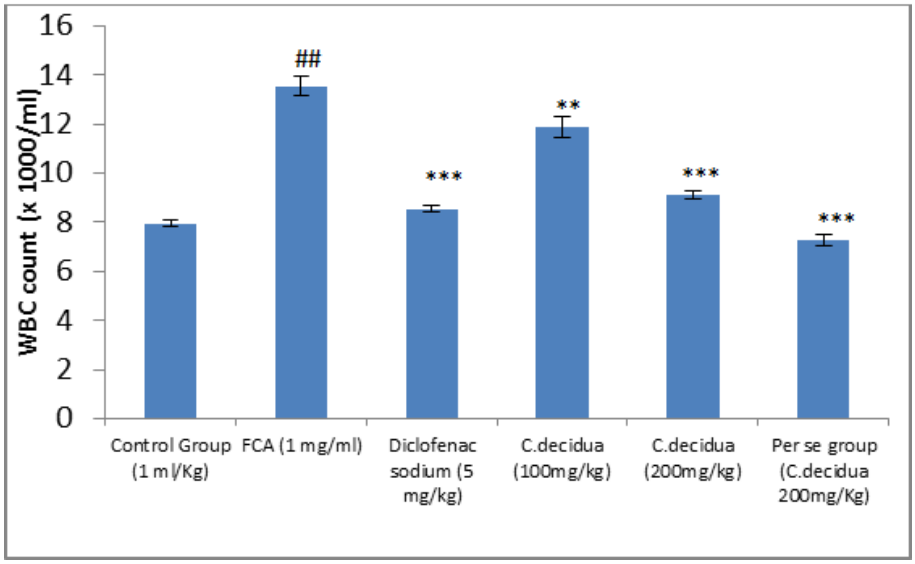

Figure 7. Effect of *Capparis decidua* on WBC count test in FCA-induced arthritic rats; Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison. $^{\#\#}P < 0.001$ as compared to control. ***P < 0.001 as compared to FCA.

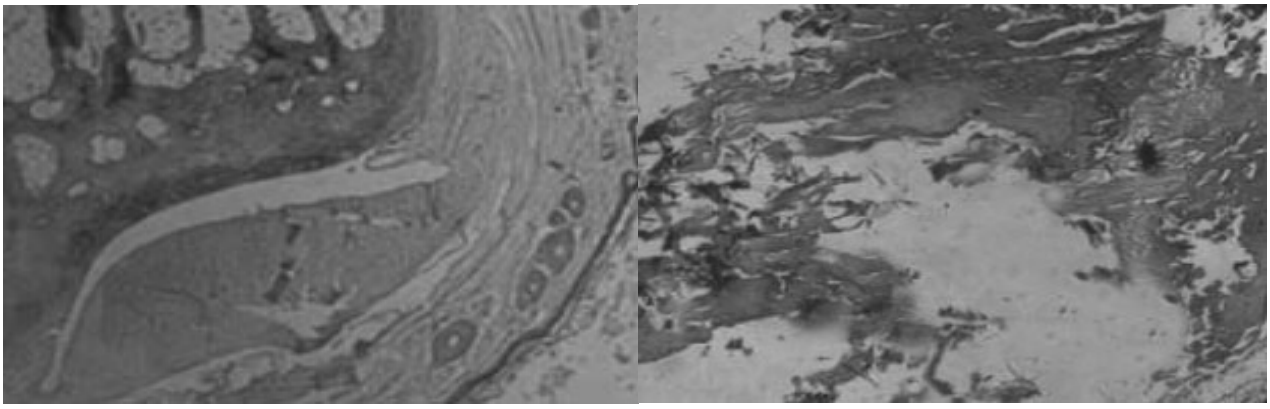

Figure 8.1 Figure 8.2

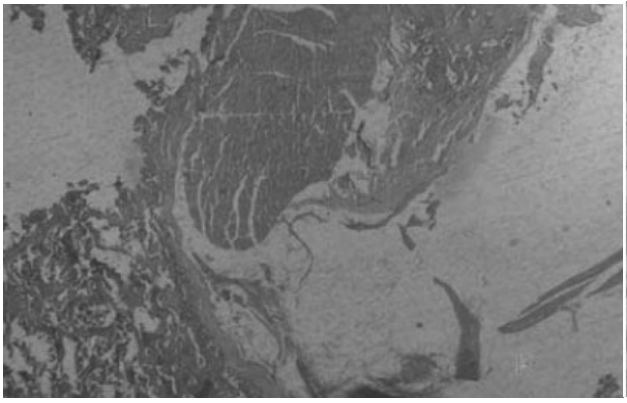

Figure 8.3

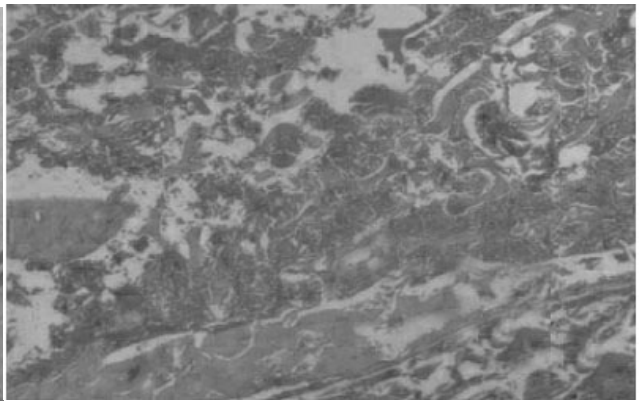

Figure 8.4

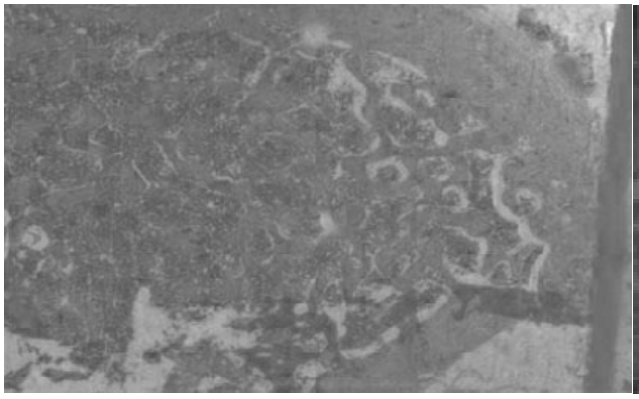

Figure 8.5

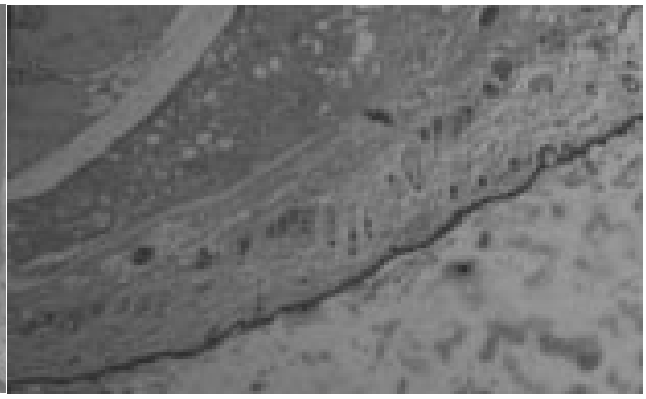

Figure 8.6

Figures 8.1-8.6. Effect of hydroalcoholic extract of *C. decidua* on histolopathology of ankle joint in experimental rats 8.1) Ankle joint synovial membrane structure in normal control rat; 8.2) Severe inflammation with synovial hyperplasia and increased vascularity, oedematous inflammation, inflammatory cell infiltrate of neutrophills in FCA induced arthritis rats; 8.3) Synovial membrane structure re-organising with decrease in oedema and inflammation in Diclofenac sodium rats; 8.4) Moderate healing in the synovial membrane in *C. decidua* extract 100mg/kg; 8.5) Decrease in inflammation with decrease in oedematous spaces, restructuring of synovial membrane with noticeable reduction of histological injury in *C. decidua* extract 200 mg/kg; 8.6) Ankle joint synovial membrane structure in per se groups rats.

4. Discussion

Numerous restorative plants give alleviation of manifestations in rheumatoid joint inflammation whose impacts are tantamount to that of accessible regular therapeutic agents [26]. Acute toxicity study revealed the non-toxic nature of the extract at the dose of 4g/kg. Limb swelling, proliferative synovitis, inflammatory cell infiltration and erosion of the bone and cartilage structure are clinical discoveries related to human arthritis and FCA-induced arthritis rat. Attributable to this likeness in pathologic highlights, the FCA-induced arthritis rat is a widely used model of rheumatoid arthritis in evaluating the efficacy of anti-inflammatory drugs [27]. In the present study, hydroalcoholic extract of *C. decidua* (100 and 200mg/kg) and per se treatment showed an anti-arthritic effect in the inflammatory parameter like paw volume. *C. decidua* extract significantly ($P<0.001$ at 100 & 200mg/kg) decreased the inflammation compared to the FCA treated the group as observed by decreased paw volume. The present study revealed that paw volume rises with ankle bone hardness in FCA treated rats. The body weight of FCA treated group rats was prominently decreased compared with that of normal control rats. The results suggest that oral *C. decidua* extract (100mg/kg, 200mg/kg and per se) reduced inflammatory body weight loss in arthritis induced rats. Thus, *C. decidua* gives protective action in terms of body weight. The decrease in body weight of FCA-induced arthritic rats in the present study is because ofdecreased intestinal absorption rate. Treatment with EACA significant inhibited weight loss in arthritic rats. Thus, EACA may have the potential as a therapeutic agent used for symptomatic treatment of rheumatoid arthritis because of its anti-inflammatory action which delays progression of disease [2]. Increase in the WBC count in FCA treated group rats indicates the leukocytosis in the joint region by infiltration of neutrophil cells. This data may be affirmed by the earlier study conducted by Glenn et al, 1965[28] who reported neutrophilia and leukocytosis on day 28 post-induction.

In arthritis decreased level of hemoglobin (Hb) and red blood cells (RBCs) is caused because of the diminished reaction of the bone marrow erythropoietin and pulverization of untimely RBCs [29]. So also increment in

the level of erythrocyte sedimentation rate (ESR) is credited to the quickened arrangement of endogenous proteins including plasma proteins, for example, fibrinogen, alpha and beta globulins. Henceforth these parameters are key biomarkers that are elevated during inflammation, stress and cell necrosis [30]. In our study, treatment with *C. decidua* extract in arthritic rats significantly increased the level of RBC and Hb while it decreased the level of ESR which can be credited to its mitigating potential. Ascend in a number of platelets in FCA treated group rats also indicated the inflammatory pathogenesis in the joint region while a decrease in the number of platelets in C. decidua extract treated groups suggest the protective role of this plant in arthritis. In addition, the defensive impact of *C. decidua* in the progression of joint damage was additionally affirmed by the histopathological investigation of ankle joint. In the present study, ankle joint histopathological sections of normal control rats showed dense cellular infiltration, synovial hyperplasia alongside pannus formation. Treatment with *C. decidua* in arthritic rats showed reduced cellular infiltration, synovial hyperplasia and pannus formation in ankle joint, which suggests that *C. decidua* can effectively inhibit the disease progression in arthritic rats. Since our study has shown that hydroalcoholic extract of *C. decidua* possesses significant anti-arthritic activity in experimental animals. *C. decidua* showed the presence of numerous constituents like phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates in the present study.

Earlier studies have shown the presence of chemical constituents like n-Triacontane, n-Pentacosane, β-Carotene, Carbohydrates, Proteins, Glucosinolates, n-Triacontanol [31-32], Tetrahydropyran-2-one, 2-Carboxy-1-dimethylpyrrolidine 9 (33), Nonacosane (34), Quercitin, Isodulcite, Nonacosane, Thymol, Isopropyl isothiocyanate, Butyl isothiocyanate, 2-Hexenol (33), Phenylpropanoid, Terpenoids, Isothiocyanate, n-Alkalenes. Thomnocitrine, Kaempferol, Isorhamnetin, rhamnocitrin, rhamnetin, rhamnazin, quaternary ammonium compounds, Spermidine alkaloid (35), Isocodonocarpine, Capparine, Capparinine, Cappariline, Codonocarpine (36), Cadabacine-26-O-β-d-glucoside, Capparisine, Capparispine-26-O-d-glucoside, N-acetylated spermidine, 14-N-acetyl isocodonocarpine, 15-N-acetylcapparisine, Rutin, l-Stachydrine, β–Sitosterol, Terpenoids, Capparisesterpenolide (37), Glucocapparin, Capric acid, Monoterpenes, Sesquiterpenes, Tocopherols (38).

It could be said that the antiarthritic activity could be attributed to these phytochemical.

5. Conclusions

The study revealed that hydroalcoholic extract of *C. decidua* (100 and 200mg/kg) possess anti-arthritic activity that is mediated by its suppression of swelling and inflammation of paw, reduction of a decrease in body weight, and analyzed by hematological, and histopathological parameters. All these results thus reflect that *C. decidua* provide a pharmacological rationale for the traditional use of the plant against rheumatoid arthritis. The possible compounds that participated in the treatment of arthritis could be attributed to phytochemicals found in the *C. decidua* plant.

Acknowledgements

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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EFFECT OF HYDROALCOHOLIC EXTRACT OF *CAPPARIS DECIDUA* (FORSSK.) EDGEW ON SERUM TESTOSTERONE AND SPERMATOGENESIS IN RATS

Prashant Kumar Dhakad,[a]* Pramod Kumar Sharma[a] and Sokindra Kumar[b]

Keywords: *Capparis decidua,* aphrodisiac, serum testosterone level, body weight, secondary sexual organ weight.

The effect of *Capparis decidua(Forssk.)Edgew* aqueous extract on the Wistar rat testes was investigated with a view to evaluating the pharmacological basis for the use of *Capparis decidua* hydroalcoholic extract as an aphrodisiac. Wistar rats were divided in the following experimental groups- control group (1 mL kg^{-1}), sildenafil citrate treated (5 mg kg^{-1}), *C. decidua* (100 mg kg^{-1}), *C. decidua* (200 mg kg^{-1}), per se group (only *C. decidua* 200 mg kg^{-1}). The hydroalcoholic extract of root, stem & leaves of *C. decidua* was studied for their effect on the body and secondary sexual organ weight, spermatogenesis, and serum testosterone level male rats. The animals were allowed free access to drinking solution during the 28 days period of exposure. At the end of the experimental period, rats were sacrificed, testis, epididymis, seminal vesicles and prostate glands were excised and weighed, and serum testosterone level was recorded. The testes underwent histological examination. Oral administration of the extract in Wistar rats showed significant dose-dependent influence on serum testosterone level (P<0.001) and spermatogenic effects in extract treated rats groups by increasing the weights of secondary sexual organs(P<0.001).

* Corresponding Author
Tel. No.: +91 8510860363
E-Mail: prashant.dhakad@galgotiasuniversity.edu.in
[a] Department of Pharmacy, Galgotias University, Greater Noida, India

INTRODUCTION

Propagation of one's race is the doctrine of every single living life form. Every living creature endeavour to accomplish this through the procedure of multiplication, which is the essential procedure that empowers animal groups to speak to itself in the accompanying age as its posterity.[1]

Variations from the norm in male regenerative frameworks like impotency, erectile dysfunction and the other way around are one of the principle issues that prompt sterility. An aphrodisiac is a substance that increments sexual intimacy.[2] It has been perceived for quite some time that specific antihypertensive drugs, centrally acting sympatholytic drugs, β-antagonists, antidepressants, antipsychotics, anticonvulsants, drugs with antimuscarinic effects and diuretics, adversely affect sexual working.[3] *Capparis decidua* (Forssk.)Edgew. (Kair) is a multipurpose perennial woody plant, belonging to caper family (Capparaceae), found largely in the hot dry region of different parts of India.[4]

Capparis decidua (Forssk.) Edgew is salt-tolerant and grows along saline hard planes in the Thar Desert of India. Mature plants form extensive root systems that penetrate deeply into the soil. Leaf stipules frame into spines to decrease transpiration. The stem bark is smooth, green when youthful and turns yellow or whitish dark as it develops. The roots, fruits, and various parts of these plants with potential therapeutic advantages have been used since long time. *C. decidua* contains constituents like phenolic compounds, alkaloids, flavonoids, terpenoids, steroids, vitamins, quarternary ammonium compounds and many more phytoconstituents that are responsible for its medicinal value.[5]

Previous studies suggested that the antimicrobial effects of *Capparis decidua* (Forssk.) Edgew may be due to presence of bioactive compounds, like flavonoids, phenolics, polyamine alkaloids, glucosinolates, and vitamins that decrease the growth of microbes.[6] Roots of this plant have been used as expectorant, carminative, sudorific, thermogenic, digestive, aphrodisiac, stimulant, antibacterial, anodyne, anthelmintic and in treating constipation, lumbago, odontalgia, amenorrhoea, arthritis, dyspepsia, and dysmenorrhoea. The root bark is known to be astringent, diaphoretic, alexeteric etc. Powder or infusion of root bark is used in a cough, dropsy, palsy, gout, rheumatism, asthma, intestinal worms and intermittent fever.

Nowadays people are swinging to herbal remedies to improve this infertility issue they are effectively agreeable to normal man. Research is done to discover the plant items that can be utilized to treat this sort of infertility problems.[7]

EXPERIMENTAL

Plant Materials

The complete plant of *Capparis decidua* was collected near fresh from Jaipur, Rajasthan, India. The plant was taxonomically identified and authenticated by Prof. Kailash Agrawal, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited at the herbarium of the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. (R.No. RUBL 211645).

Experimental animals

Healthy Albino Wistar rats of both sexes weighing 150-250 g were obtained from Central Animal Facility AIIMS New Delhi. The experimental protocol was approved by Institutional Animal Ethics Committee CPCSEA No. -1149/PO/ERe/07/CPCSEA. Animals were housed under standard conditions of temperature (24±2 ˚C) and relative humidity (30-70 %) with 12:12 light: dark cycle. The animals were given standard pellet diet and water ad libitum.

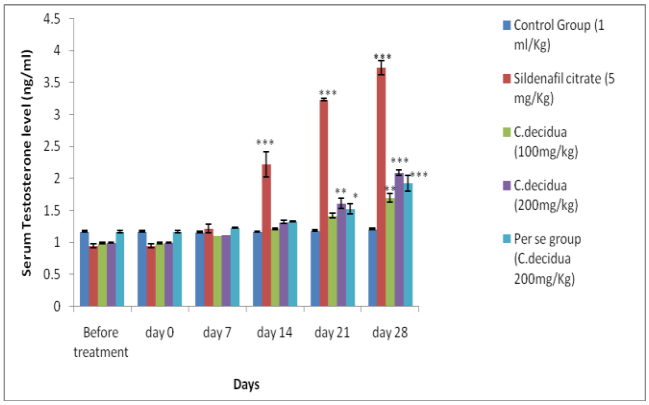

Figure 1. Effect of hydroalcoholic extract of *C. decidua* on serum testosterone level on male Wistar rats.

Preparation of test samples

The hydroalcoholic extract was dissolved in the distilled water and orally administered to the test groups. Sildenafil citrate was procured from the Cadila Pharmaceuticals Limited, Ahmadabad, Gujarat, India as a generous gift.

Experimental design

Adult male albino rats of Wistar strain were used for the experimentation. The animals were divided into 5 groups of 6 animals each and treated as follows. Group I: Control group (1 mL kg^{-1} distilled water p.o), Group 2: Sildenafil citrate treated (5 mg kg^{-1} p.o), Group 3: *C. decidua* (100 mg kg^{-1} p.o), Group 4: *C. decidua* (200 mg kg^{-1} p.o), Group 5: per se group (only *C. decidua* 200 mg kg^{-1} p.o). All the above treatments were given orally for 28 days. The serum testosterone level was determined before treatment, day 0, day 7, day 14, day 21 and 28th day. On the 29th day all the rats were sacrificed and the testis, epididymis, seminal vesicle, prostate were dissected out, surrounding blood vessels and tissues were removed and blotted free of blood and mucous. The tissues were weighed using electronic balance.

Histological studies

The testis and cauda epididymis from the opposite side was settled in Bouine's liquid, inserted in paraffin, sectioned at 5 µm thickness and stained in haematoxylin and eosin and prepared for histological investigations.[8]

Statistical analysis

All the values were reported as mean±S.E.M. Analysis of variance (ANOVA) was employed to analyze the data, while Tukey's multiple comparison tests were used to test for differences between individual treatments groups using Graph pad prism software version 5.0. $P<0.05$ was considered statistically significant.

RESULTS

A dose-dependent increase in serum testosterone concentration were observed on the 21st and 28th day of the study in *C. decidua* extract (100 mg kg^{-1}) ($P<0.01$), *C. decidua* extract (200 mg kg^{-1}) ($P<0.001$), per se group (only *C. decidua* extract 200 mg kg^{-1}) ($P<0.001$). While sildenafil citrate group showed an increase in serum testosterone level on 14th, 21st and 28th day of the study as compared to control group ($P<0.001$) (Figure 1).

The body weight has increased in all the experimental animal groups. This increase is 6.5 % in control rats whereas it is 32.3, 21.61, 25.20 and 14.54 %, respectively in the rats treated with Sildenafil citrate, *C. decidua* extract (100 mg kg^{-1}), *C. decidua* extract (200 mg kg^{-1}), per se group (only *C. decidua* extract 200 mg kg^{-1}). Sildenafil citrate group showed a 7.6 % increase in testis weight, a 6.94 % increase in seminal vesicle weight, an 8.91 % increase in weight of epididymides and a 9 % in prostate gland weight.

Table 1. Effect of hydroalcoholic extract of *C. decidua* on body weight and secondary sexual organ weight on male wistar rats.

Treatment groups	Body weight (g)		Weight of organs on 28th day (mg 100 g^{-1} of body weight)			
	Day 0	Day 28	Testes	Seminal vesicle	Epididymides	Prostate
Control Group (1 mL kg^{-1})	107.5	114.5±0.76	950.83±0.6	415±1.42	746.16±0.47	281.33±0.8
Sildenafil citrate (5 mg kg^{-1})	103.16	136.5±0.67***	1023.66±0.71***	443.83±1.08***	812.66±0.88***	306.66±0.49***
C. decidua (100 mg kg^{-1})	101	122.83±0.6***	1014.5±0.76***	434.33±1.28***	797.16±0.6***	296.83±0.6***
C. decidua (200 mg kg^{-1})	103.83	130±0.57***	1024.83±0.6***	449±1.53***	814.5±0.76***	316.5±0.76***
Per se group (*C. decidua* 200 mg kg^{-1})	103.16	118.16±0.94*	970.16±0.87***	419.83±0.94	762±0.96***	289±0.96***

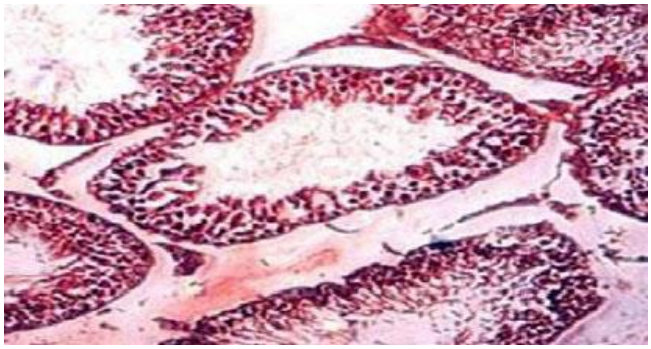

Figure 2. Control rat showing normal seminiferous tubules with normal spermatogenesis.

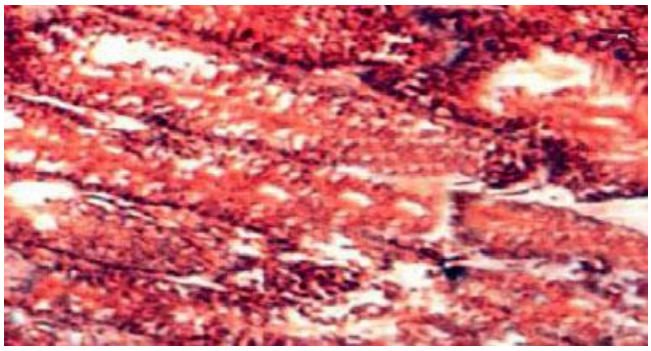

Figure 3. Sildenafil citrate treated rats showing increase in the size of seminiferous tubules, increase in the spermatogonia, spermatocytes and spermatids and spermatozoa.

The *C. decidua* extract (100 $mgkg^{-1}$) group showed an increment of 6.69 % in testis weight, a 4.65 % increase in seminal vesicle weight, a 6.83% increase in the weight of epididymides and a 5.50 % increase in prostate gland weight. Likewise, the *C. decidua* extract (200 $mgkg^{-1}$) group showed an elevation of 7.78 % in testis weight, an 8.19% increase in the weight of the seminal vesicle, a 9.15 % increase in the weight of the epididymides and a 12.50 % increase in prostate gland weight. Per se group (only *C. decidua* extract 200 $mgkg^{-1}$) showed an increment of 2.03 % in testis weight, a 1.16 % increase in seminal vesicle weight, a 2.12 % increase in the weight of epididymides and a 2.72 % increase in prostate gland weight after 28 days of treatment compared to the control group (Table 1).

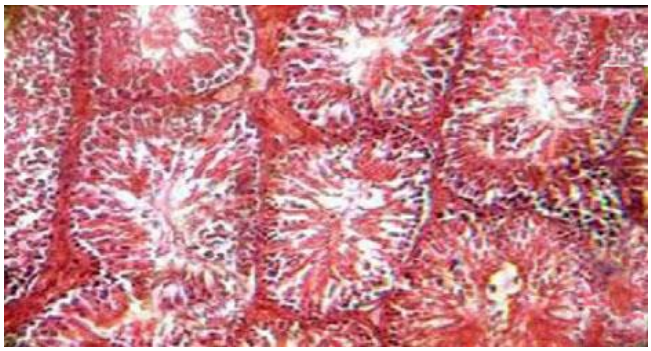

Figure 4. *C. decidua* (100 $mgkg^{-1}$) hydroalcoholic extract treated rat showing all types of spermatogenic elements and spermatozoa in the lumen.

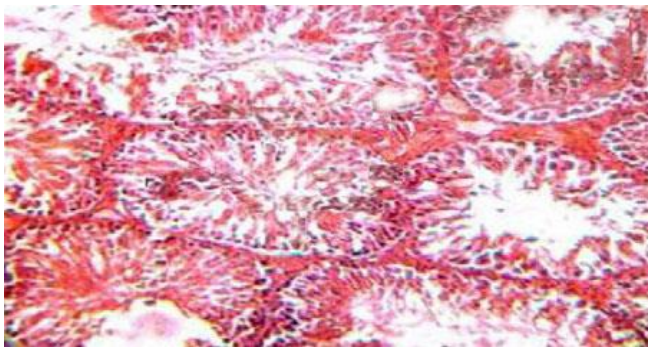

Figure 5. *C. decidua* (200 $mgkg^{-1}$) hydroalcoholic extract treated rat showing increased number of spermatogonia, spermatocytes and spermatids and more number of spermatozoa in lumen.

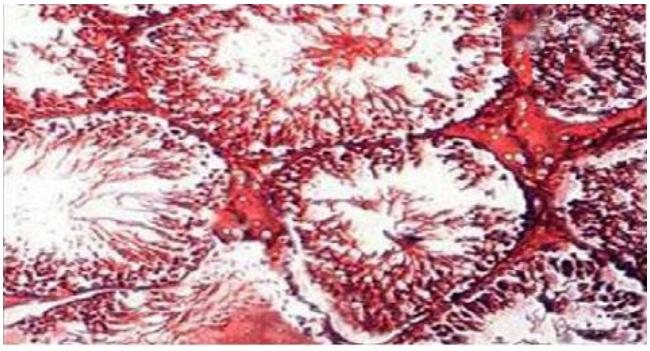

Figure 6. Per se group (200 $mgkg^{-1}$ of hydroalcoholic extract of *C. decidua*) showing moderate number of spermatogenic elements.

Histological examination demonstrated the control group with typical testicular structures, in confirmation with spermatogenesis. A noteworthy impact on spermatogenesis was noted following 28 days of treatment with the extract. The weight and size of the testis were found more in the plant extract treated groups. The germinal epithelium cells seemed, by all accounts, to be hyperactive. Substantial quantities of various cells at various phases of spermatogenesis were apparent. Sertoli cells were enlarged, highly processed, and rich in nutrients as appeared by very granulated cytoplasm. The expanding in the volume of the two cells and nuclei was strongly suggestive of steroid synthesis under the direct or indirect impact of the extract. The blood vessels of testis were slightly enlarged. Expanded spermatogenesis was obvious from the vast number of spermatozoa in the seminiferous tubules and was additionally appeared by the expansion in spermatogenic components compared with control group. (Figure 2-6).

DISCUSSIONS

In the present study administration of *C. decidua* extracts have stimulated the activity of testis and accessory organs. A significant ($P<0.001$) increase in testosterone level was found in the extract treated animals compared with control. It demonstrates that the extract has an impact at the endocrine level. Testosterone is the significant male gonadal hormone, and it is created by the interstitial Leydig cells in the testis. It is additionally the real factor for androgenicity.

A specific concentration of androgens is required for the initiation and maintenance of spermatogenesis and for the start and support of spermatogenesis and for the incitement of development and the working of the prostate and original vesicles. The expansion in testosterone level may improve androgen-dependent parameters such as mating behavior and the maintenance of spermatogenesis.[9-12]

Out of three extracts administered *C. decidua* (200 $mgkg^{-1}$) extract proved to be profoundly stimulant, *C. decidua* (100 $mgkg^{-1}$) extract is a medium stimulant and Per se group (*C. decidua* extract 200 $mgkg^{-1}$) is less stimulant in increasing the weight of testis and male reproductive accessory organs. There is likewise an advance in spermatogenesis as found in the expansion of spermatogenic components in the testis which might be because of the higher accessibility of pituitary follicle stimulating hormone (FSH), as FSH is known to invigorate the spermatogenesis. Both FSH and LH are important for meiosis and generation of spermatids. The possible increment in the number of spermatogonia, spermatocytes, and spermatids might be credited because of the expanded accessibility of FSH and LH in *C. decidua* extracts treated rats. The androgen synthesis in the testis is dependent on pituitary LH and FSH. The expanded weight in the accessory organ in treated rats demonstrates the extract may stimulate the FSH and LH release and testosterone production.[13,14]

Past phytochemical examines have demonstrated the *C. decidua*plant extract contains alkaloids, steroids, phenolic compounds, terpenoids, tannins, glycosides, flavonoids, and saponins. Saponins have been appeared to be responsible for endothelium-dependent nitric oxide release causing relaxation of the rat aorta. Nitric oxide is a noteworthy physiological boost for penile vasculature and trabecular smooth muscles, all necessary for penile erection.[15]

In this study, the investigation of different sexual parameters has approved the customary faith in the viability of the root, stem, and leaves of *C. decidua* for treating sexual dysfunctions. The outcomes additionally show the conceivable utilization of extract of *C. decidua* as an herbal alternative to the allopathic medicines that are gaining popularity for the treatment of sexual dysfunction.

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Antiarthritic Potential of *Capparis Decidua* (Forssk.) Edgew on FCA Induced Arthritis in Wistar Rats

Dhakad Prashant Kumar[1*], Sharma Pramod Kumar[1] and Kumar Sokindra[2]

[1]*Department of Pharmacy, School of Medical & Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh 201306, India.*

[2]*RV Northland Institute (Pharmacy), Pharmacology Research Lab, Greater Noida-II, Gautam Budh Nagar, Uttar Pradesh 203207, India.*

[*]Correspondence:
Prashant Kumar Dhakad, Department of Pharmacy, School of Medical & Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India, E-mail: dhakadprashant654@gmail.com.

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ABSTRACT

The present investigation was designed to evaluate anti-arthritic potential of hydroalcoholic extract of leaves, root and stem of Capparis decidua. The anti-arthritic activity was evaluated using Freund's complete adjuvants (FCA) induced arthritic models in Wistar rats. The arthritic study was carried out on basis of parameters including arthritic score, antinociceptive study, motor incoordination test, paw joint diameter, and biochemical parameters like serum AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase) and total protein levels. Phytochemical analysis of C.decidua extract was done to assess the various phytoconstituents present in C.decidua. The results of C.decidua extract administration significantly (P<0.001) significantly decreased the arthritis which was evident with arthritis score, joint diameter, pain threshold. Also improvement in fall of time, and biochemical parameters suggested the antiarthritic role of C.decidua extract. The results indicate that hydroalcoholic C.decidua extract (100 mg/kg and 200mg/kg) showed a potent protective role against FCA induced arthritic rats which could be attributed to phytoconstituents present in C.decidua and its effect is comparable to the standard drug diclofenac sodium.

Keywords

Anti-arthritic, *Capparis decidua*, Wistar rats, Phytoconstituents.

Introduction

Rheumatoid arthritis (RA) is a fundamental immune system sickness, portrayed by synovial hyperplasia and constant irritation, which inevitably brings about joint obliteration and functional disability [1]. It can quickly advance into multisystem irritation with joint harm in this way causing torment, swelling, and demolition of ligament and bone, which could influence personal satisfaction [2]. Joint aggravation is a involves the role of various signaling molecules originated by mast cells, leukocytes, and macrophages and in addition by the activation of complement factors, which causes edema formation as a result of leakage of liquid and proteins and collection of leukocytes at the provocative site. Different non-steroidal anti-inflammatory drugs (NSAID's) are broadly utilized clinically for rheumatoid joint inflammation. Be that as it may, regardless of their incredible number, their remedial viability is by all accounts hampered by the nearness of various undesired, and regularly genuine, symptoms. It would, in this way, be exceptionally alluring to discover less dangerous choices, and some therapeutic botanicals may be contender for such options [3]. It stays vital to assess the capability of therapeutic plants with a specific end goal to distinguish painkillers creating intense impacts and initiating couple of unfavorable responses.

Capparis decidua Edgew family Capparidaceae, is an important medicinal plant in Indian system of medicine, used in treatment of ailments like digestive disorders, sudorific, gout, constipation, flu, cough, dropsy, asthma, palsy, and odontalgia. *C.decidua* roots are known to act as thermogenic, sudorific, carminative, expectorant, digestive, stimulant, aphrodisiac, antibacterial, anthelmintic, anodyne and efficient in arthritis, dyspepsia, constipation, dysmenorrhoea. Root bark is used in rheumatism, gout, dropsy, palsy, asthma, gastrointestinal worms and high fever. Several phytoconstituents belonging to category alkaloids, glycosides, flavonoids, phenolic compounds, quarternary ammonium compounds, steroids and volatile oil has been reported from

different parts of this plant. Like Capparisinine, Isocodonocarpine, Capparidisine, Spermidine alkaloid, capparinine and Capparine have been isolated from Caper roots. Codonocarpine, capparisine, capparipine-26-O-d-glucoside and cadabacine-26-O-d-glucoside have also been isolated from dry root bark of *Capparis decidua* [4]. As per our literature survey, there are no scientific evidences available on antiarthritic study of this plant. Hence, in this study we have evaluated the antinociceptive, motor inco-ordination, arthritic index, joint diameter and biochemical study of hydroalcoholic extract of root, stem and leaves of *Capparis decidua*.

Material and Methods

Drugs and Chemicals

Freund's complete adjuvant (FCA) was obtained from Sigma-Aldrich Ltd. (USA). Diclofenac sodium was procured as gift sample from Afton Pharma, Gujarat, India. All other chemicals and reagents used for study were of analytical grade procured from approved organization.

Plant material and preparation of extracts

The complete plant of *Capparis decidua* was collected fresh from Jaipur, Rajasthan, India. The plant was taxonomically identified and authenticated by Prof. Kailash Agrawal, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited at the herbarium of the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India (R.No.-RUBL 211645). *C.decidua* root, stem and leaves were washed with tap water followed by distilled water and then cut and dried under the shade. The dried plant parts were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. The dried and powdered plant material was Soxhlet extracted with water and ethyl alcohol (99.9%) in the ratio of 30:70. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered and concentrated at 48˚C by keeping on a water bath and weight of residue was recorded. The percentage yield of hydroalcoholic extract was found to be 42.8%. The collected extract was stored in a sterile container for further use.

Experimental animals

Female Wistar rats (100-150g) were purchased from AIIMS, New Delhi and maintained in animal house under standard conditions: temperature (24 ± 1◦C), relative humidity (45-50%), 12 hrs (light) and 12 hrs (dark) cycle and fed with standard food pellets and water ad libitum. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. The study was approved by Institutional Animal Ethics Committee (Registration No.-1149/PO/ERe/07/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

Acute toxicity studies

The acute toxicity of the extract was studied in adult female Wistar rats as per OECD guideline no. 425. They were divided into five groups each consisting of five rats. The suspension of the extract was administered orally at four different doses of 500, 1000, 2000 and 4000 mg/kg, respectively, to different groups of rats separately. Control animals received 10 ml/kg of distilled water orally. The animals were observed continuously for the initial 4 hrs for behavioral changes and mortality and intermittently for the next 6 hrs and then again at 24 hrs and 48 hrs after dosing. The behavior parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration [5].

Preliminary phytochemical screening of *C.decidua*

The hydroalcoholic extract of *C.decidua* was analyzed for the presence of pharmacologically active constituents such as phenols, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and carbohydrates.

Freund's complete adjuvant-induced arthritis

Arthritis was be induced to all the groups of animals except normal control group by single intra-dermal injection of 0.1 mL of Freund's Complete Adjuvant (FCA) containing 1 mg.mL-1 Mycobacterium tuberculosis H37Ra suspension in sterile paraffin oil into a foot pad of the left hind paw of female rats. The rats were anesthetized with ether inhalation prior to and during adjuvant injection, as the very viscous nature of the adjuvant exerts difficulty while injecting. Treatment with hydroalcoholic extract of *C.decidua*, Diclofenac and normal control (Distilled water) was started on the 14th day after arthritis induction and continued for 28 days. The paw volume of all the animal groups was measured by plethysmograph at 1, 4, 10, 14, 17, 21, 24 and 28 after the injection of Freund's complete adjuvant [6].

The animals were divided into six groups consisting of six animals per group

Group I: Normal control group (distilled water 1 ml/Kg p.o) (non-arthritic), (n=6)

Group II: FCA injected arthritic control; (n=6)

Group III: Arthritic animals treated with Diclofenac Sodium (5 mg/kg/day), (n=6)

Group IV: Arthritic animals treated with hydroalcoholic extracts of *C.decidua* (100 mg/kg body weight/day p.o), (n=6)

Group V: Arthritic animals treated with hydroalcoholic extracts of *C.decidua* (200 mg/kg body weight/day p.o), (n=6)

Group VI: Per se group (normal group where only plant extract with 200 mg/kg will be administered p.o).

Anti-arthritic effect of hydroalcoholic extract of *C.decidua* was evaluated on arthritic score, anti-nociceptive activity, motor incoordination test, and joint diameter on following days 1, 4, 10, 14, 17, 21, 24 and 28. On day 28 the animals were anesthetized with ether and the blood was withdrawn by tail vein for the estimation of various biochemical parameters in rats.

Arthritic score

The morphological feature of the arthritis like redness, swelling and erythema will be monitored by set visual criteria as follows: normal paw= 0, mild swelling and erythema of digits = 1, swelling and erythema of the digits = 2, severe swelling and erythema = 3, gross deformity and inability to use the limb = 4 on respective days [7].

Anti-nociceptive activity

The apparatus consists of a hot plate on which the rats will be placed for testing (Eddy's Hot Plate Method). Pain threshold will be determined by the latency for nociceptive response (withdrawal of any paw) with a maximum cut-off time 15 sec for all groups [8].

Motor incoordination test

Motor incoordination will be evaluated by Rota-rod apparatus. Rats will be placed on the rotating rod of device for 1 min. The time taken for the falling of rats from the roller, during the period of 1 min will be recorded [8].

Measurement of joint diameter

Joint diameter was measured using a digital Vernier caliper (Mitutoyo, Japan) on day 0 before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28 [20]. The change in joint diameter was calculated as the difference between the final and initial joint diameter [9].

Biochemical parameters

On day 28, blood of the rats was withdrawn by tail vein and serum was used for the estimation of serum AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase) and total protein levels.

Statistical Analysis

The data were represented as a mean ± standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test where P<0.001 was considered statistically significant using Graph Pad Prism version 5.03 software.

Results

Preliminary phytochemical studies

Preliminary qualitative phytochemical analysis of hydroalcoholic extract of *C.decidua* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates.

Acute toxicity studies

The oral administration of hydroalcoholic extract of *C.decidua* did not provoke any gross behavioral changes or manifestations of toxic symptoms such as increased or decreased motor activity, loss of right reflex, ataxia, clonic convulsions, muscle relaxation spasticity, tremors, tonic extensions, lacrimation, salivation, weight loss, watery diarrhea, writhing and urination over a period of 48 h. The hydroalcoholic extract of *C.decidua* was found to be non-lethal even at the maximum single dose of 4.0 g/kg. The dose of hydroalcoholic extract of *C.decidua* was selected on this basis and as per the earlier studies conducted by Goyal et al. [10] where 100 mg/kg and 200 mg/kg of *C.decidua* showed significant results (P<0.05) without any toxic effects at these doses.

Effect on arthritic score

All the groups of animals administered with FCA started showing signs of clinical inflammation i.e. swelling and rigidity in one or more hind paws. The first manifestation of disease was erythema of one or more ankle joints followed by involvement of the metatarsal and interphalangeal joints. There was an initial development in the manifestations of inflammation from day 1 of administration to day 14, followed by a brief decrease in the inflammatory signs from day 14 to 28. A dose dependent decrease in inflammation was seen at *C.decidua* (200mg/kg & per se group; P<0.001), 100 mg/kg (P<0.01) and diclofenac treated group from day 14 to day 28 as compared to FCA treated group (Figure 1).

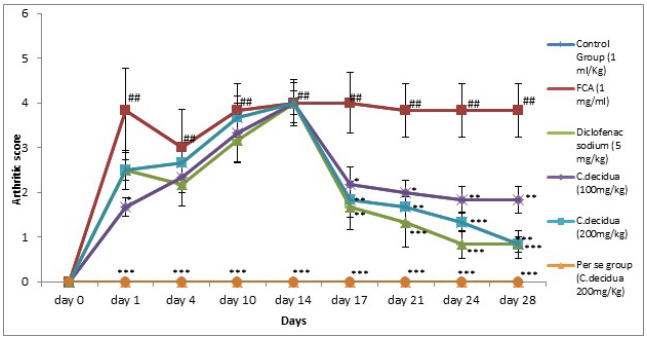

Figure 1: Effect of *Capparis decidua* on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

Effect on nociceptive threshold

There was consistent decrease in paw withdrawal threshold observed in FCA group rats compared to control animals and pain threshold was observed to be lowest on day 28. *C.decidua* treated (100 and 200 mg/kg), diclofenac treated group significantly (P<0.001) increased the pain threshold from day 14 to day 28, whereas per se group also showed significant (P<0.001) result in the pain threshold reponse as compared to FCA group animals (Figure 2).

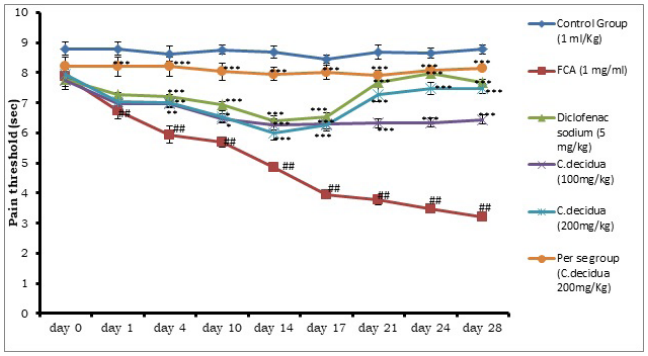

Figure 2: Effect of *Capparis decidua* on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

Effect on fall off time

Average fall off time in rota rod test was determined for the assessment of motor in-coordination. Administration of FCA

results in the decrease in fall off time in the FCA treated group as compared to the control group. *C.decidua* treated (100 and 200 mg/kg), significantly (P<0.001) increased fall off time from day 14 till day 28 as compared to the FCA control group while diclofenac (5 mg/kg) treated group also showed significant (P<0.001) increase in fall off time but lesser than *C.decidua* (200mg/kg) as compared to FCA group animals (Figure 3).

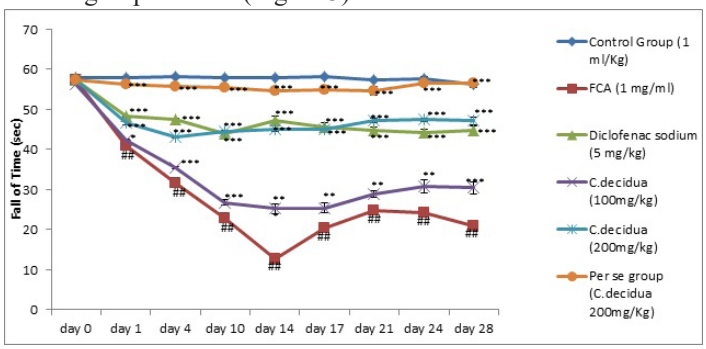

Figure 3: Effect of *Capparis decidua* on fall of time in motor incoordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

Effect on paw joint diameter

There was significant (P<0.001) increase in joint diameter of rats of all the groups from day 1 till day 14 treated with FCA compared to control group. *C.decidua* (100 and 200 mg/kg) significantly (P<0.01 and P<0.001, respectively), decreased the joint diameter from day 14 till day 28 as compared to FCA group. Diclofenac (5 mg/kg) treated group also showed significant reduction in paw diameter as compared to FCA group rats (Figure 4).

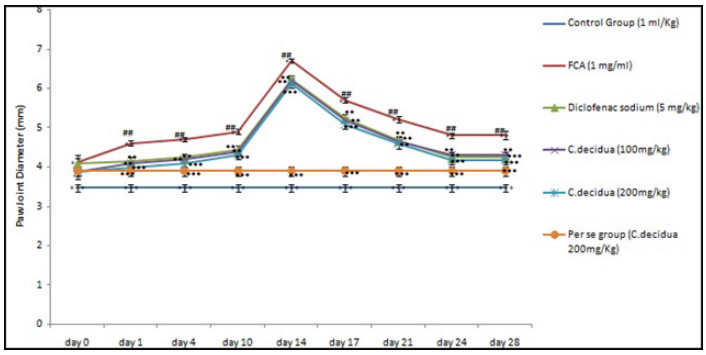

Figure 4: Effect of *Capparis decidua* on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA. **P<0.01 as compared to FCA. *P<0.05 as compared to FCA.

Effect on biochemical parameters

As a result of FCA-induced arthritis, the serum levels of AST, ALT and ALP were increased significantly (P<0.001) and total protein level was decreased significantly (P<0.001) in FCA group. These enzyme levels were altered by treatment with *C.decidua* (100 and 200 mg/kg), and diclofenac (5 mg/kg) group. The level of AST, ALT and ALP were significantly (P<0.001) decreased by treatment with *C.decidua* (100 and 200 mg/kg), and diclofenac 5 mg/kg and the level of total protein was significantly (P<0.001) increased in *C.decidua* (100 mg/kg; P<0.01, 200mg/kg; P<0.001) and diclofenac group (P<0.001) as compared to FCA group (Figures 5-8).

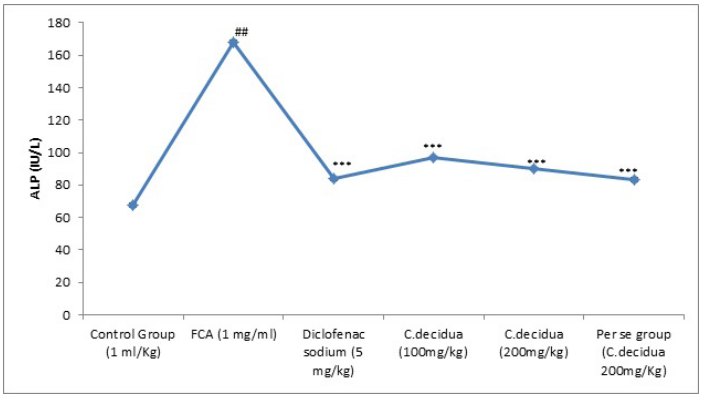

Figure 5: Effect of *Capparis decidua* on ALP (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

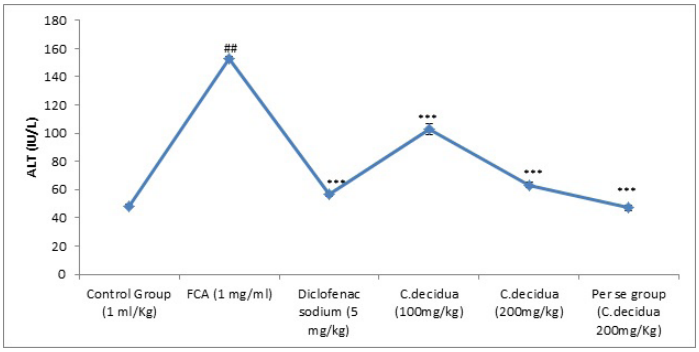

Figure 6: Effect of *Capparis decidua* on ALT (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

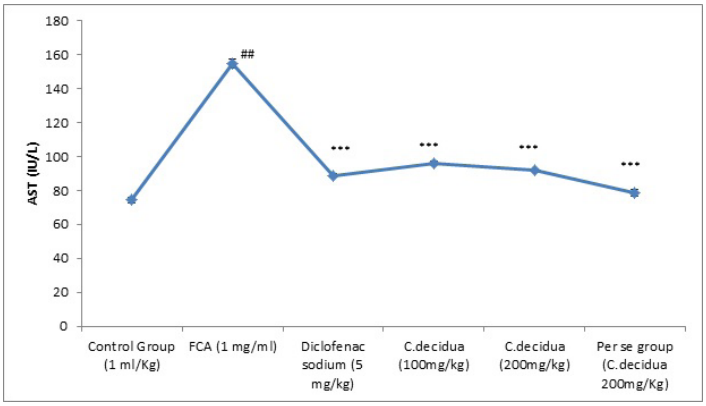

Figure 7: Effect of *Capparis decidua* on AST (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

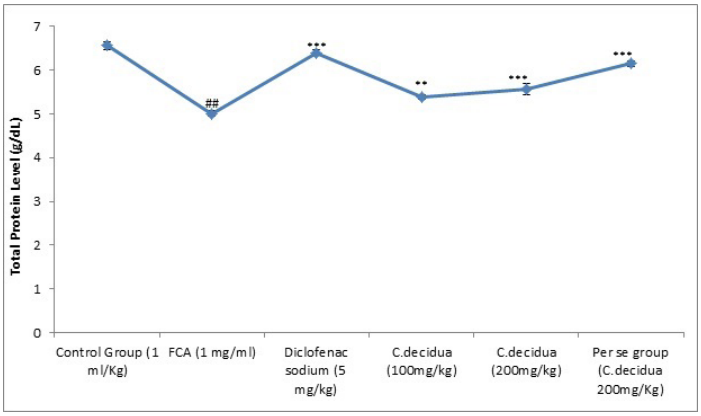

Figure 8: Effect of *Capparis decidua* on Total Protein Level (g/dL) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

Discussions

In the present study, anti-arthritic effect of *C.decidua* was additionally affirmed by Freund's Complete Adjuvant arthritis in rats. The FCA model is an entrenched rat model to study the inflammation [11]. FCA comprises of inactivated and dried mycobacterium, which adequately fortifies cell intervened insusceptibility and eventually drives the immunoglobulin generation and further creation of prostaglandins. The diclofenac, a non-steroidal anti-inflammatory drug was utilized for examination since it is usually recommended for the treatment of joint inflammation and its activity is primarily through the hindrance of cyclooxygenase and prostaglandin creation [12,13]. In the present investigation diclofenac sodium kept the spread of adjuvant instigated joint pain which is predictable with past reports of different scientists [14,15]. Acute toxicity study uncovered the non-poisonous nature of the extract at the dose of 4000 mg/kg. In the present examination, hydroalcoholic extract of *C.decidua* (100 and 200 mg/kg) treatment showed anti-arthritic effect in all the arthritic parameters. It significantly decreased the inflammation compared to the FCA group as observed by decreased paw joint diameter (Figure 4) and arthritic score (Figure 1). The present study revealed that paw joint diameter increases with ankle stiffness in FCA subjected rats. The analgesic effect of *C.decidua* (100 and 200 mg/kg) in rats with FCA induced arthritis is also marked as evident by the increase in pain threshold (Figure 2). Muscle grip strength of FCA group rats markedly reduced and in *C.decidua* (100 and 200 mg/kg) treated groups the fall of time in motor incoordination test (Figure 3) significantly increased suggesting the antiarthritic activity of hydroalcoholic etract of *C.decidua*.

In the present study, the single intradermal injection with FCA (0.1 mL) significantly (P<0.001) elevated the serum ALP, AST and ALT level and decreased the total protein level. Evaluation of the serum levels of ALP, AST and ALT provides an excellent and simple tool to measure the anti-arthritic activity of the drug. The activities of aminotransferases and alkaline phosphatase rises significantly in arthritic rats, since these are good markers of liver and kidney disorders which is also considered a feature of adjuvant arthritis. Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as kinins in inflammatory process [16]. The administration of *C.decidua* (100 and 200 mg/kg) hydroalcoholic extract significantly (P<0.001) decreased the level of ALP, AST and ALT and increased the level of total protein that confirms the anti-arthritic activity of the extract.

The anti-arthritic effect of *C.decidua* hydroalcoholic extract set up in this investigation could be ascribed to the nearness of flavonoids, triterpenoid, saponins, tannins and steroids detected after phytochemical screening of the *C.decidua*. Triterpenoids are known to repress histamine discharge from mast cells and exert anti-inflammatory effects. Non-specific anti-arthritic activity might be because of the consolidated impact of the distinctive phytoconstituents display.

Conclusion

The present study confirms the anti-arthritic activity of hydroalcoholic extract of *C.decidua* which is mediated by its antinociceptive effect, anti-inflammatory effect, muscle grip strength and improvement in biochemical parameters. Be that as it may, additionally studies are needed to identify the possible phytoconstituent(s) responsible for the activity, which would be of use in age-related diseases like arthritis.

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Effect of Hydroalcoholic Extract of *Capparis decidua* (Forssk.) Edgew on Sexual Behavior of Male Rats

Prashant Kumar Dhakad[1,*], Pramod Kumar Sharma[1], Sokindra Kumar[2]

[1]Department of Pharmacy, Galgotias University, Greater Noida, 201310, Uttar Pradesh, India
[2]R.V. Northland Institute (RVNI), Chithera, Dadri, Greater Noida-II, 203207, Uttar Pradesh, India

Abstract *Capparis decidua* has traditionally been recommended as an aphrodisiac agent. Although, it's medicinal use has not been scientifically proved. The present study therefore evaluated the effects of hydroalcoholic extract of *C. decidua* roots, stem and leaf on sexual behavior in normal male rats. The hydroalcoholic extract of *C. decidua* was administered (100 mg/kg, 200mg/kg) to different groups of male wistar rats for 28 days. The female rats were brought in estrous phase before the mating in each group of male rats. Standard group was administered sildenafil citrate at 5mg/kg orally for 28 days. Control group was administered only distilled water. All the experimental groups i.e *C. decidua* 100mg/kg, 200 mg/kg, per se group (only *C. decidua* 200 mg/kg), standard group(sildenafil citrate 5mg/kg) were compared with the control group for parameters evaluated for sexual behavior study. *C. decidua* at 100mg/kg, 200 mg/kg, and per se treated groups produced significant arousal of sexual activity in male rats. They significantly increased the frequencies of mount, intromission frequency. The mount latency and intromission latency were significantly reduced but ejaculation latency was prolonged significantly. The results indicated that hydroalcoholic extract of *C. decidua* enhanced sexual behaviour in male rats. The enhanced sexual behavior in male rats at the doses of 100 and 200 mg/kg body weight may be due to the presence of phytochemicals like alkaloids, saponins, flavonoids as these phytochemicals has role in increasing sexual strength, androgen enhancing, and antioxidant activity.

Keywords Aphrodisiac, *Capparis decidua*, Sexual Behavior, Sildenafil Citrate, Phytochemicals

1. Introduction

Sexual intercourse holds importance in biological and social relationships in human life. Male erectile dysfunction influences not only sexual behavior, but also quality of life. Erectile dysfunction also called as "impotence", is the repeated failure to get or keep up a sufficiently firm erection of penis to allow coitus[1]. The ascent in populace in developing countries is overwhelming and this strengthens the requirement for viable anti-conception medication measures. The synthetic drugs available in market for birth control show severe side effects like hormonal imbalance, increased blood pressure, and weight gain and increased risk of cancer. Subsequently, it is necessary to change these drugs by safe and effective plant-origin drugs [2]. Caper is a perennial shrub and is the common name of the Capparis genus belonging to Capparidaceae family. This genus contains several plant species (about 250) [3]. *Capparis decidua* (Forsk.) Edgew. (Family: Capparidaceae) is a xerophytic shrub, discovered broadly in the western parts of Pakistan, India and a portion of the Asian nations[4]. It is broadly distributed in surrendered dry terrains presented to extraordinary radiations where yearly temperatures run from 18 to 48 ˚C. It is a little bush reaching up to height of 5 m and possess dense and thin branches [5]. In light of its xerophytic versatile nature this plants develops well under the brutal climatic states of parched locales. Roots of *C. decidua* have been utilized as an aphrodisiac agent by traditional people in dry regions of Thar deserts [6]. The dried fruits are used as an ingredient in diabetes treating medications and inexperienced green berries are utilized in meals preparations such as pickles owing to the historical perception that it possesses medicinal properties [4]. The roots, flowers, and fruits of *C. decidua* have been in use on the grounds that it acts against infectious diseases without any side effects. Roots acts as carminative, thermogenic, expectorant, anodyne, anthelmintic, sudorific, stimulant, digestive, antibacterial, and useful in arthritis, constipation, dysmenorrhoea, odontalgia, dyspepsia, amenorrhoea and lumbago [6]. Enough phytochemical work has been done as such far on *C. decidua*, which has been accounted for containing β-sitosterols, aliphatic constituents, indoles, isocodonocarpine, oxygenated heterocyclic compounds,

diterpene alcohol, tannins, β-carotene, minerals, vitamin C and large quantities of alkaloids, e.g., stachydrine and spermidine alkaloids [2]. The aim of present work is to investigate the effect of hydroalcoholic extract of *C. decidua* on sexual behavior of male rats using multiple parameters like ejaculation latency, intromission latency, intromission frequency, mount latency, mount frequency.

2. Material and Methods

2.1. Plant Material and Extraction

The complete plant of *Capparis decidua* was collected fresh from Jaipur, Rajasthan, India. The plant was taxonomically identified and authenticated by Prof. Kailash Agrawal, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited at the herbarium of the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India (R.No.-RUBL 211645). *C. decidua* root, stem and leaves were washed with tap water followed by distilled water and then cut and dried under the shade. The dried plant parts were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. The dried and powdered plant material was Soxhlet extracted with water and ethyl alcohol (99.9%) in the ratio of 30:70. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered and concentrated at 48˚C by keeping on a water bath and weight of residue was recorded. The percentage yield of hydroalcoholic extract was found to be 42.8%. The collected extract was stored in a sterile container for further use.

2.2. Experimental Animals

Healthy Albino Wistar rats of both sexes weighing 150-250 g were obtained from Central Animal Facility AIIMS New Delhi. The experimental protocol was approved by Institutional Animal Ethics Committee CPCSEA No. - 1149/PO/ERe/07/CPCSEA. Animals were housed under standard conditions of temperature (24±2˚C) and relative humidity (30%-70%) with 12:12 light: dark cycle. The animals were given standard pellet diet and water ad libitum.

2.3. Chemicals

The hydroalcoholic extract of *C. decidua* was dissolved in the distilled water and orally administered to the test groups. Sildenafil citrate was procured from the Cadila Pharmaceuticals Limited, Ahmadabad, Gujarat, India as a generous gift. Ethinyl oestradiol and progesterone were purchased from Sigma Chemical Co. ,USA).

2.4. Phytochemical Screening

The hydroalcoholic extract of *C. decidua* was analyzed for the presence of pharmacologically active constituents such as phenols, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and carbohydrates [7]. Phytochemical results have been shown in Table 1.

2.5. Animal Groups and Extract Administration

Thirty male rats were randomly divided into five groups of 6 rats each and were orally administered the following: Group 1 (control), 10 ml/kg of distilled water orally in sexually active male rats; group 2 (standard), suspension of sildenafil citrate 5 mg/kg body weight orally in sexually sluggish male rats; groups 3 and 4, received suspension of the hydroalcoholic extract of *C. decidua* at 100, 200 mg/kg body weight orally in sexually sluggish male rats; group 5 (Per se) received suspension of the hydroalcoholic extract of *C. decidua* at 200 mg/kg body weight orally only to note the effect of extract only in sexually active male rats. Oral administration was carried out using a metal oropharyngeal cannula. Five rats in each group were monitored for sexual behavior after their daily doses on days 0, 7, 14, 21 28. The experiments on animals were conducted in accordance with the principles of Institutional Animal Ethical Committee.

2.6. Acute Toxicity Studies

The acute toxicity of the extract was studied in adult male wistar rats. They were divided into five groups each consisting of five rats. The suspension of the extract was administered orally at four different doses of 500, 1000, 2000 and 4000 mg/kg, respectively, to different groups of rats separately. Control animals received 10 ml/kg of distilled water orally. The animals were observed continuously for the initial 4 h for behavioral changes and mortality and intermittently for the next 6 h and then again at 24 h and 48 h after dosing. The behavior parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration [8].

2.7. Sexual Behavior Study

Prior to the drug treatment, the male rats were trained separately with normal adult female rat for sexual experience. At that point, the male rats were partitioned into sexually active and sexually inactive groups, in view of their copulatory conduct. A male was considered sexually dynamic when it endeavored to mount any female brought into the cage. The normal mountings in ordinary male rats were observed to be 4-10 of every 5 min. The animal showing below 4 mounts was considered as inactive. Sexually inactive male rats were selected for extract treated groups and standard group in the present study. The female

sexual behavior is restricted to the estrous phase, that agrees with ovulation and during this time animal is said to be in heat. The estrous female stirs sexual enthusiasm for male rat by physical changes in the genital district and the creation of pheromones. These are sexual fragrances found in rats that deliver sensational sex-chasing conduct in rats. The female rats react to each mount with a lordosis reaction. This reaction happens when the female is responsive to mounting male and comprises of an angling of the back to a curved position with deviation along the side and the neck extended. The female rat with estrous cycle was affirmed by vaginal spread technique. A dropper with a drop of distilled water was brought into the rats's vagina and the discharges were gathered and were seen under microscope. Estrous cycle was affirmed when half or a greater amount of the cells were cornified. The sexually inactive male rats were divided into three groups and each contains five animals while control group and per se group includes sexually active male rats. Standard group (sildenafil citrate at 5 mg/kg p.o), hydroalcoholic extract of *C. decidua* at a dose of 100mg/kg (p.o.) group and hydroalcoholic extract of *C. decidua* at a dose of 200mg/kg (p.o.) treated groups includes the sexually inactive rats. The female animals were artificially brought into oestrus (heat) as the female rats allow mating only during the estrus phase. They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The exceedingly responsive female (in estrous stage) was brought into the home cage of the male rats and the accompanying male sexual behavioral parameters were recorded amid a time of 30 min: Latency (time) of first mount, number of mounts, latency of first intromission, number of intromission, latency of ejaculation (time from intromission to ejaculation), number of ejaculations. All the groups were tested for copulatory behavior on 0, 7th, 14th, 21st and 28th days [9].

2.8. Statistical Analysis

The data were represented as a mean ± standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P<0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software.

3. Results

3.1. Preliminary Qualitative Phytochemical Screening

Preliminary qualitative phytochemical analysis of hydroalcoholic extract of *C. decidua* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates (Table 1).

3.2. Effect of Acute Toxicity Studies

The oral administration of hydroalcoholic extract of *C. decidua* did not provoke any gross behavioral changes or manifestations of toxic symptoms such as increased or decreased motor activity,loss of right reflex,ataxia, clonic convulsions,muscle relaxation spasticity, tremors, tonic extensions, lacrimation, salivation, weight loss, watery diarrhea, writhing and urination over a period of 48 h. Hydroalcoholic extract of *C. decidua* was found to be non-lethal even at the maximum single dose of 4.0 g/kg. Dose of hydroalcoholic extract of *C. decidua* was selected on this basis and as per the earlier studies conducted by goyal et al 2009 [2] where 100 mg/kg and 200 mg/kg of *C. decidua* showed significant results ($P<0.05$) without any toxic effects at these doses.

3.3. Effect of Sexual Behavior Study

The observations of sexual behavior are presented in Table 2-6. Treatment with hydroalcoholic extract of *C. decidua* at different doses influenced the behavior of the treated animals in a dose-dependent manner. All the experimental groups significantly affected sexual behavior as compared with the control. *C. decidua* extract at the dose of 200 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.05$) on day 21 and 28, Intromission Frequency (IF) on day 21($P < 0.05$) and day 28 ($P < 0.01$), Ejaculatory Latency (EL) on day 21 ($P < 0.01$) and day 28 ($P < 0.01$) and caused significant reduction in the Mounting Latency (ML) ($P < 0.01$) on day 28, Intromission Latency (IL) ($P < 0.01$) on day 21 and 28, as compared to control group. The dose of 200 mg/kg of the extract significantly increased the MF on day 14 ($P < 0.05$), day 21($P < 0.01$), day 28 ($P < 0.001$); IF on day 14 ($P < 0.05$), day 21 and 28 ($P < 0.001$); EL on day 7 (($P < 0.01$), day 14($P < 0.01$), day 21($P < 0.001$), day 28($P < 0.001$) and significantly decreased the ML on day 7($P < 0.01$), day 14($P < 0.01$), day 21($P < 0.001$), day 28($P < 0.001$); IL on day 7($P < 0.01$), day 14($P < 0.05$), day 21($P < 0.001$), day 28($P < 0.001$), in comparison with the control group. Per se group showed significant increase in MF on day 14($P < 0.05$), day 21($P < 0.01$), day 28($P < 0.01$); IF on day 28 ($P < 0.05$); EL on day 14 ($P < 0.05$), day 21($P < 0.001$), day 28($P < 0.001$) and significantly decreased the ML on day 7($P < 0.05$), day 21($P < 0.001$), day 28($P < 0.001$); IL on day 7($P < 0.01$), day 21($P < 0.001$), day 28($P < 0.001$) as compared to control group. Standard drug treated group gave highly significant increase in MF on day 7($P < 0.01$), day 14($P < 0.001$), day 21($P < 0.001$), day 28($P < 0.001$); IF on day 7 ($P < 0.05$), day 14($P < 0.001$), day 21($P < 0.001$), day 28($P < 0.001$); EL on day 7, 14, 21 and 28 ($P < 0.001$) and significant decrease in ML and IL on day 7, 14, 21 and 28 ($P < 0.001$) as compared to control.

Table1. Representation of result of phytochemical studies in the hydroalcoholic extract of *C. decidua*

S.No	Chemical test	Phytochemicals present
1	Test for phenolic compounds- *C. decidua* extract dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. The appearance of bluish black color indicated the presence of phenolic compounds.	Showed the presence of phenolic compounds
2	Test for flavonoids- Few drops of dilute sodium hydroxide solution were added to the *C. decidua* extract (0.5 ml) to give intense yellow color which disappears after addition of dilute hydrochloride acid showed the presence of flavonoids.	Showed the presence of flavonoids
3	Test for terpenoids- The extract (0.5 mg) of *C. decidua* was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Formation of the reddish-brown ring at the interface indicated the presence of terpenoids.	Showed the presence of terpenoids
4	Test for saponins- *C. decidua* extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min using hands. Formation of 2 cm layer of foam layer indicated the presence of saponins.	Showed the presence of saponins
5	Test for alkaloids- About 50 mg of *C. decidua* extract was shaken with few ml of dilute hydrochloric acid and filtered. Few drops of Wagner's reagent were added to the side of the test tube. The appearance of reddish-brown precipitate indicated the presence of alkaloids.	Showed the presence of alkaloids
6	Test for cardiac glycosides- *C. decidua* extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface is a feature of cardenolide deoxy sugar and appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.	Showed the presence of cardiac glycosides
7	Test for steroids- *C. decidua* extract (1 gm) was dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The color of the upper layer changed to red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.	Showed the presence of steroids
8	Test for carbohydrates- Molisch test: To 2–3 ml of the aqueous. *C. decidua* extracts added two drops of alpha-naphthol solution in alcohol, shaken and added conc. H2SO4 from the sides of the test tube. Violet ring was formed	Showed the presence of carbohydrates
9	Test for tannins- *C. decidua* extract (1 gm) dissolved in water in a test tube and diluted with chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully to the side of the test tube to the solution. A green color was formed which showed the presence of tannins	Showed the presence of tannins

Table 2. Effect of hydroalcoholic extract of *C. decidua* on mounting frequency in male rats

Treatment Groups	Mounting frequency (MF)				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	13.5±0.88	13.5±0.88	13±0.73	13.83±1.08	14.66±0.95
Sildenafil citrate (5 mg/Kg)	19.5±0.67	20±0.57**	20.5±0.67***	21.16±0.70***	22.5±0.76***
C. decidua (100mg/kg)	14.83±1.08	15.16±0.75	15.83±0.87	18.16±0.47*	18.83±0.47*
C. decidua (200mg/kg)	16.83±1.54	17.16±1.66	18±1.85*	19.33±1.41**	21±1.21***
Per se group (*C. decidua* 200mg/Kg)	18±1.18	18±1.18	18.5±0.92*	19±0.77**	19.5±0.76**

Tabular values are expressed as mean ± SEM, n = 6 (number of animals in each group); significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P <0.01; ***P < 0.001.

Table 3. Effect of hydroalcoholic extract of *C. decidua* on mounting latency in male rats

Treatment Groups	Mounting latency (ML)				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	135.96±2.93	130.16±2.75	116.25±2.05	110.45±2.20	93.36±1.37
Sildenafil citrate (5 mg/Kg)	109.14±4.06	97.28±2.43***	80.44±2.81***	75.67±1.42***	61.42±1.64***
C. decidua (100mg/kg)	144.09±2.96	124.72±2.8	111.57±4.78	98.67±1.65	84.82±1.71**
C. decidua (200mg/kg)	118.84±2.67	110.45±3.49**	99.17±3.22**	84.29±2.37***	70.63±1.48***
Per se group (*C. decidua* 200mg/Kg)	120.16±3.09	113.50±4.13*	105.39±2.80	87.32±2.39***	77.14±1.20***

Tabular values are expressed as mean ± SEM, n = 6 (number of animals in each group); significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P <0.01; ***P < 0.001.

Table 4. Effect of hydroalcoholic extract of *C. decidua* on Intromission latency in male rats

Treatment Groups	Intromission latency(IL)				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	146.74±2.80	140.30±2.64	126.11±2.03	120.39±2.18	103.08±1.37
Sildenafil citrate (5 mg/Kg)	116.97±3.79	107.53±2.52***	87.70±2.40***	86.06±1.30***	71.68±1.71***
C. decidua (100mg/kg)	154.17±2.98	133.33±2.73	122.84±4.60	108.94±1.95**	94.84±1.68**
C. decidua (200mg/kg)	129.34±2.84	121.99±3.33**	109.09±3.26*	94.36±2.31***	80.64±1.45***
Per se group (*C. decidua* 200mg/Kg)	129.74±2.66	123.39±4.18**	113.79±3.69	98.78±2.53***	87.14±1.18***

Tabular values are expressed as mean ± SEM, n = 6 (number of animals in each group); significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P <0.01; ***P < 0.001.

Table 5. Effect of hydroalcoholic extract of *C. decidua* on Intromission frequency in male rats

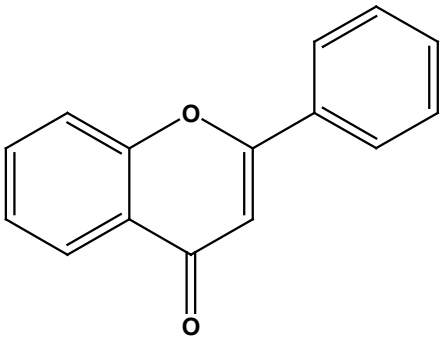
Treatment Groups	Intromission frequency (IF)				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	9.5±0.99	11.16±0.54	11.16±0.60	11.66±0.84	11.16±0.65
Sildenafil citrate (5 mg/Kg)	14.16±1.17	15.16±0.87*	17.66±0.84***	19.83±1.30***	21.66±1.61***
C. decidua (100mg/kg)	10.83±0.60	11.16±0.70	13.83±1.45	16.16±0.70*	16.5±0.76**
C. decidua (200mg/kg)	11.66±1.20	13.33±0.66	15.33±1.02*	17.5±0.67***	19.5±0.76***
Per se group (*C. decidua* 200mg/Kg)	10.16±1.01	11.33±1.14	12.16±0.83	12.83±0.83	15.33±0.66*

Tabular values are expressed as mean ± SEM, n = 6 (number of animals in each group); significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P <0.01; ***P < 0.001.

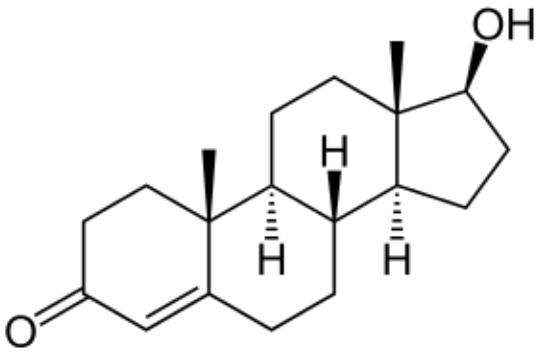
Table 6. Effect of hydroalcoholic extract of *C. decidua* on Ejaculation latency in male rats

Treatment Groups	Ejaculation latency (EL)				
	day 0	day 7	day 14	day 21	day 28
Control Group (1 ml/Kg)	176.16±3.63	177.66±4.82	180.5±4.08	187.66±3.98	197.33±4.01
Sildenafil citrate (5 mg/Kg)	261.83±18.62	265.5±19.39***	270.33±20.80***	289.83±9.47***	299.16±9.05***
C. decidua (100mg/kg)	209.16±7.73	212.5±7.81	221±7.85	235±7.88**	243.83±7.05**
C. decidua (200mg/kg)	237.66±8.60	244±8.65**	253.16±8.68**	261.5±6.58***	274±7.02***
Per se group (*C. decidua* 200mg/Kg)	220.33±7.33	230.83±7.98	239.33±8.32**	249.16±10.31***	259.16±10.64***

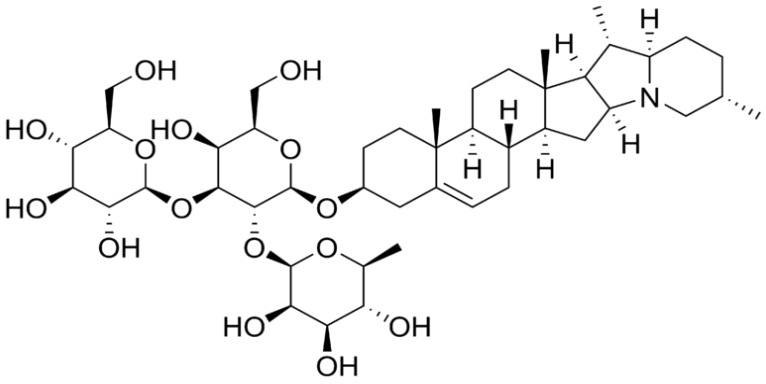
Tabular values are expressed as mean ± SEM, n = 6 (number of animals in each group); significant difference compared from control to extract treated groups and standard group. *P < 0.05, **P <0.01; ***P < 0.001.



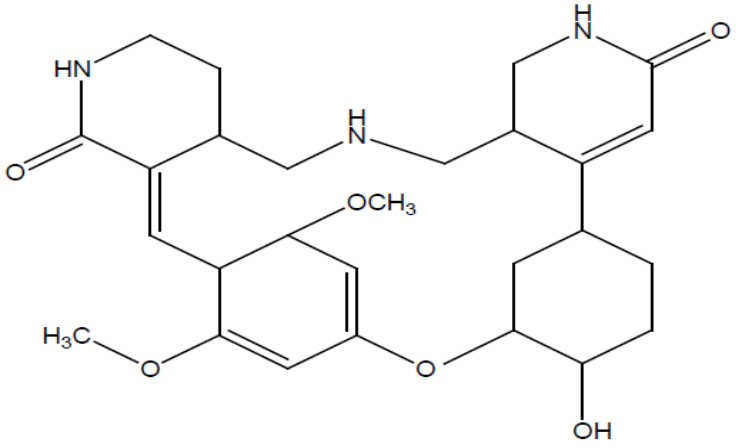
(A) Flavonoid



(B) Steroid



(C) Saponin



(D) Capparisine alkaloid

Figure (A-D). Representation of possible phytoconstituents responsible for the aphrodisiac activity found in *C. decidua* plant

4. Discussions

In the present study, hydroalcoholic extract of *C. decidua* was tested in animal experimentation for its effect on sexual behaviour, and sildenafil citrate was used as the standard referent. Mating behaviour test revealed that the extract of *C. decidua* significantly increased the Mounting Frequency (MF) and Intromission Frequency (IF) as compared to control but less than that of the standard drug. The (MF) and (IF) are considered as the exponents of sexual desire and potency. So, it indicates that the test drug possesses a sexual function improving effect. The extract of *C. decidua* significantly increased the EL as compared to control group animals, whereas a highly significant increase was observed with the standard drug sildenafil citrate. The *C. decidua* extract was found to produce a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control while a highly significant decrease was found in ML of animals treated with sildenafil citrate. This is also an evidence of the sexual function improving effect of the *C. decidua* extract. The significant increase in the Ejaculatory Latency (EL) indicates that the extract and standard drug prolonged the duration of coitus. These findings demonstrate that the extract produces a striking improvement of general sexual execution of rats in experimental groups. Moreover the proceptive behaviours were seen in the animals like darting, hopping and lordosis by female rats and precopulatory behavior in male rats as well which implicates the sexual arousal between opposite sex rats. Mount Frequency and Intromission Frequency are useful factors of sexual strength, sexual desire and potency. The number of mount (MF) reflects sexual motivation, and rise in the number of intromission (IF) shows the efficiency of erection.Some of the medicinal plants are effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins [10].In the previous studies it is seen that Dehydroepiandrosterone (DHEA), a major circulating steroid (Figure B) in the plasma, and a common precursor for both androgens and estrogens and its subsequent conversions to testosterone and its metabolites responsible for the effective masculine behavior in rats [11].The involvement of saponins (Figure C) in the biosynthesis of DHEA boosts the level of testosterone and therefore triggers the sexual desire in male rats [12]. Steroidal nature of saponins makes it possible to act as intermediary in androgen synthesis where saponin binds to hormone receptor and undergoes conformational

change to yield androgen production. Similarly the flavonoids (Figure A) due to its antioxidant property alter androgen levels and are responsible for the enhanced male sexual behavior. Alkaloids (Figure D) are reported to have ergogenic properties act on central nervous system by causing vasodilation of the blood vessels through the production of endothelium dependent releasing factor i.e. nitric oxide and allowing erection or arouses steroidogenesis in the testes of the animals. Alkaloids also act on peripheral nervous system by relaxing Corpus cavernosum smooth muscle in the penis of the male rats[13].

In the previous studies ethanolic extract of rhizomes Curculigo orchioides evaluated for effect on sexual behavior in rats. 100 mg/kg of extract change significantly the sexual behavior pattern assessed by parameters such as mating performance, mount frequency and mount latency. The rhizome extract markedly affected sexual behavior of rats as seen in reduction of mount latency, an increase in mount frequency and enhanced attractability towards female rats [14].During the phytochemical screening of extract of *C. decidua* there was occurrence of flavonoids, alkaloids, saponins and many other phytochemicals. Thus, the resultant aphrodisiac activity of the test drug might be attributed to phytochemicals like flavonoids, alkaloids, saponins. Moreover, further research is also needed for the distinguishing proof of its dynamic constituents responsible for sexual function improving activities and the mechanism by which it increases sexual function.

5. Conclusions

Overall, our results have revealed that the hydroalcoholic extract of *C. decidua* at the doses of 100 and 200mg/kg body weight could be used as a stimulator of sexual behaviour in male rats. The present study thus supports the acclaimed aphrodisiac use of this plant in herbal medicine. The aphrodisiac effect of the plant extract may be due to the presence of alkaloids, saponins, flavonoids through a multitude of central and peripheral ways.

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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A Review on Phytochemical Studies and Biological Potential of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae)

Prashant Kumar Dhakad[1,*], Pramod Kumar Sharma[1], Sokindra Kumar[2]

[1]Department of Pharmacy, Galgotias University, India
[2]R.V. Northland Institute (RVNI), Chithera, Dadri, Greater Noida-II, India

Abstract Cucurbits are edible crops found in the Cucurbitaceae family. Interest in plant-based biological compounds has now awakened throughout the world and hence the literature data in this area is significant. The Cucurbitaceae family is distributed in the tropical and subtropical countries. The plants of this family are superb fruit crops rich in vitamins, nutrients, and minerals that very good for health. A number of plants belonging to this family have been reported so far and one among them is *Citrullus colocynthis (L.)* Schrad. All the parts of this plant (root, stem, leaf, fruits, and seeds) are utilized in the traditional system of medicine. A plethora of research is going on this plant species to discover new active moiety and to establish their medicinal importance. The present review gives updated information about the phytochemistry and pharmacological activities of *Citrullus colocynthis (L.)* Schrad. established so far. As per the research so far among different cucurbitacins, cucurbitacin E is known to be found profoundly in *Citrullus colocynthis (L.)* Schrad. Some of the biological activities reported for this plant include antioxidant activity, antimicrobial activity, anti-diabetic activity, anti-hyperlipidemic activity, analgesic activity, anti-ulcer activity, anticonvulsant activity and insecticidal activity.

Keywords Cucurbitaceae Family, *Citrullus colocynthis*, Traditional System, Phytochemistry, Biological Activities

1. Introduction

Cucurbitaceae family is one of the best genetically assorted accumulations of restorative plants in the plant kingdom. Most plants of this family are dry season tolerant, intolerant to wet, frost-sensitive and ineffectively drained soils [1]. In the course of the most recent two decades, India and China have been the biggest cucurbit makers took after by Russia, United States of America, Egypt and Republic of Iran. *Citrulluscolocynthis (L.)* Schrad. is a Cucurbitaceae family plant[1]. The plant is generally accessible in the Sahara and Arabian deserts, Sudan and a Southern piece of Asia including Pakistan, India and Southern Islands. The fruit is intense and globular with a smooth surface. It is hard and has a skin around it and contains 200–300 seeds/gourd. Seeds are small (6mm in length), ovoid, compressed, smooth and brownish when ripe. Seeds constitute about 75% of the weight of fruit of *Citrullus colocynthis* [2]. It is a non-tough, herbaceous lasting vine, extended from the base. The stems are precise and harsh; the leaves rough, 2-4 inches long, with 3-7 profound lobes; and solitary light yellow blossoms are found. Each plant generates 15-30 round fruits, with 3-4inches in diameter, green with undulate yellow stripes, getting to be noticeably yellow all finished when dry. Seeds are 1/4 inch or less long, smooth and caramel when riped [3]. In the Vedic literature, it is mentioned that "There is no man on this planet that is inept and there is no plant which has no medical use. Where everything is available, actually, a man to oversee them legitimately is rarely accessible [4]. Practically speaking, a plant is called restorative plant, when it is very used in the system of medicine [4]. Nowadays, therapeutic plants get attention to researchers because of their special significance in safety of humanity. The curative properties of therapeutic plants are predominantly because of the presence of different chemical constituents of various compositions which exists as secondary metabolites. A few dynamic synthetic constituents of C. colocynthis plant were surveyed. They are grouped as saponins, carbohydrates, tannins, glycosides, alkaloids, flavonoids and essential oils. Plant-based characteristic constituents can be obtained from any part of the plant like leaves, roots, flowers, stems, fruits, and seeds. Various plant secondary metabolites

including flavonoids and cucurbitacins have already been accounted for from C. colocynthis [5]. *Citrullus colocynthis* (L.) Schrad. has a wide range of therapeutic and nutritional uses. Traditionally this plant is used in the treatment of diseases like cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases.

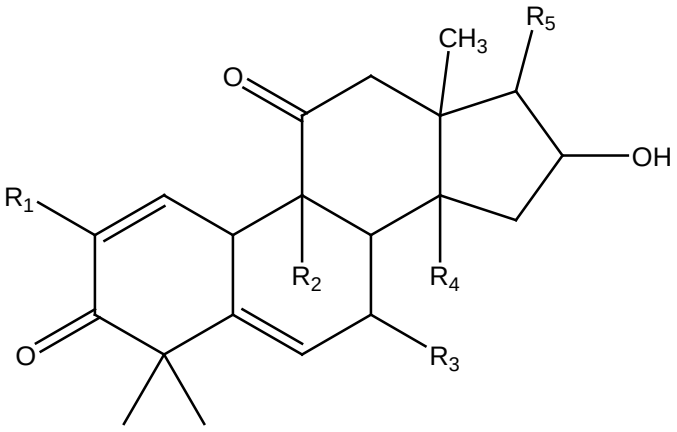
The data of phytochemical studies and biological activities of *Citrullus colocynthis* (L.) Schrad. plant parts were collected through authenticated sources like Google Scholar, PubMed, ScienceDirect etc.

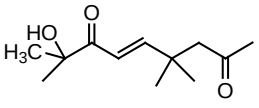
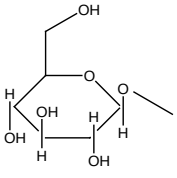
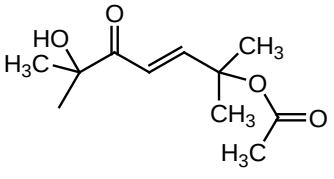
2. Phytochemical Studies

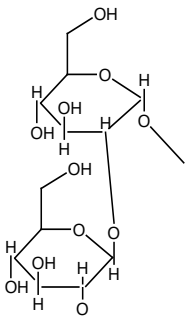
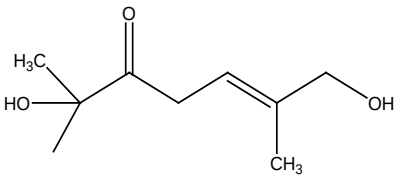
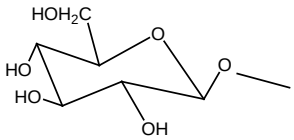
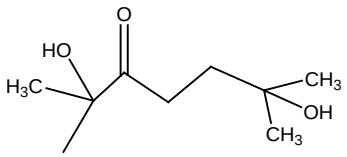
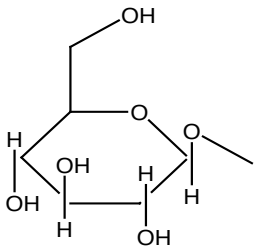
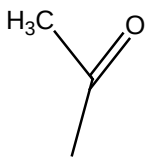
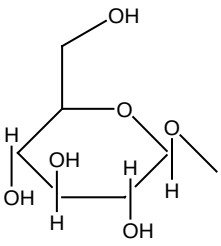
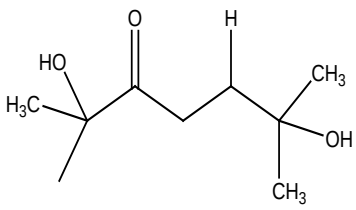
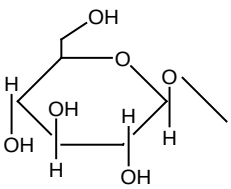
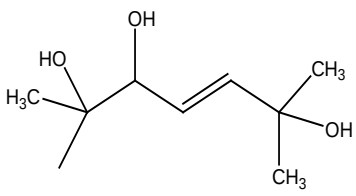
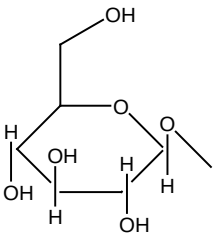
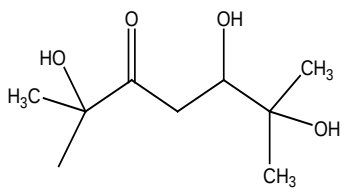
A few bioactive compounds of *Citrullus colocynthis (L.)* Schrad. fruit have been elucidated in the studies so far. They are included as carbohydrates, alkaloids, fatty acids, glycosides, flavonoids and essential oils. The cucurbitacins are prevalently found in the Cucurbitaceae family. As per chemical structures, cucurbitacins can be divided into 12 categories, yet all are not found in *Citrullus colocynthis (L.)* Schrad. Because of the cytotoxic conduct, cucurbitacins seem to assume an important part in medicate disclosure, especially in anticancer medication advancement. Among different cucurbitacins, cucurbitacin E (compound a) was discovered richly in *Citrulluscolocynthis (*L.) Schrad. fruit pulp. Colocynthoside A (compound b) and colocynthoside B (compound c), were isolated from the methanolic concentrate of the fruits. Different cucurbitacins secluded from the butanol part were, cucurbitacin L 2-O-β-D-glucopyranoside (compound d), hexanocucurbitacin I 2-O-β-D glucopyranoside (compound e), cucurbitacin K 2-O-β-D glucopyranoside (compound f) and khekadaengoside E (compound g), cucurbitacin J 2-O-β-D glucopyranoside (compound h), cucurbitacin I 2-O-β-D glucopyranoside (compound i). Some flavonoid glycosides e.g., isoorientin 30-O-methyl ether (compound j), isovitexin (compound k) &isosaponarin (compound l)and two cucurbitacin glycosides e.g. 2-O-β-D-glucopyranosyl cucurbitacin L & 2-O-β-D-glucopyranosyl cucurbitacin I were also identified in butanol fraction of the methanolic extract of *Citrullus colocynthis (L.)*Schrad. fruits. The major fatty acids found in *Citrullus colocynthis (L.)* Schrad. seed oil includes palmitic (8.1–17.3%) and stearic acids (6.1–10.5%) that form principal saturated fatty acids of this oil Linoleic and oleic acids are the primary monounsaturated fats, and this high content of linoleic acid (50.6–60.1%) in seed oil, which is an essential unsaturated fat, makes this oil restoratively profitable. The unsaturated fat profile of the seed oil uncovers that it falls in the class of linoleic-oleic acid oils and closely resembles a few other vegetable oils. In this way, the *Citrullus colocynthis (L.)* Schrad. oil, similar to some other cucurbit seed oils, is probably going to have potential uses as a cooking oil. Previous studies reported that the seed oil composition of this plant was like that of safflower oil, with an aggregate of 80– 85% unsaturated fats. An investigation detailing the physical-compound portrayal and the fatty acid composition of the fixed oil of the seeds revealed that it is a decent wellspring of characteristic cancer prevention agents like uncovered that it is a decent wellspring of characteristic cancer prevention agents like natural antioxidants e.g., α-tocopherol, γ-tocopherol and β-carotene with respective composition of 45.1, 435 and 0.18 mg/kg. Many examinations revealed the presence of alkaloids in the *Citrullus coloccynthis* fruits however just a couple of reports are accessible on the isolation and identification of individual alkaloids. A study was done in 1973 in which choline and two unidentified alkaloids from fruit pulp of *Citrullus colocynthis (L.)* Schrad was isolated. *Citrullus colocynthis (L.)* Schrad. is a superb wellspring of various amino acids as methionine, arginine, and tryptophan. The biological files its protein quality has been depicted as: "lower than soybean however similar to or higher than generally oilseeds." *Citrullus colocynthis (L.)* Schrad. fruits and seeds possess many vitamins and minerals that play an important role in the diet. The potential of *Citrullus colocynthis (L.)* Schrad. seed as a source of calcium and niacin is encouraging to the low milk-consuming zones of the world. *Citrullus colocynthis (L.)* Schrad. seeds contain ash-2.00g/100g, protein-13.19g/100 g, moisture- 4.91 g/100 g, fat-18.59 g/100 g and mineral such as Calcium-569 mg/100g, Potassium-465 mg/100g, Magnesium- 210 mg/100g, Phosphorous 30.0 mg/100g, Sodium- 11.9 mg/100g, Iron- 11.6 mg/100, Copper- 5.1 mg/100g & Zinc- 1.1 mg/100g.[2] The details of chemical constituents elucidated till now have been described in **Table No. 1 and Figure 1.**

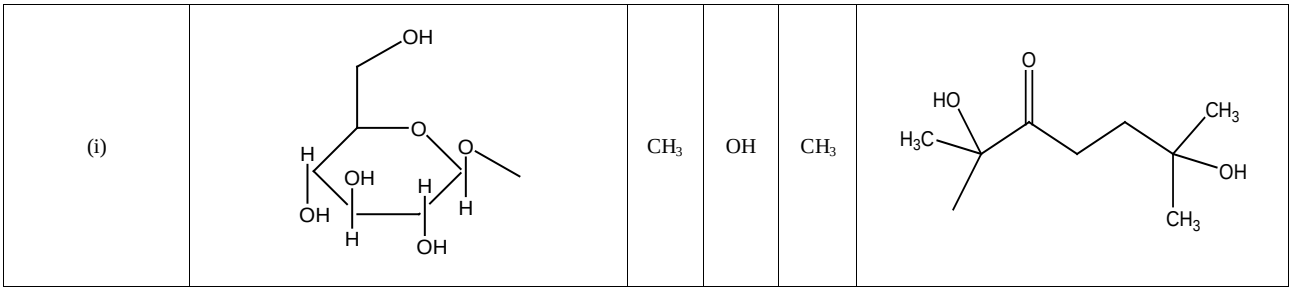
Table 1. Representation of different chemical constituents found in various plant parts extracts of *Citrullus colocynthis (L.)*Schrad

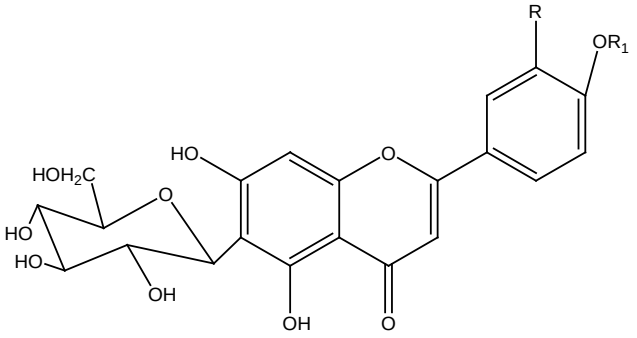
S. No	Plant Part	Extract	Chemical constituent	References
1.	Fruit	Methanolic extract	Ursolic Acid and Cucurbitacin E 2-O-β-D-glucopyranoside	[6]
2.	leaf	Ethyl acetate extract of *Citrullus colocynthis (L.)*Schrad. leaves	25-p-coumaroyl-3′- acetyl-2-O-β-D- glucocucurbitacin I & 6′-acetyl-2-O-β-D-gluco-cucurbitacin	[7]
3.	Whole plant	Methanolic extract	colocynthins A, B & C along with β-sitosterol, 3-O-β-D-glucopyranoside, elaterinide, and bryoamaride	[8]
4.	Fruit	Methanol extract	4-methylquinoline	[9]
5.	Leaf, stem, fruit, and root	Soxhlet extracted in 80% methanol and then re-extracted with petroleum ether, diethyl ether, and ethyl acetate	Quercetin	[10]
6.	Fruits	Butanol fraction of the hydro-methanolic (70%) extract of the fruits	3'-O-methyl ether and two cucurbitacin glycosides, 2-O-β-D-glucopyranosyl-cucurbitacin L & 2-O-β-D-gluco-pyranosyl-cucurbitacin	[11]
7.	Whole plant	Chloroform extract	2-O- β -D-glucopyranosyl-cucurbitacin E, 2-O-β-D-glucopyranosyl-cucurbitacin I, 2-O- β -D-glucopyranosyl-cucurbitacin L and 2-O-β-D-glucopyranosyl-(22 -27)-hexanor-cucurbitacin I	[12]
8.	Fruits	Ether extract	elatericin B (II) (cucurbitacin I) dihydroelatericin B (III) and tetrahydroelatericin B (IV), elaterinidell	[13]
9.	Fruits	Chloroform: Methanol(1:1)ratio	cucurbitacin E and cucurbitacin I glycosides	[14]
10.	Fruits	Butanol extract	2-O-D-glucopyranosyl-cucurbitacin E	[15]
11.	Fruits	Fruit extract	2-O- β-D- glucopyranosylcucurbitacins I, J, K, and L	[16]



Compounds	R_1	R_2	R_3	R_4	R_5
(a)	OH	CH_3	H	H	
(b)		OH	OH	CH_3	

(c)		CH_3	OH	CH_3	
(d)		CH_3	H	CH_3	
(e)		CH_3	OH	CH_3	
(f)		CH_3	OH	CH_3	
(g)		CH_3	OH	CH_3	
(h)		CH_3	OH	CH_3	





Compounds	R	R_1
(j)	OCH_3	H
(k)	H	H
(l)	H	glucosyl

Figure 1. Chemical structures of various phytoconstituents found in *Citrullus colocynthis (L.)*Schrad. (a–l): (a) Cucurbitacin E; (b) Colocynthoside A; (c) Colocynthoside B; (d) Cucurbitacin L 2-O-β-D glucopyranoside; (e) Hexanocucurbitacin I 2-O-β-D glucopyranoside; (f) Cucurbitacin K 2-O-β-D glucopyranoside; (g) Khekadaengoside E; (h) Cucurbitacin J 2-O-β-D glucopyranoside; (i) Cucurbitacin I 2-O-β-D glucopyranoside; (j) Isoorientin 30-O-methyl ether ; (k) Isovitexin; (l) Isosaponarin

3. Biological Activities

Citrullus colocynthis (L.)Schrad. has the conventional use in treatment for cancer, carcinoma, endothelioma, leukemia, tumors of the liver, spleen, and eye [17]. A decoction of the entire plant, made with the juice of fennel is said to help indurations of the liver. Roots may likewise be utilized as a laxative and for treatment of rheumatism, urinary diseases, jaundice and in snake poison [17]. *Citrullus colocynthis (L.)Schrad*. is broadly utilized as a part of society prescription for quite a long time and as a vitality source too such as oilseed and biofuel. The leaves are diuretic and utilized as a part of the treatment of jaundice and asthma. The root is used as a treatment measure during inflammation of the breasts, rheumatism, joint pains, and amenorrhea and is also used externally in uterine torments and ophthalmia [17]. The fruit is pungent, cooling laxative, antipyretic, anthelmintic and carminative. They are used in cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia's and throat diseases while fruit pulp acts as antiepileptic, purgative, diuretic and used against gonorrhea[17].The biological role of *Citrullus colocynthis (L.) Schrad*. has been attributed in **Table No. 2.**

3.1. Antioxidant Activity

In experimental studies on fruits of *Citrullus colocynthis* the total phenolic content, was found to be 0.74% of gallic acid equivalents of phenolic compounds and the total flavonoid content was 0.13% of catechin equivalent of fresh mass of C. colocynthis fruit extract. The free radical scavenging effect of fruit extract of this plant on the 2,2-diphenyl-1-picrylhydrazyl radical was found to be 88.0±2.7% ($p < 0.005$), at concentration of 2500 $mgmL^{-1}$ while scavenging effects of ascorbic acid, BHA and α-tocopherol were found to be 50 $mgmL^{-1}$ of 89.5±1.1, 83.2±1.1 and 67.5±0.8% ($p < 0.05$) respectively [18].

3.2. Antihyperlipidemic Effect

Citrullus colocynthis pulp and the seeds showed significant antihyperlipidemic results on New Zealand rabbits. The hypercholesterolemic control rabbits remained hypercholesterolemic throughout the experimental time but serum cholesterol and triglyceride in the groups administered with both seeds and pulps extracts of C. *colocynthis* were reduced ($p<0.05$). The reduction of LDL-C in the groups receiving the pulp extracts and 100 mg/kg seed extract were significant ($p<0.05$). The impact of *C. colocynthis* on the blood lipid profile in rabbits might

be because of high measures of saponins in *C. colocynthis* which diminished cholesterol levels by lessening the ingestion of cholesterol, expanding the repulse of feces estriol and looseness of the bowels because of expanded peristalsis [19].

3.3. Antifertility Effects

In this study, 50% ethanol extract of *Citrullus colocynthis* (L.) Schrad. administered orally to male albino rats for evaluation of antifertility effects. Prominently reduced cauda epididymis sperm density and motility, a number of pups, fertility, and circulatory levels of testosterone were seen in all treatment groups. The weights of testes, epididymis, seminal vesicle and prostate were significantly reduced in groups receiving 100 mg/kg/day C. colocynthis extract for periods of 20, 40, and 60 days, respectively. The concentration of testicular cholesterol significantly increased, and sialic acid, protein, and alkaline phosphatase concentrations diminished. The histological analysis of the testis showed degenerative modifications in the seminiferous cells, cytolysis, and the lumen filled with eosinophilic substance. Hence 50% ethanol extract of *C. colocynthis* fruit actuated reversible antifertility activity in male rats because of antiandrogenic nature [20].

3.4. Antiulcer Activity

Anti-ulcer activity of Citrullus colocynthis fruits showed positive results against pylorus ligation induced ulcers in male Wistar rats. Aqueous and ethanolic extracts of *Citrullus colocynthis* fruits administered at doses of 200 mg/kg and 400 mg/kg evaluated parameter like- pH, gastric volume, free acidity, total acidity, the percentage inhibition of ulceration and ulcer index. Ethanolic and aqueous extracts at 400 mg/kg indicated anoteworthy ($p<0.001$) decline in the total acidity, free acidity, and gastric volume. It showed also significant ($P<0.001$) decrease in ulcer score index and a number of ulcers in pylorus ligation ulceration model [21].

3.5 Anticonvulsant Activity

In the present study, the anticonvulsant activity of hydroalcoholic extract of *Citrullus colocynthis* Fruit involves the significant role of benzodiazepine and opioid receptors. *Citrullus colocynthis* pulp extract demonstrated a measurable significant reduction in the convulsions and increase in latency period of convulsions instigated by pentylenetetrazole in mice. Extract with doses 25 and 50 mg/kg prolonged the onset of seizures and decreased the duration compared with control group. Anticonvulsive effect increased dose-dependently with following doses 10, 25, and 50 mg/kg. The primary activity of the pentylenetetrazole-instigated seizure is decreasing Ɣ-aminobutyric acid level in the cerebral cortex region [22].

3.6. Antimicrobial Effect

Antimicrobial activity of the leaf extract of *Citrullus colocynthis* was carried out using agar disc diffusion technique against sixteen bacteria and six fungal strains. Phytochemical analysis showed the presence of active constituents like phenols, tannins, and flavonoids. Antimicrobial activity of extracts was compared with the standard Gentamicin (10µg/disc) & piperacillin (100µg/disc). Aqueous extract of the *Citrullus colocynthis* showed high antibacterial action against *Staphylococcus aureus* and *E. coli* and less impact against *Klebsiella pneumoniae* and *Bacillus subtilis* but, methanolic extracts showed better antibacterial action against *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella typhi* [23].

3.7. Antifungal Activity

The present study evaluated the antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit against different Candida and Aspergillus strains. Anti-Aspergillus and Anti-Candidal actions were studied by disc diffusion and broth macrodilution methods. All tested parasitic strains indicated sensitivity to the extract. The minimum fungicidal concentration and minimal inhibitory concentration values ranged from 3.125 to 25 mg/ml and 1.56 to 12.5 mg/ml respectively. The high antifungal action was seen against *A. niger* and *A. fumigatus* as compared to the strains of *C. krusei* and *C. guilliermondii* [24].

3.8. Antibacterial Activity

In this experiment, the antibacterial effect of *Citrullus colocynthis* fruits and leaves extracts against a standard (ATCC 25923) and isolated strains of *Staphylococcus aureus* from novobiocin treatment patients was assessed utilizing disc diffusion method. Phytochemistry of this plant showed constituents like tannins, saponins, alkaloids, flavonoids, and glycosides. The ethanolic extract showed a significant inhibitory activity against S. aureus as compared to aqueous extract in a dose-dependent manner [25].

3.9. Insecticidal Effect

Insecticidal effect of Cucurbitacin E Glycoside (2-O-β-D-glucopyranosyl cucurbitacin E) isolated from *Citrullus colocynthis* was tested against Aphis craccivora. Different extracts of *Citrullus colocynthis* fruits (methylene chloride, n-hexane, ethanol, and chloroform)

against A. craccivora were studied in this experiment. The highest insecticidal effect (LC 11003 ppm) was obtained from the ethanol extract [15].

3.10. Antibacterial and Anticandidal Activity

In vitro antibacterial and anticandidal activity of aqueous and diluted acetone extracts of *Citrullus colocynthis* Schrad. MIC and MBC/MFC were assessed in plant roots, stems, leaves and three maturation stages of its fruit and seeds against gram-negative and gram-positive bacteria like Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis and Candida spp. like candida glabrata, candida albicans, Candida parapsilosis and candida kreusei. The most elevated minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) were obtained from the fruit aqueous extracts (MIC 0.20 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* and 0.10 mg/ml against *Candida albicans* and *Candida glabrata*) and obtained lowest activity from the plant root extracts [26].

3.11. Hypoglycemic Activity

The hypoglycemic effect of root of C. *colocynthis* on the biochemical parameters of normal and alloxan-induced diabetic rats was studied in this experiment. Aqueous extract of roots of *Citrullus colocynthis* demonstrated critical decrease in glucose level (58.70%) when contrasted with chloroform (34.72%) and ethanol extracts (36.60%) ($p<0.01$). The aqueous extracts indicated positive change in parameters like serum creatinine, body weight, serum protein and urea as well as lipid profile and furthermore reestablished the serum level of conjugated bilirubin, bilirubin total, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) [27].

3.12. Antihyperglycaemic Effect

Antihyperglycaemic effects of the alkaloidal, glycosidic, saponin and aqueous extracts of the rind of *Citrullus colocynthis* on the plasma glucose levels were studied in normal rabbits and the action of saponin extract on the fasting blood sugar levels were studied in alloxan-induced diabetic rabbits. Oral dosage of aqueous extract of *Citrullus colocynthis* (300 mg/kg) in normal rabbits produced noteworthy decrease in plasma glucose after 1 h and was exceptionally huge after 2, 3 and 6 h. The action was more articulated with saponin extract which decreased the glucose levels (fasting) after 1 and 2 h and significantly ($p<0.001$) after 3 and 6 h [28].

3.13. Antidiabetic Activity

Antidiabetic action of petroleum ether fruits extract of *Citrullus colocynthis* against Streptozotocin initiated hyperglycemic rats was assessed after oral administration of two distinct doses (300 and 500 mg/ kg) of *Citrullus colocynthis*. Phytochemical investigations revealed following chemical constituents like alkaloids, terpenes, saponins and glycosides. Administration of petroleum ether extract of *Citrullus colocynthis* fundamentally enhanced body weight of diabetic rats in a dose and time-dependent manner. *Citrullus colocynthis* showed antidiabetic action through stimulation of β-cells of islets of Langerhans by releasing more insulin and this effect were brought about by constituents like glycosides, saponins, flavonoids [29].

3.14. Analgesic Activity

In the following experiment, immature fruit aqueous extracts of *Citrullus colocynthis obtained* from various populations of Tunisia was evaluated for analgesic activity. The alkaloid level differs from the *Citrullus colocynthis* population. All extracts displayed analgesic activity at different doses without inducing acute toxicity. Studies also reveal that steroids and iridoids which are present in this plant may contribute to a better performance. The immature fruits from Medenine region showed highest analgesic activity. The lowest activity was seen for Hammamet region fruits (90.86%). This immature fruit exhibited a high activity at very low aqueous extract doses (0.1 mg/Kg and 2 mg/Kg) [30].

Table 2. Representation of biological activities shown by various plant parts of *Citrulluscolocynthis (L.)*Schrad. and phytoconstituents responsible for biological activity

S. No	Plant Part	Extract	Biological activity	Possible constituents responsible for activity	References
1.	Fruit	Methanolic extract	Antioxidant activity	Phenolic compounds	[18]
2.	Pulp and seeds	Hydro-methanolic extract	Anti-hyperlipidemic effect	Saponins	[19]
3.	Fruit	50% Ethylalcoholic extract	Anti-fertility effects	Decrease in Cholesterol levels	[20]
4.	Whole Plant	Ethanolic& aqueous extracts	Anti-ulcer activity	Flavanoids, saponins, alkaloids, and tannins	[21]
5.	Whole Plant	Hydroalcoholic extract	Anticonvulsant Activity	Flavonoids	[22]
6.	Leaf	Aqueous & Methanolic extracts	Antimicrobial effect	Alkaloids, Tannins, Flavonoids	[23]
7.	Fruit	Hydroalcoholic extract	Antifungal activity	Glycosides and resins, colocynthin and colocynthin alkaloids	[24]
8.	Fruits & leaves	Ethanolic extract	Antibacterial activity	Alkaloids, flavonoids, and glycosides	[25]
9.	Whole Plant	Butanolic extract	Insecticidal activity	Cucurbitacin E Glycoside	[15]
10.	Stems, roots, leaves & maturation stages of its seeds & fruit	Aqueous and diluted acetone	Antibacterial and anticandidal activity	Tannins, steroids, pigments and flavonoids, alkaloids, iridoids	[26]
11.	Roots	Aqueous extract	Hypoglycaemic activity	Glycosides (saponin glycosides), triterpenoids, alkaloids, flavonoids, and resins	[27]
12.	Rind of fruits	Aqueous extract	Anti-hyperglycemic effect	Saponin, glycosides	[28]
13.	Fruits	Petroleum ether fruits extract	Antidiabetic effect	Saponins, flavonoids, and glycosides	[29]
14.	Fruits	Aqueous extract	Analgesic effect	Alkaloids, iridoids, flavonoids, steroids	[30]

4. Discussions

Traditionally *Citrullus colocynthis (L.) Schrad.* has been utilized as a plant of medicinal significance at various nativities in different parts of the world. Experimental studies have proven the presence of various phytochemical compounds in the whole of the plant including fruits, seeds, root, and shoot. With the literature survey done in this review cucurbitacins form the most abundant compounds found in this plant species. Some other constituents like phenolic compounds, saponins, flavonoids, alkaloids, tannins, glycosides, triterpenoids, resins, and steroids are also present in this plant. Their structures have also been elucidated in different experimental studies as mentioned in the present review. As far as the pharmacological studies are concerned, till now only the above-mentioned activities (Table 2) have been evaluated. These include antioxidant activity, anti-hyperlipidemic effect, anti-fertility effects, anti-ulcer activity, anticonvulsant activity, antimicrobial effect, antifungal activity, antibacterial activity, insecticidal activity, anticandidal activity, antidiabetic effect, and analgesic effect. Still, a lot of research is needed on *Citrullus colocynthis (L.) Schrad*. regarding phytochemistry and biological evaluations of certain activities which have been mentioned in the literature and also being practiced by the traditional people.

5. Conclusions

In the present study, the authors reported the phytochemical constituents and biological activities of the *Citrullus colocynthis (L.) Schrad.* Several plants parts like leaf, stem, roots, fruits, and seeds have been studied extensively by eminent scientists and researchers. Moreover, fruit holds much biological and chemical significance as far as the above study is concerned. Biological activities showed by different parts of this plant display a multidisciplinary usage of this plant in treating several diseases. Although a number of compounds and many pharmacological activities have been elucidated in this plant species still more research is needed to be done as many traditional uses been reported so far are required to be authenticated by research.

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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EDITORIAL OPEN ACCESS

Phytochemical analysis and pharmacological spectrum of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae)

Prashant Kumar Dhakad, Pramod Kumar Sharma, Sokindra Kumar

INTRODUCTION

Ethno medicinal studies play a major role to highlight the endemic plant species notably for the invention of recent crude medicine. Documentation of native medicinal information of ancient plant species has resulted in development of variety of recent medicine [1]. Medicinal plants are found through varied habitats and landscapes. Rajasthan state is understood for a fashionable floral diversity with 1911 wild species including 780 genera and 154 families. The most skillfully accustomed herbal medicines used by tribes of Rajasthan are plants of Fabaceae, Euphorbiaceae, Asteraceae, Apiaceae, Cucurbitaceae, Acanthaceae, Papaveraceae, Capparidaceae and Solanaceae families [2].

Chemical constituents of plants are of utmost importance for the discovery of therapeutic agents and in establishing the medicinal value of traditional plants [3]. *Citrullus colocynthis* (L.) is a member of family Cucurbitaceae, a xerophyte with huge medicative importance and a decent supply of valuable oil. It is cosmopolitan throughout Asia as well as India. It is normally referred to as bitter apple, or colosynth is employed as an abortifacient, cathartic, purgative and vermifuge, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, neoplasm and as an insectifuge. It additionally act as a conventional medicines for inflammatory disease, diabetes, inflammatory disorders, and gastralgia [4].

Citrullus colocynthis is annual or perennial (in wild), herbaceous, bearing monoecious type flowers, pepo fruit and numerous seed. Its fruits are used as robust laxative [5]. This plant produces fruits known as as colocynth apples that are the same as the common *Citrullus vulgaris* and tastes bitter. It possesses solitary sterile flowers and an oversized, fleshy perennial root, that rises to from slender, tough, angular, vine-like stems and branched tendrils [6].

Aqueous pulp extract of *Citrullus colocynthis* fruits is used for treatment of kidney, liver related diseases. Isolated phenolic compounds have antioxidative and antineoplastic properties by absorption and neutralization of free radicals [7]. *Citrullus colocynthis* fruits are known for pain relieving, cathartic, anti-inflammatory, antioxidative, anti-diabetic effects. Cucurbitacins are reported to be the main constituent of fruits of this plant [8]. Infusion prepared from the seed as well as fruit of this plant are indeed recommended to diabetic patients. Since this plant has promising effect on diabetic patients and it is known that antidiabetic plants contains alkaloids, polyphenols, polysaccharides, gums and glycans [9].

This literature survey involves the documentation of data from 1950–2017 from authenticated sources like Google scholar, ScienceDirect and PubMed regarding phytochemical studies and pharmacological activities of various parts of *Citrullus colocynthis* plant. Figure 1 shows the fruits and leaves of *Citrullus colocynthis* lying in desert region while Figure 2 shows the dried fruits and seeds portion of *Citrullus colocynthis* plant.

PHYTOCHEMICAL STUDIES

Isolated compounds ursolic acid and cucurbitacin E 2-O-β-D-glucopyranoside in the methanolic fruit extract of *Citrullus colocynthis* showed antimicrobial activity. In

Prashant Kumar Dhakad[1], Pramod Kumar Sharma[2], Sokindra Kumar[3]

Affiliations: [1]Assistant Professor, Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India; [2]Professor, Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India; [3]Professor, R V Northland Institute (Pharmacy), Pharmacology Research Lab, Greater Noida-II, Gautam Budh Nagar, Uttar Pradesh, India.

Corresponding Author: Prashant Kumar Dhakad, Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Uttar Pradesh 201306, India; Email: dhakadprashant654@gmail.com

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an experimentation isolated compounds, cucurbitacin and colocynthis from the ethanolic root extract of this species proved to be hepatoprotective against carbontetra chloride induced toxicity in experimental animals [10].

In an investigation identified with *Citrullus colocynthis* leaf extract two new triterpene glycosides were isolated from an ethyl acetic acid derivation concentrate of leaves of this plant alongside four known cucurbitacins. Compound structures were designed through spectroscopic information utilizing NMR and mass spectrometry. Two new cucurbitacins isolated were-25-p-coumaroyl-3′-acetyl-2-O-β-D-glucocucurbitacin I and 6′-acetyl-2-O-β-D-glucocucurbitacin E. The later coumaroyl cucurbitacin subordinate demonstrated huge particular cytotoxic action towards colorectal cell lines [11].

In an experimental study, the methanolic extract of the *Citrullus colocynthis* plant was divided into fractions soluble in hexane, chloroform, ethyl acetate, butanol, and water. Column chromatography of the ethyl acetic acid soluble fraction showed three new bitter principles named colocynthis A, B and C along with β-sitosterol, 3-O-β-D-glucopyranoside, elaterinide, and bryoamaride, respectively. New compounds were glycosides gave positive Molisch test result, as well as Salkowski and Liebermann–Burchard color reactions for triterpenes.

Figure 1: *Citrullus colocynthis* plant with leaves and fruits in the desert region.

Figure 2: Dried fruits and seeds of *Citrullus colocynthis* plant.

The compound colocynthin A was obtained as grayish indistinct amorphous solid and infrared spectrum showed bands for OH (3400 cm^{-1}), C¼O (1715 cm^{-1}), conjugated C¼O (1680 cm^{-1}), and olefinic (1610–1650 cm^{-1}) functionalities. The HR-FAB-MS (positive-ion mode) gave a $[M+H]^+$ crest at m/z 659.3422, showing the atomic recipe $C_{36}H_{50}O_{11}$, and also a fragment ion $[M-162+H]^+$ at m/z 497.2832 because of the loss of the glucose moiety. Colocynthin B also obtained as grayish formless strong compound. The HR-FAB-MS of Colocynthin B gave signal at 691.3681 $[M+H]^+$, and in conjunction with the ^{13}C-NMR information, the sub-atomic formula decided as $C_{37}H_{54}O_{12}$. Colocynthin C was obtained as a grayish nebulous strong. The atomic equation got as $C_{37}H_{54}O_{11}$ by HR-FAB-MS in the positive-particle mode, which gave an $[M+H]^+$ peak at m/z 675.3732 ($C_{37}H_{55}O^+_{11}$; calc. 675.3744) [12].

In a study conducted in the chloroform portion of the methanol concentrate of *C. colocynthis* natural products, dynamic constituent was isolated by silica gel segment chromatography and preparative HPLC. Structural analysis was done using spectroscopy including EI/MS, ^1H NMR, ^{13}C NMR, COSY, DEPT, and HMQC NMR and then by direct comparison with an authentic reference compound. The dynamic compound segregated was 4-methylquinoline. Spectroscopy detailed around 4-methylquinoline ($C_{10}H_9N$); EI/MS (70 eV) m/z M^+ 143 (100, base peak), 135 (40), 105 (39), 107 (40), 79 (46), 51 (13); ^1H NMR (CD_3 OD, 600 MHz) δ 2.61 (s), 7.26–7.27 (d, J = 6.7 Hz), 7.42–7.43 (t, J = 6.9 Hz), 7.63–7.75 (t, J = 83.1 Hz), 8.00–8.05 (d, J = 35.7 Hz), 8.09–8.11 (d, J = 7.3 Hz), 8.42– 8.43 (d, J = 1.6 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 150.6, 148.2, 135.5, 130.0, 128.5, 128.1, 126.5, 124.4, 122.9, 19.6 [13].

Amid the examination of *Citrullus colocynthis* in vivo (leaf, stem, fruit and root) and in vitro callus a flavonoid quercetin was obtained. Rf value (0.82) of quercetin separated from extract samples resembles the Rf value of standard quercetin and in addition characteristic infrared spectral peaks were superimposable with individual standard reference mixes of quercetin. The HPLC parameter showed retention time of 3.475 min which matched with that of standard quercetin. Quercetin was present both in vivo and in vitro samples of *Citrullus colocynthis* [14].

In an investigation the reversed-phase preparative investigation of the butanol portion of the methanol concentrate of *C. colocynthis* fruits gave three flavonoid glycosides, isosaponarin, isovitexin and isoorientin 3'-O-methyl ether and two cucurbitacin glucosides, 2-O-β-D-glucopyranosylcucurbitacin L and 2-O-β-D-gluco-pyranosylcucurbitacin I. An ESIMS mass spectrum of isosaponarin showed [M+H]+ (positive ion mode) ion peak at m/z 595, M_r = 594 and obtained molecular formula as $C_{27}H_{30}O_{15}$. An ESIMS mass spectrum of isovitexin obtained as $[M+H]^+$ ion peak at m/z 433, M_r = 432 and $C_{21}H_{20}O_{10}$. An ESIMS mass range of isoorientin 3'-O-methyl ether demonstrated $[M+H]^+$ ion peak at m/z

463, M_r = 462 and $C_{22}H_{22}O_{11}$. While ESIMS mass range of 2-O-β-D-gluco-pyranosylcucurbitacin I gave $[M+H]^+$ (positive ion mode) particle peak at m/z 677, suggesting M_r = 676 and understanding for $C_{36}H_{52}O_{12}$ and ESIMS mass spectrum of 2-O-β-D-glucopyranosylcucurbitacin L demonstrated $[M+H]^+$ (positive ion mode) ion peak at m/z 679,Mr = 678 and $C_{36}H_{54}O_{12}$ [15].

The chloroform concentrate of the defatted *Citrullus colocynthis* plant was fractionated to result four glycosides as, 2-O-β-D-glucopyranosyl-cucurbitacin E, 2-O-β-D-glucopyranosyl-cucurbitacin I as the major product, 2-O-β-D-glucopyranosyl-cucurbitacin L and a novel glycoside 2-O-β-D-glucopyranosyl-(22-27)-hexanorcucurbitacin I. Structural studies of cucurbitacins were done using ^{1}H and ^{13}CNMR spectra. The NMR spectra of first three compounds resemble with free aglycones while EIMS spectra for same compounds did not deliver noticeable parent particles. But FABMS showed observable parent ions either: as $[M+1]^+$ or $[M + Na]^+$ ions. The spectral evidence lead to the structural assignment of 2-O-B-D-glucopyranosyl-(22-27)-hexanorcucurbitacin I, which was the only degraded cucurbitacin glycoside reported till 1988. This compound was isolated as an amorphous powder, and investigation demonstrated a sub-atomic particle crest at m/z 585 $[M (C_{30}H_{42}O_{10}) + Na]^+$ in the FAB mass spectrum, and ketonic carbonyl absorption (1690 cm^{-1}) in the infrared spectrum [16].

In this study alcoholic concentrate of *Citrullus colocynthis* was extracted with chloroform and the product of this extraction after maintaining the pH 5.2–5.4 and addition of elaterase enzyme showed the formation of elaterin in the sediment. Further experimental studies segregated a white crystalline substance distinguished as Elatericin B (II) (cucurbitacin I). Thereafter two more compounds were isolated as dihydroelatericin B (III) and tetrahydroelatericin B (IV) with petroleum ether and ether. A yellow substance solidified out of from ether solution distinguished as the glycoside elaterinide ll [17].

During the gas chromatography-mass spectrometry spectral investigation of methanolic extract of *Citrullus colocynthis* 33 bioactive phytochemical compounds were obtained by investigating the retention time molecular weight, peak area and molecular formula. Spectral analysis of *C. colocynthis* revealed the existence of the methyl 6-oxoheptanoate, hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, methyl ester, dodecanoic acid, 3-hydroxy, benzofuran,2,3-dihydro, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 12,15-octadecadiynoic acid, methyl ester, (5ß)pregnane-3,20ß-diol, 14α,18α- [4-methyl-3-oxo-(1-oxa-4-azabutan, 3-N,Ndimethyllaurylammonio) propanesulfonate, 2H-1-benzopyran-3,4-diol,2-(3,4-dimethoxyphenyl)-3,4dihydro-6-met, 11,13-dihydroxy-tetradec-5-ynoic acid, methyl ester, cyclopenta [1,3] cyclopropa [1,2] cycloheptan-3(3aH)-one,1,2,3b,6,7, 4-(2,4,4-trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one, 1-tetradecanamine,N,N-dimethyl, α-D-glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw.3)-ß-D-fructo, acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-,9-octadecenamide,(z)-,butyrophenone,2´,3,4´,6´-tetramethyl-,ethyl 5,8,11,14,-eicosatetraenoate, 9,12,15-octadecatrienoic acid, 2,3,-dihydroxypropyl ester, (Z,Z,Z)-,1H-cyclopropa [3, 4] benz [1,2-e] ezulene-5,7b,9,9a 476.241018tetrol,1a,1b,4,4a, 9,12,15-octadecatrienoic acid, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25,-triol,(3ß,5Z,7E)-,9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-, triazido-(1,2,3,4,5-pentamethylcyclopenta-2,4-dienyl)-german, ethyl iso-allocholate, α-N-Normethadol, octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, phthalic acid, decyl oct-3-yl ester, 1,2-benzenedicarboxylic acid, bis-(8-methylnonyl)ester, phthalic acid, di(6-ethyl-3-octyl) ester, y-tocopherol, 1,4-ethanonaphthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10 and vitamin E [6].

In this study the chromatographic purification obtained from *C. colocynthis* fruits extract resulted in cucurbitacin E and cucurbitacin I glycosides. These compounds demonstrated promising outcomes against in vitro cytotoxic action against hepatoma cell line (HepG2) and mice-bearing tumor of Ehrlich's ascites carcinoma (EAC). The in vivo study showed the tendency of both compounds prolonging the survival time, life span and normalize the biochemical parameters of the infected mice with EAC. The two compounds had strong inhibitory effect onHepG2 with IC50 3.5 and 2.8 nmol/ml individually [18].

During investigation of *Citrullus colocynthis* fruits using different solvents (n-hexane, methylene chloride, chloroform and ethanol) showed the presence of six compounds, and the most bottomless of them had retention time (Rt.) 4.8 min. (69.3%). This compound was purified by utilizing Florisil® column section stepwise eluted with various blends of methanol: chloroform. Mass examination of the compound demonstrated the atomic particle peak at m/z 719. The elemental analysis demonstrated molecular recipe to be $C_{38}H_{55}O_{13}$ and I.R., proton and ^{13}C NMR analysis recognized the compound as 2-O-β-D-glucopyranosylcucurbitacin E [19].

PHARMACOLOGICAL ACTIVITIES

Traditional uses

Citrullus colocynthis (Linn.) Schrad is an imperative therapeutic plant of Cucurbitaceae family. It is recognized plant in the ethnical medicine and was utilized by individuals in country zones as a laxative, anti-diabetic and bug spray. *Citrullus colocynthis* has a valuable impact in sciatica and gout. It is valuable as douche during colic, sciatica, spinal pain, and loss of motion distresses. *C. colocynthis* oil obtained by boiling pulp with sesame or olive oil is externally used for ear pains, tinnitus, toothache, and male pattern baldness. The leaf of *C.*

colocynthis has laxative impact and furthermore utilized as a part of epilepsy. External application of leaf of this plant is useful in treating inflammation and bleeding. The root goes about as a powerful antitoxin for scorpion and snail nibbles. The most popular traditional use of *C. colocynthis* fruits and seeds is in diabetes treatment [20].

Antioxidant activity

Methanolic fruit extract of *Citrullus colocynthis* showed the total phenolic content as 0.74% of gallic acid equivalents of phenolic and the total flavonoid content as 0.13% of catechin equivalents. The free radical scavenging effect of fruit extract of this plant on the 2,2-diphenyl-1-picrylhydrazyl radical found to be 88.0±2.7% ($p < 0.005$), at concentration of 2500 $mgmL^{-1}$ while scavenging effects of ascorbic acid, BHA and α-tocopherol found to be 50 $mgmL^{-1}$ of 89.5±1.1, 83.2±1.1 and 67.5±0.8% ($p < 0.05$) respectively. The level of H_2O_2 scavenging action of was observed to be 62.7±3.5 ($p < 0.001$) at 2500 mg mL^{-1}, and antioxidant activity of BHA and α-tocopherol was 89.3±3.1% ($p < 0.05$) and 94.5±2.5% ($p < 0.05$), respectively with concentration 50 mg mL^{-1}. The most astounding antioxidative and free radical scavenging capacity of the fruit extract was seen at 2500 mg mL^{-1} concentration [21].

Antihyperlipidemic effect

Citrullus colocynthis pulp and the seeds were investigated for the antihyperlipidemic effects on New Zealand rabbits. The hypercholesterolemic regimen of *Citrullus colocynthis* essentially expanded the measure of LDL-C, blood cholesterol, triglyceride, HDL-C and glucose ($p<0.05$). The reduction of low density lipoprotein-cholesterol in the groups administered with pulp extracts and 100 mg/kg of seed extract found significant ($p<0.05$). High density lipoprotein-cholesterol decrease was found in the groups administered with diet containing the standard regimen, along with cholesterol (0.5%) and 100 mg/kg of *Citrullus* pulp extract as well as with diet having standard treatment, with cholesterol (0.5%) and 100 mg/kg of *Citrullus* seed extract. The impact of *C. colocynthis* on the blood lipid profile in rabbits might be because of high measures of saponins in *C. colocynthis* which diminished cholesterol levels by lessening the ingestion of cholesterol, expanding the repulse of feces estriol, and looseness of the bowels because of expanded peristalsis. In this trial, the utilization of *C. colocynthis* came about huge diminishment of total serum cholesterol and LDL-C in groups administered with extracts [22].

Antifertility effects

The present investigation of *C. colocynthis* 50% ethanolic extract suppresses sperm density and motility and fertility of rats. But fruit extract administration showed a serious and reversible restraint of sperm fertility and density. The sperm density approached to around 10 million/mm^3 in all treatment groups as compared to 46.5 million/mm^3 in the vehicle-treated group. The weights of testicles, epididymis, original vesicles, and ventral prostate extraordinarily decreased after *C. colocynthis* treatment in the different groups due to the antiandrogenic nature of the drug recommending androgen imbalance and inhibition of the androgen generation by the testicles. Hence 50% ethanol extract of *C. colocynthis* fruit actuated reversible antifertility activity in male rats because of antiandrogenic nature [23].

Antiulcer activity

The present examination explored the antiulcer capability of ethanolic and aqueous extracts of *Citrullus colocynthis* plant. Ethanolic and aqueous extracts at 400 mg/kg indicated noteworthy ($p<0.001$) diminish in the total acidity, free acidity and gastric volume. The pH of the gastric juice significantly ($p<0.001$) ascended at the dose of 400 mg/kg. It indicated additionally significant ($p<0.001$) diminish in number of ulcer score index & ulcers using pylorus ligation ulceration model. *Citrullus colocynthis* fruit extracts exhibited a significant antiulcer activity in experimental male Wistar rats. Ethanolic extract indicated preferable hostile than aqueous extract [24].

Anticonvulsant activity

This examination researched the *Citrullus colocynthis* fruit extract as anticonvulsive in the treatment of seizures. Pentylenetetrazole induced convulsions were made in albino mice pretreated with fruits extract of 10, 25, 50, and 100 mg/kg dose. 25 and 50 mg/kg of hydroalcoholic extract delayed the beginning of seizures and diminished the duration in comparison to control group. *Citrullus colocynthis* pulp extract demonstrated a measurable significant reduction in the seizures term and increment in latency period of seizures instigated by pentylenetetrazole in mice. Anticonvulsive effect increased dose dependently with following doses 10, 25, and 50 mg/kg. The primary activity of the pentylenetetrazole-instigated seizure is diminishing Ɣ-aminobutyric acid level in the cortex [8].

Antimicrobial effect

Antimicrobial effect of aqueous extract of the *Citrullus colocynthis* demonstrated high antibacterial action against *Staphylococcus aureus* and *E. coli* and significantly less impact against *Klebsiella pneumoniae* and *Bacillus subtilis*. While, methanolic extracts of this plant showed significant antibacterial action against *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella typhi*, considerably less activity against Streptococcus faecalis and there was no impact against *Proteus vulgaris*, *Vibrio cholera* and *Proteus mirabilis*. The methanolic extract also indicated high antifungal activity against

Aspergillus fumigatus, *Mucor* sp., and *Aspergillus flavus*, *Candida albicans*, *Penicillium* sp., and *Rhizopus* sp. did not demonstrated any antifungal action. The outcomes acquired in the investigation propose the antimicrobial role of *Citrullus colocynthis* in treating diseases caused by the test organisms [25].

Antifungal activity

The study assessed the antifungal action of hydroalcoholic extract of *Citrullus colocynthis* fruits against various *Aspergillus* and *Candida* strains. Activity was determined utilizing broth of macrodilution and disc diffusion methods. All tested parasitic strains indicated sensitivity to the extract. The growth restraint value of the fruits extract indicated high antifungal action against *A. niger* and *A. fumigatus* and a lesser impact against *C. krusei* and *C. guilliermondii*. The minimum fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) values ranged from 3.125–25 mg/ml and 1.56–12.5 mg/ml respectively [26].

Antibacterial activity

In the present examination, the antibacterial effect of *Citrullus colocynthis* was studied. The antibacterial activity of *Citrullus colocynthis* fruits and leaves extracts against standard (ATCC 25923) and isolated strains of *Staphylococcus aureus* from novobiocin treatment patients were assessed utilizing disc diffusion method. The inhibitory impacts of these extracts were compared to novobiocin (standard antibiotic). The ethanolic extract indicated inhibitory activity as compared to aqueous extract against *S. aureus*. 5 mg/mL fruits ethanolic extract demonstrated comparative inhibitory impact with novobiocin against standard strain. The present research proposed that one of the concoction segments in ethanolic concentrate, for example, alkaloids, flavonoids and glycosides had an intense antibacterial impact significantly more than novobiocin, particularly against hospital isolated strains [27].

Insecticidal activity

A glycoside Cucurbitacin E separated from *Citrullus colocynthis* was examined for insecticidal activity against *Aphis craccivora* with extraction with extraction acquired from various solvents like methylene chloride, chloroform 50, ethanol and *n*-hexane. The ethanolic extract demonstrated the most noteworthy insecticidal effect (LC 11003 ppm) against *A. craccivora*. After further extraction of the deposit staying after vanishing of ethanolic extract with nine solvents, the butanol portion demonstrated the most noteworthy insecticidal effect (LC3123.10 ppm). This insecticidal strength of *C. colocynthis* extract is because of the presence of active ingredients like glycosides, saponin, and alkaloids. Overall analysis, conclude that this compound showed an insecticidal effect against *Aphis craccivora* [19].

Antibacterial and anticandidal activity

Citrullus colocynthis aqueous and diluted acetone extracts of stems, roots, leaves and maturation stages of its seeds and fruit) demonstrated activity against every single microbial strain such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* and different *Candida* spp. i.e., *Candida krusei*, *Candida parapsilosis*, *Candida albicans* and *Candida glabrata*. The most elevated minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) were obtained from the fruit aqueous extracts (MIC 0.20 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* and 0.10 mg/ml against *Candida albicans* and *Candida glabrata*) and obtained lowest activity from the plant root extracts [28].

Hypoglycemic activity

A study showed presence of saponin glycosides, triterpenoids, alkaloids, flavonoids and resins in aqueous extract of roots of *Citrullus colocynthis* which lessened the glucose level (58.70%) when compared with ethanolic (36.60%) and chloroform (34.72%) extracts ($p< 0.01$). Assumed mechanism behind the lessening in the blood glucose levels of diabetic rats treated with the extracts is due to stimulation of residual pancreatic mechanism or by increment in fringe use of glucose. The water extracts of *Citrullus colocynthis* enhanced the parameters like serum urea, body weight, serum creatinine and serum protein additionally lipid profile and furthermore reestablished the serum level of bilirubin add up to serum glutamate oxaloacetate transaminase (SGOT), conjugated bilirubin, serum glutamate pyruvate transaminase (SGPT) and antacid phosphatase [29].

Antihyperglycaemic effect

Oral dosage of aqueous extract of *Citrullus colocynthis* (300 mg/kg) in normal rabbits produced noteworthy decrease in plasma glucose after 1 h and exceptionally huge after 2, 3 and 6 h. The hypoglycemic impacts of tertiary and quaternary alkaloids, glycoside and saponin segments introduce in this plant at a measurements (50 mg/kg p.o) were studied in normoglycemic rabbits. The alkaloidal extract did not essentially bring down the blood glucose levels while the glycosidic extract fundamentally brought down the fasting glucose levels after 2 and 3 h and exceptionally huge after 6 h. The action was more articulated with saponin extract which decreased the glucose levels (fasting) after 1 and 2 h and significantly ($p<0.001$) after 3 and 6 h [30].

Antidiabetic activity

Antidiabetic action of petroleum ether fruits extract of *Citrullus colocynthis* against Streptozotocin initiated hyperglycemic rats was assessed after oral administration of two distinct doses (300 and 500 mg/

kg) of *Citrullus colocynthis*. Additionally subacute impact i.e., antihyperglycemic effect was seen on seventh and in addition day-14 of the analysis. Administration of petroleum ether extract of *Citrullus colocynthis* fundamentally enhanced body weight of diabetic rats in a dose and time dependent manner. The total hemoglobin and glycosylated hemoglobin levels ($p < 0.01$) was also restored by administration of extracts. The investigation reported that petroleum ether extract of *Citrullus colocynthis* demonstrated critical pharmacological action towards bringing down blood glucose in diabetes [31].

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Keywords: Cucurbitaceae family, Cucurbitacins, Phytochemistry, Pharmacological activites

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Authors declare no conflict of interest.

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