Research Article

Stem Cell & Regenerative Medicine

Antiarthritic Potential of *Capparis Decidua* (Forssk.) Edgew on FCA Induced Arthritis in Wistar Rats

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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).

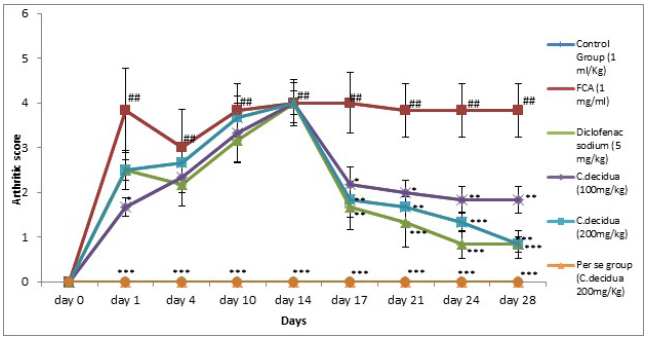

Figure 1: Effect of *Capparis decidua* on arthritic score in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.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

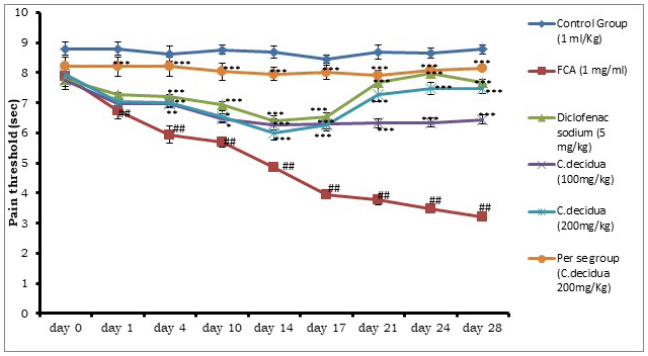

Figure 2: Effect of *Capparis decidua* on anti-nociceptive study (pain threshold) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<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).

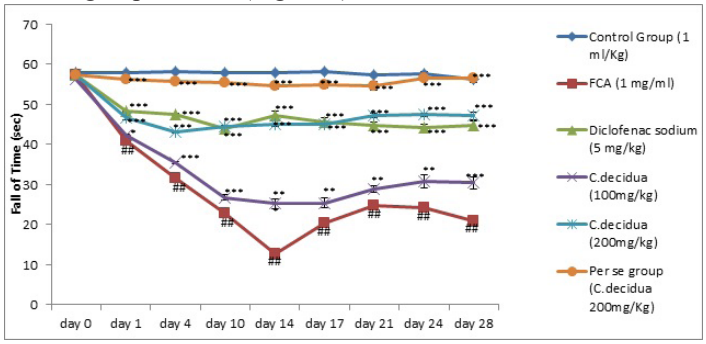

Figure 3: Effect of *Capparis decidua* on fall of time in motor incoordination test in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P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

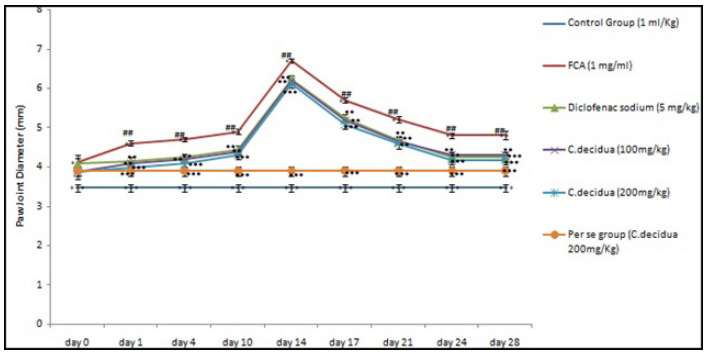

Figure 4: Effect of *Capparis decidua* on paw joint diameter (mm) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared 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).

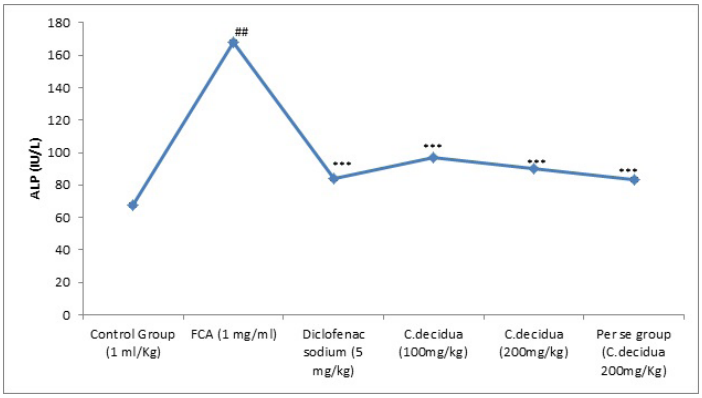

Figure 5: Effect of *Capparis decidua* on ALP (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

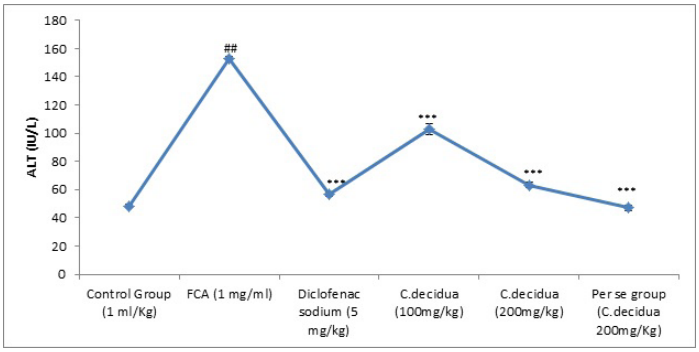

Figure 6: Effect of *Capparis decidua* on ALT (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

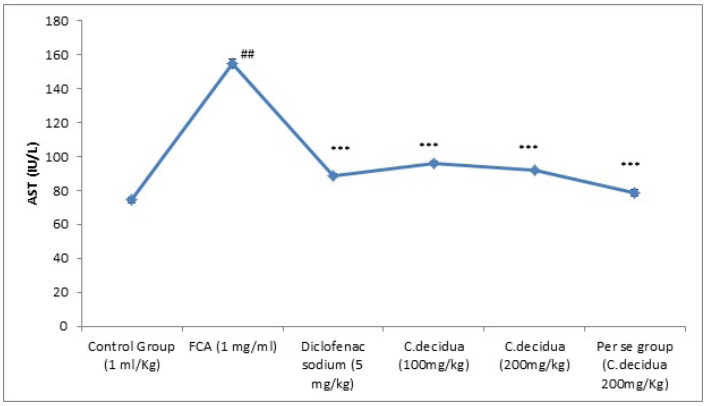

Figure 7: Effect of *Capparis decidua* on AST (IU/L) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared to FCA.

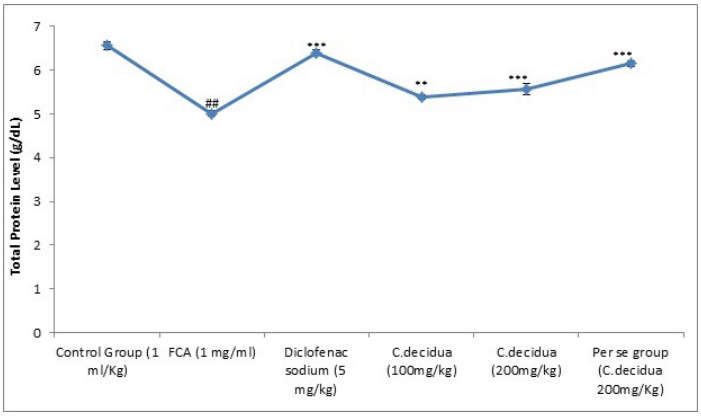

Figure 8: Effect of *Capparis decidua* on Total Protein Level (g/dL) in FCA-induced arthritic rats. Data are expressed as mean ± S.E.M. (n = 6). Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests for comparison.##P<0.001 as compared to control. ***P<0.001 as compared 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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