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

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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 ulcerogenicity 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, hiccup, and asthma [5]. *Capparis decidua* (Forssk.) Edgew. contains constituents like flavanoids, phenolic compounds, steroids, alkaloids, vitamins, quaternary 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, Capparidecuduasterol, capparisterol, capparisditerpenyl ester. Previous studies conducted on various parts of this plant showed numerous pharmacological activities like antibacterial, antifungal, antiparasitic [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 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-arthritis 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 (UGO Basile, Italy) on day 0 before FCA injections and thereafter on day 1, 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 5 μ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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.

Figure 3. Effect of *Capparis decidua* on ESR count mm/hr test in FCA-induced arthritic rats; Data are expressed as mean $\pm$ 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 $\pm$ 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 5. Effect of *Capparis decidua* on Platelet count (x 1000 cells/mm³) test in FCA-induced arthritic rats; Data are expressed as mean $\pm$ 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 RBC count test in FCA-induced arthritic rats; Data are expressed as mean $\pm$ 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 treated.

Figure 7. Effect of *Capparis decidua* on WBC count test in FCA-induced arthritic rats; Data are expressed as mean $\pm$ 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 histopathology 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 neutrophils 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 of decreased intestinal absorption rate. Treatment with EACA significantly 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-arthritis 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, Isodulcitol, Nonacosane, Thymol, Isopropyl isothiocyanate, Butyl isothiocyanate, 2-Hexenol (33), Phenylpropanoid, Terpenoids, Isothiocyanate, n-Alkalenes, Thymocitrin, Kaempferol, Isorhamnetin, rhamnocitrin, rhamnetin, rhamnazin, quaternary ammonium compounds, Spermidine alkaloid (35), Isocodonocarpine, Capparine, Capparidine, Cappariline, Codonocarpine (36), Cadabacine-26-O- β -d-glucoside, Capparidine, Capparispine-26-O-d-glucoside, N-acetylated spermidine, 14-N-acetyl isocodonocarpine, 15-N-acetylcapparidine, Rutin, l-Stachydrine, β -Sitosterol, Terpenoids, Capparisterpenolide (37), Glucocapparine, 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-arthritis 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.

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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