WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

Volume 1, Issue 2,309-325. Research Article ISSN 2277 – 7105

ANTI-ARTHRITIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *RANDIA DUMETORUM* FRUITS IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS

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Article Received on
25 March 2012,

Revised on 15 April 2012,

Accepted on 23 April 2012

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ABSTRACT

Randia dumetorum has multiple applications in traditional medicine because it exhibit Analgesic, Antifertility, Antibacterial, Anti-inflammatory, Antiulcer, and Protective effects. However, no study on the anti-arthritic activity of *Randia dumetorum* has been reported in vivo. The present study was undertaken to determine efficacy of madecassoside (MA) against Freund's complete adjuvant-induced arthritis (CFA) in female rat. Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation. It is characterized by hyperplasia of synovial cells in affected joints, which ultimately leads to the destruction of cartilage and bone. We investigated therapeutic efficacy of *Randia dumetorum* in treating Rheumatoid Arthritis (RA) using Freund's complete adjuvant-induced arthritis (CFA) animal model. Arthritis was induced in female Wistar rats by intradermal injection of Freund's complete adjuvant (CFA). CFA rats were treated daily with oral administration of different doses of Methanolic extract of *Randia dumetorum*(RD), beginning on the day after the onset of arthritis (day 21st, the therapeutic treatment) until day 28th. The results showed that treatment with *Randia dumetorum* markedly reduced paw swelling and arthritic index even in the established CFA. Radiologic and histopathologic changes in the arthritic joints were also significantly reduced in the *Randia dumetorum* -treated versus vehicle-treated rats. Moreover,

Rheumatoid factor was significantly reduced in *Randia dumetorum* treated group in compared to disease control group. Hence, our studies demonstrate safety, and effectiveness of *Randia dumetorum* as an anti-arthritic agent, which makes *Randia dumetorum* a strong candidate for further research on rheumatoid arthritis (RA).

Keywords: *Randia dumetorum*, Rheumatoid arthritis, Freund's complete adjuvant-induced arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, relapsing autoimmune disorder that is characterized by pain, synovial membrane inflammation and restricted joint movement due to tissue damages.[1] In RA, bone deformations and disability of joint function occurred due to progressive erosion of articular cartilage in synovial joint via generation and infiltration of autoantibodies in it, leading to severe pain. Around 1% of the population of the world is suffering from RA.[2]

Amongst the various experimental animal models of arthritis, induction of arthritis by Freund's complete adjuvant (FCA) is one of the standardized method which mimics the human pathophysiological state including chronic swelling in multiple joints due to accumulation of inflammatory cells, joint cartilage erosion, bone destruction and used to investigate the activity of various potent anti-inflammatory and anti-arthritic agents.[3,4] Synthetic chemical moieties such as non-steroidal anti-inflammatory drugs (NSAIDs) including ibuprofen, aceclofenac, naproxen, etc. are utilized to minimize the degree of pain. Furthermore in combination with the steroid hormones like cortisone and prednisone are effective for its treatment.[5,6] But they fail to produce long-term response treatment for this disease.[7] However long-term treatment with this drug may cause serious side effects, such as gastrointestinal ulcergenicity and renal morbidity.[8] There are many treatments like disease modifying antirheumatoid arthritic drugs (DMARDs) including methotrexate, cyclosporin A, leflunomide as well as anti-cytokine therapy such as infliximab, adalimumab, etc. available for RA [9] but around 25–30% of patients fail to respond to this treatment.[10] However, besides their high cost, it also associated with severe well-known side effect including gastrointestinal irritation, cardiovascular complication, hematologic toxicity and nephrotoxicity, which limit their utility in treatment of RA.[11,12]

Owing to these shortcomings, there is need to have a more effective and safe therapeutic strategy to treat RA. By virtue of its safety and efficacy, plants still hold their own unique

place. Hence, there is need of systematic approach to find out the herbs which possess anti-inflammatory and antiarthritic potential. Many authors have previously reported antiarthritic activity of many natural herbs which contain the anti-inflammatory and anti-nociceptive effects including the roots of Anemarrhena asphodeloides, Achyranthes japonica, Aralia continentalis, Larrea divaricata, Ocimum sanctum, etc.[3,13,14]

Randia dumetorum occurs in almost throughout India up to 4,000 ft attitude. It is seen in Gujarat, Tamilnadu, forest of Dehradun, Suralik range, Bengal, Bihar, Orrisa & South Maharashtra and costal districts of south India. It is also cultivated in dry deciduous forests in India for medicinal purpose.[15] The methanol extract showed the presence of glycosides, randioside A, mollisidial triterpenoid glycosides and randianin, six saponins-dumetoronins A to F.[16] Saponins named as Dudumentoronin from fruit pulp of *Randia dumetorum* Dumetoronin A, B, C, D, E and F etc. A hemolytic triterpenoid saponins that is Randianin, from fruit of R. dumetorum.[17] It cures abscess, ulcers, inflammation, wounds, tumours, skin diseases and have antibacterial activity. It is believed by many practitioners that the pulp of fruit also have anthelmintic properties, and also used as an abortiffcient as folklore remedy.[16] It is relieve pain of bruises and bone aches during fevers and to disperse abscesses. The aqueous extract of the root bark of the tree is used as an active insecticide.[18]

MATERIALS AND METHODS

Animals

Protocol of the study was passed by Institutional Ethics Committee of C. U Shah College of Pharmacy and Research, Wadhwan. The study was carried out with adult female Wistar rats weighing 180–300 g. Animals were acclimatized to experimental conditions in cages and kept under standard environmental conditions (22 ± 3◦C; 12/12 h light/dark cycle). Rats were allowed to feed and water ad libitum.

Plant Material

Dried fruits of *Randia dumetorum* were purchased from local market of Surendranagar, Gujrat, India. The fruits were identified and authenticated at Botany Department of Gujarat University by Dr. H. A. Solanki.

Preparation of the Extract

The fruits were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to soxhlet extraction with methanol for 72 hours at a temperature 50-60ºC. The

extract was concentrated by removal of the solvent. Extract was freeze dried and stored in the vacuum desiccators until further use.

Phytochemical screening

Phytochemical analysis of the major phytoconstituents of the plant extracts were undertaken using standard qualitative methods. [19]

Freund' Adjuvant Induced Arthritis In Rats:

The method used in the present study was described by Pearson and Wood, 1963.[20] Female wistar rats with an initial body weight of 200 to 300g were taken and divided into six groups each containing six animals. On day zero, all rats were injected into the sub plantar region of the left hind paw with 0.1ml of complete Freund's adjuvant (FA). This consists of 6mg *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6mg/ml.[20] Dosing with the test and standard compounds were started on the same day and continued for 12 days according to the following schedule:

Table 1 Dosing Shedual purposely from day 13th to 21st the animals were not be dosed with the test compound or the standard.

Evaluation of the development of arthritis

Rats were inspected daily for the onset of arthritis characterized by edema and/or erythema in the paws. The incidence and severity of arthritis were evaluated using a system of arthritic

Sr. No.	Group	No. of Animals	Treatment	Dose
1	Group 1 Normal Control	6	Normal Saline neither FA treated nor drug treated	----
2	Group 2 Disease Control	6	Freund's adjuvant (CFA) Distilled water	0.1ml (6mg/ml)
3	Group 3 Standard Treatment	6	Dexamethasone + CFA	5mg/kg CFA 0.1ml
4	Group 4 Methanolic Extract	6	Methanolic extract of *Randia dumetorum* Fruit + CFA	RD 100mg/kg CFA 0.1ml
5	Group 5 Methanolic Extract	6	Methanolic extract of *Randia dumetorum* Fruit + CFA	RD 200mg/kg CFA 0.1ml
6	Group 6 Methanolic Extract	6	Methanolic extract of *Randia dumetorum* Fruit + CFA	RD 300mg/kg CFA 0.1ml

scoring, and measurement of bi-hind paw volumes every 2 or 3 days beginning on the day when arthritic signs were first visible. Animals were observed for presence or absence of nodules in different organs like ear, fore paw, hind paw, nose and tail. Animal were score 0 for absence and 1 for presence of nodules. 5 was the potential maximum of combined arthritic score per animal. Hind paw volume was measured using plethysmometer. Paw volumes of both hind limbs were recorded from 21st day to 45th day at four day interval using mercury column plethysmometer. [21]

Total leukocyte Count and Neutrophile count

Blood samples were collected by puncturing the retro-orbital plexus into heparanized vials and analysed for total leucocyte counts (TLC) and differential leucocyte counts (DLC).[22]

Rheumatoid factor:

The latex turbidimetry method was used in the present study using RF TURBILATEX KIT of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml. The reading of RF factor of all the groups obtained was compared with the control animals and was expressed as IU/ml RF.[23]

Spleen Index:

Rats were sacrificed by cervical dislocation & Spleens were removed. All the spleens of rats were weighed immediately after dissection. The spleen indexes were calculated by using the following formula:[24]

$$\text{Spleen Index} = \frac{\text{spleen weight of CFA rat/body weight of CFA rat}}{\text{spleen weight of normal rat/body weight of normal}}$$

Radiography:

Female wistar rats were sacrificed on 45th day of collagen administration and legs were removed and placed on formalin containing plastic bag. This plastic bag was kept at a distance of 90 cm from the X-ray source was and Radiographic analysis of arthritic and treated animal hind paw were performed by X-ray machine with a 300-mA exposition for 0.01 s. An investigator blinded for the treatment regimen performed radiograph score. The following radiograph criteria were considered: These scores (destroyed or intact joint) were used as a quantal test for bone necrosis. Radiographs were carefully examined using a stereo microscope and abnormalities were graded as follows:

(i) Periosteaic reaction, 0 - 3 (None, Slight, Moderate, Marked);

(ii) Erosions, 0 - 3 (None, Few, Many Small, Many Large);

(iii) Joint space narrowing, 0 - 3 (None, Minimal, Moderate, Marked);

(iv) Joint space destruction, 0 - 3 (None, Minimal, Extensive, Ankylosis).

Bone destruction was scored on the patella as described previously.[25]

Histological processing and assessment of arthritis damage:

Rats were killed by ether anesthesia. Knee joints were removed and fixed for 4 days in 4% formaldehyde. After decalcification in 5 % formic acid, the specimens were processed for paraffin embedding tissue sections (7 µm thick) and were stained with haematoxylin and eosin, or safranin. An experienced pathologist, unaware of the different drug treatments scored the condition of tibiotarsal joints.[26-29]

Statistical analysis:

Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by student t test. The values $P < 0.05$ were regarded as significant and the values $P < 0.01$ were considered as highly significant.

RESULTS

Phytochemical screening

Preliminary phytochemical results showed the Presence coumarin, steroid, phenol, glycosides, terpenoid, Saponin and tannin.

Paw Volume

When compared with Normal Control rats, the Disease Control rats showed significant increase ($P < 0.001$) in the paw volume after seven days of sub plantar FCA administration. In the primary phase of the arthritis i.e. form day 4 to 7, there was non-significant deceased in the paw volume was observed. Rats treated with *Randia dumetorum* (100, 200 and 300 mg/kg) showed significant and dose-dependent attenuation ($P < 0.01$ and $P < 0.001$ respectively) in paw volume from day 12 onward as compared to Disease Control rats. Rat treated with Dexamethasone (5 mg/kg) significantly decreased ($P < 0.001$) paw volume from day 12 to 28 to as compared to control rats (Fig. 1,2).

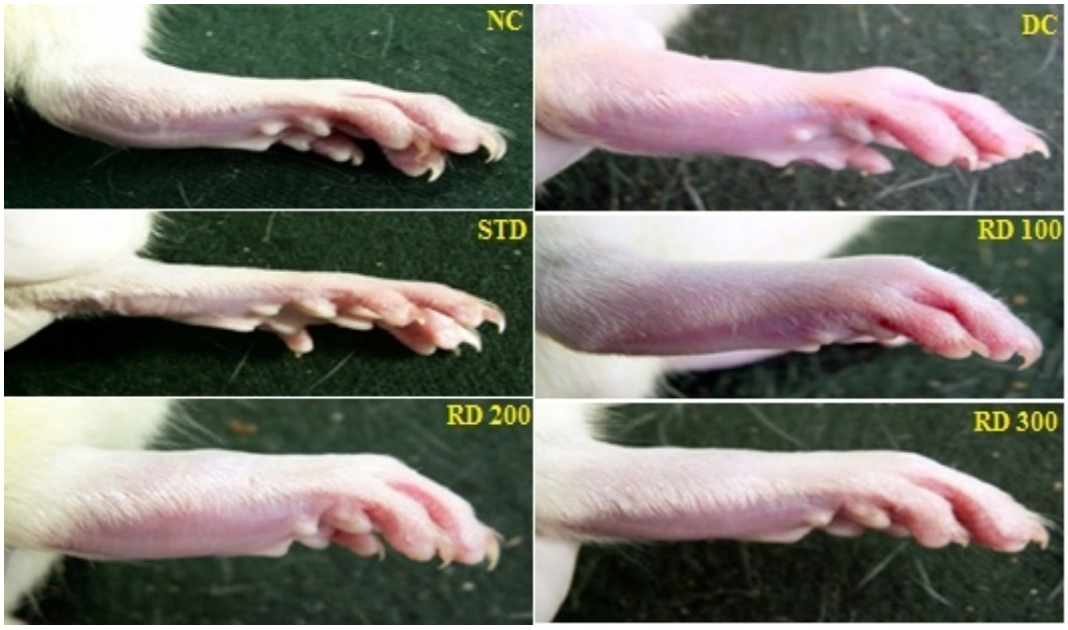

FIG 1 Effect of Methanolic extract of *Randia dumetorum* on Paw Edema.

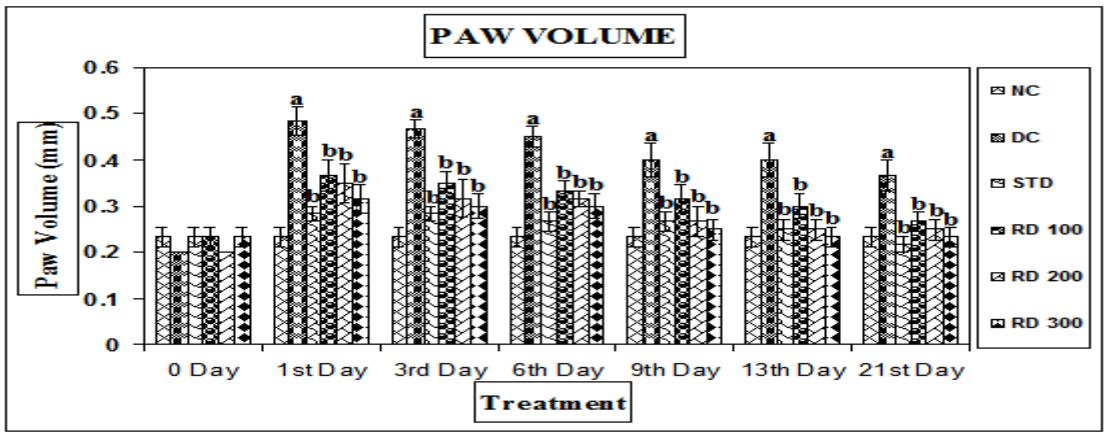

Fig 2 Effect of Methanolic extract of *Randia dumetorum* on Paw Volume.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

Arthritic Index

Sub plantar administration of FCA results in significant increased (P < 0.001) in arthritic score in all FCA treated rats (DC) as compared to Normal rats(NC) and this had shown a biphasic response. There was decreased in the arthritic score from day 4 to 7, however, this change was not significant. The arthritic score was significantly increased from day 7 to 12 in control rats which remained significantly increased till the end of the study i.e. up to 28th day as compared to Normal rats (NC). Rats treated with *Randia dumetorum* (100, 200 and 300

mg/kg) showed significant and dose dependant decreased in arthritic score ($P < 0.05$,0 $P < 0.01$ and $P < 0.001$ respectively) from day 12 onward till the end of the study as compared to control rats (Fig. 3).

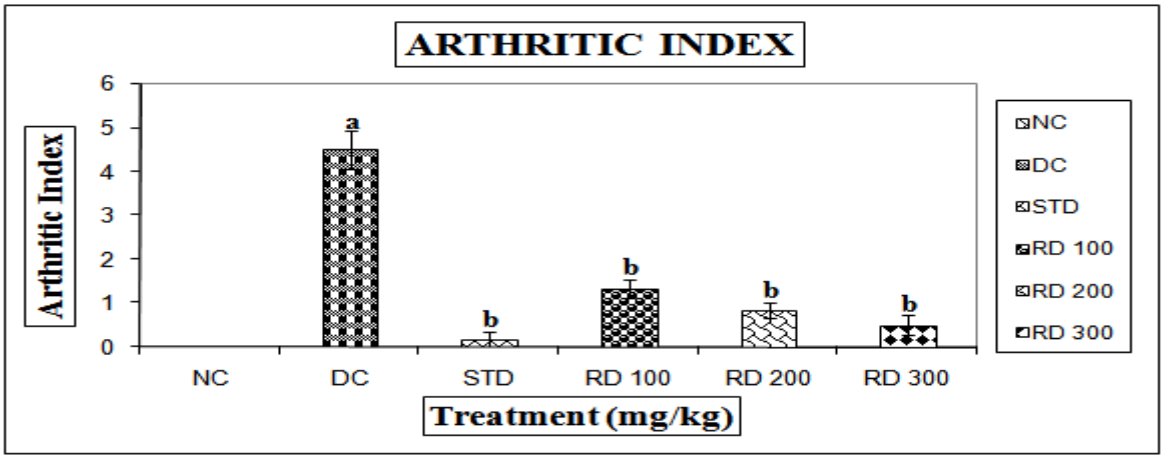

FIG 3 Effect of Methanolic extract of *Randia dumetorum* on Arthritic Index.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

Hematological Parameter

There was significant increase ($P < 0.001$) in WBCs in Disease Control (DC) rats as compared to Normal rats(NC). Rats treated with *Randia dumetorum* (100, 200 and 300 mg/kg) showed significant decrease ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) in WBCs when compared to Disease Control rats (DC). Whereas treatment with Dexamethasone (5mg/kg) significantly decrease WBCs ($P < 0.05$ and $P < 0.01$ respectively) as compared to control rats (FIG 4,5).

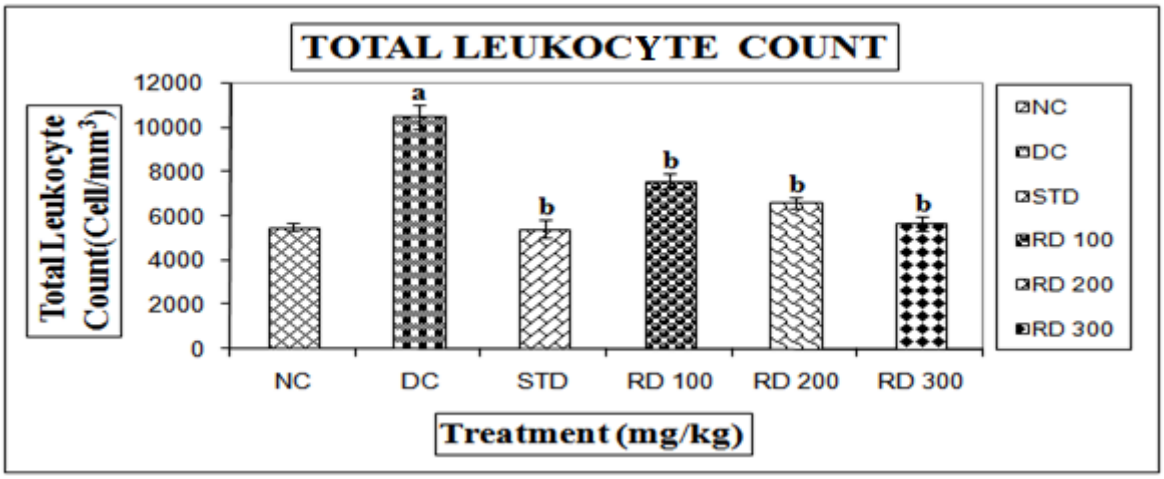

Fig 4 Effect of Methanolic extract of *Randia dumetorum* on Total Leukocyte Count.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

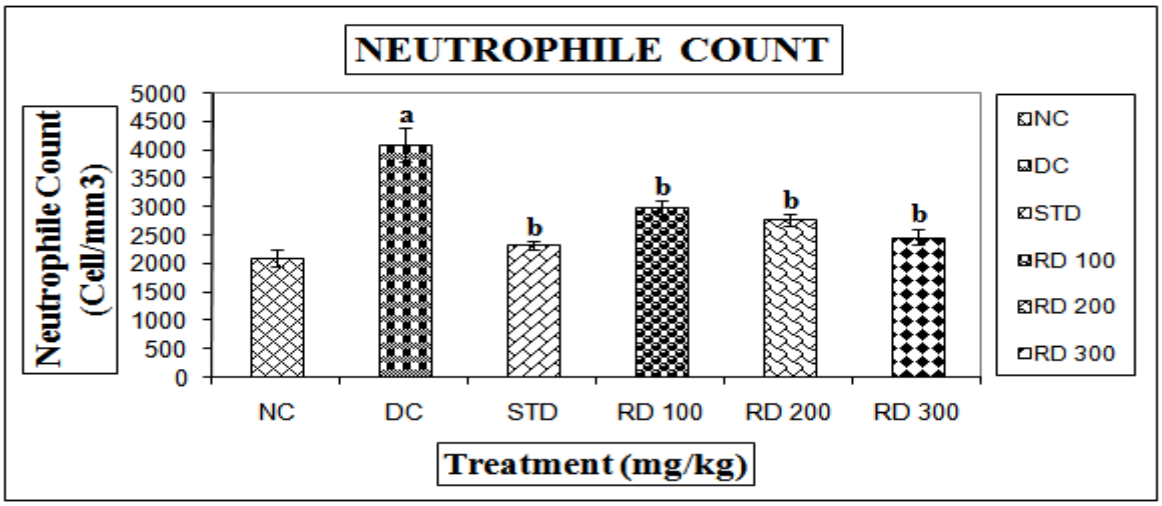

Fig 5 Effect of Methanolic extract of *Randia dumetorum* on Neutrophile Count.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

Rheumatoid factor & Spleen Index

As shown in Fig.6,7, Rheumatoid factor & Spleen Index were significantly decreased in treatment with *Randia dumetorum* (100 mg/kg, 200 mg/kg and 300mg/kg) and Dexamethasone (5 mg/kg) treated animal as compare to Disease Control(DC) treatment.

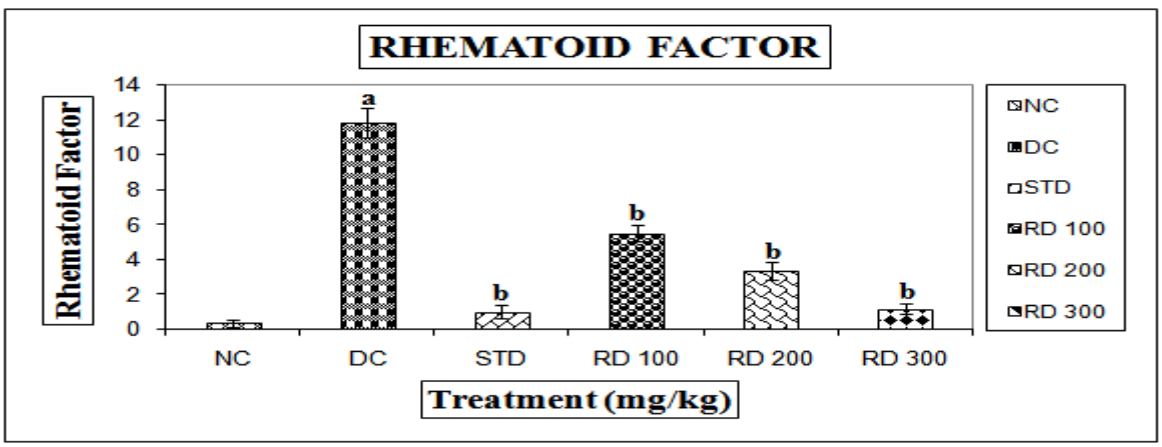

Fig 6 Effect of Methanolic extract of *Randia dumetorum* on Rheumatoid Factor.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

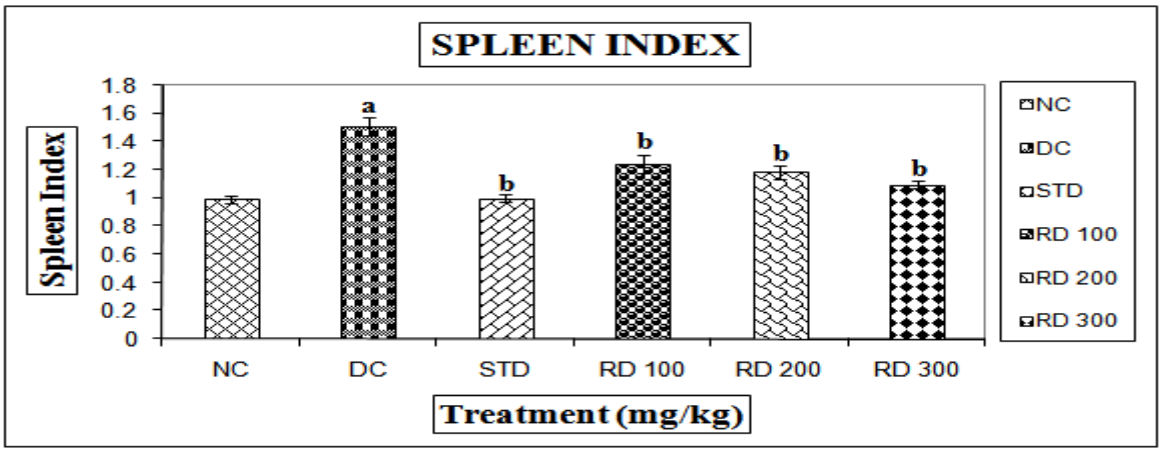

Fig 7 Effect of Methanolic extract of *Randia dumetorum* on Spleen Index.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

ESR

ESR level in rats treated with FCA was significantly increased (P < 0.001) as compared to Normal Control rats (NC). There was significant decrease in ESR in rat treated with *Randia dumetorum* (100, 200 and 300 mg/kg, P < 0.01 and P < 0.001 respectively) as compared to Disease Control rats (DC). Whereas, treatment with Dexamethasone (5 mg/kg) also significantly attenuated this increased (P < 0.001) level of ESR as compared to Disease Control rats (FIG 8).

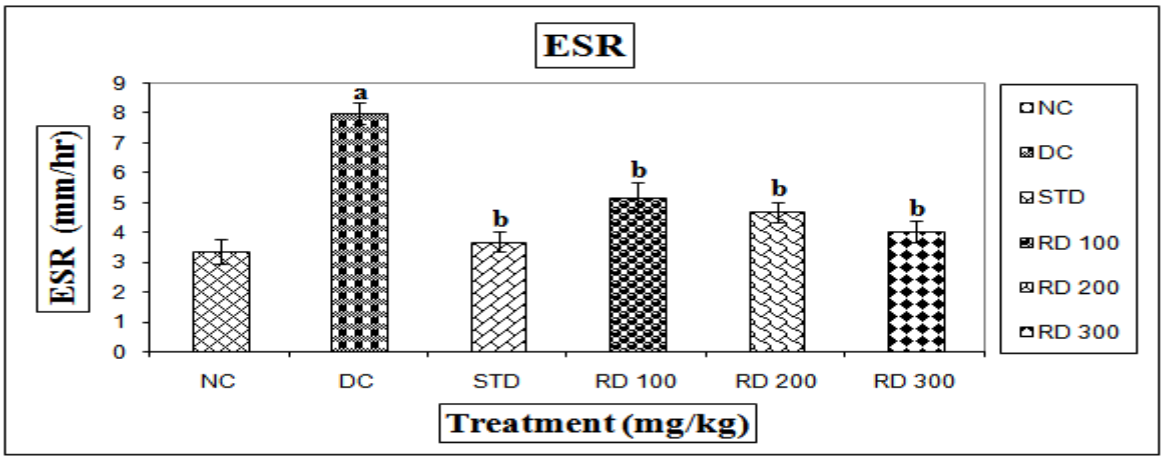

Fig 8 Effect of Methanolic extract of *Randia dumetorum* on ESR.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

Radiological Analysis

As shown in Fig.9,10, Bone destruction, which is a common feature of arthritis, was examined by radiological analysis. Collagen administered rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudowidening of all joint spaces. In contrast, in rats treated with RD attenuate abnormalities consisted of asymmetric soft tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws.

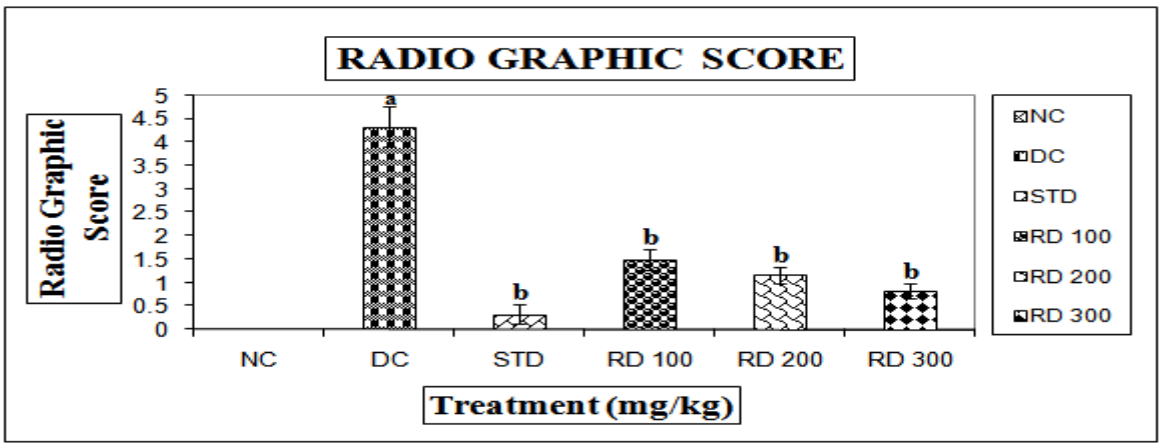

Fig 9 Effect of Methanolic extract of *Randia dumetorum* on Radiology Examination.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with normal control group. "**b**" indicate P value<0.01, when compared with disease control group. Statistical analysis was done by One –way ANOVA followed by post hoc Tukey test.

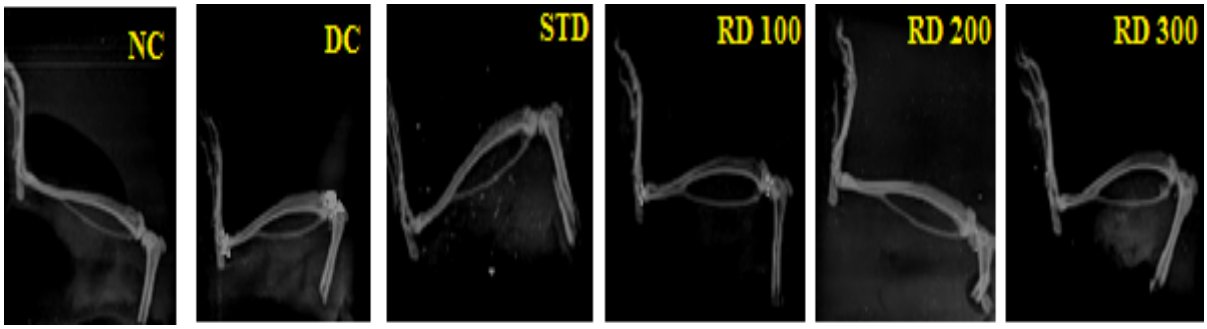

Fig 10 Effect of Methanolic extract of *randia dumetorum* on Radiographs of joints.

Histopathological Evaluation

Histopathological evaluation of the tibiotarsal joint of Disease Control rats (DC) showed massive influx of inflammatory cells, synovial hyperplasia with mono and poly-morphonuclear cells accumulation in the joint and edema associated with granuloma formation. It also shows the presence of higher degree of necrosis (Fig. 11). In the tibiotarsal joint Normal rats (NC), there was presence of normal connective tissue structure with the absence of necrosis present. It does not show any evidence of lymphocytic infiltration (Fig. 11). Treatment with Dexamethasone (5 mg/kg) showed normal connective tissue of tibiotarsal joint with the presence of lower degree of edema. There was absence of necrosis as well as lymphocytic infiltration (Fig. 11). Tibiotarsal joint of rats treated with *Randia dumetorum* (100, 200 & 300 mg/kg) showed presence of mild necrosis with low amount of mono and poly-morphonuclear cells accumulation. It does not show any sign of granuloma formation but mild edema was present (Fig. 11).

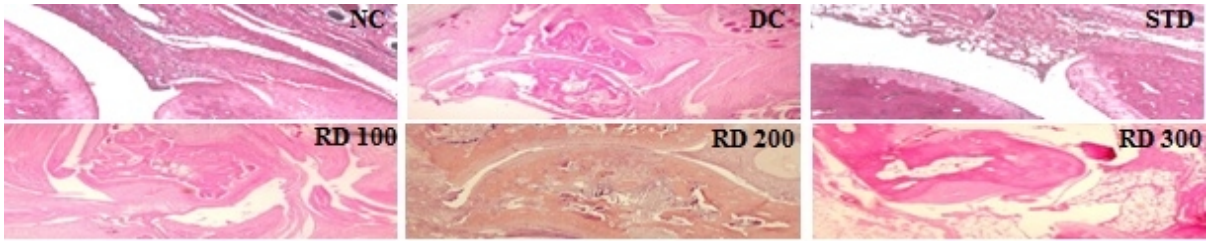

Fig 11 Effect of Methanolic extract of *Randia dumetorum* on histopathology of synovial joint.

DISCUSSION

Freund's Complete Adjuvant- induced arthritis are well established rat model and has been widely used from many years for evaluation of anti-inflammatory and anti-arthritic potential of various agents.[30,31] An array of changes occurred after the administration of FCA in rats including joints swelling, infiltration of inflammation cells, bone destruction, joint cartilage erosion and remodeling which results in the destruction of joint integrity and function disability. In present investigation, treatment with *Randia dumetorum* methanolic extract (RD) exerts its anti-inflammatory potential via acting on various inflammatory mediators.

Freund's Complete Adjuvant is a inactivated and dried mycobacteria which are mainly responsible for stimulation of cell-mediated immunity which ultimately increased the production of certain immunoglobulins. FCA induced arthritis is a primary and secondary chronic arthritis. [31] Primary is inflammatory phase where generation of prostaglandin occurs and secondary immunological state in which autoantibodies is generated.

Release of various inflammatory mediators including cytokines (IL-1B and TNF-alpha), MCSF, interferon's and Platelet derived growth factor (PDGF) are responsible for the initiation of pain along with swelling of the limbs and joints, bone deformations and disability of joint function.[32] Significant increased in the paw thickness after subplantar administration of FCA is reflecting the status of arthritis. Treatment with *Randia dumetorum* significantly decreased the thickness of paw via inhibition of release of inflammatory mediators, indicating its anti-inflammatory potential in FCA induced arthritis.
In FCA induced arthritis, arthritis score is index of the joint inflammation after immunization.[33] A selective reduction in the arthritis score distinguishes the immunosuppressive effects of *Randia dumetorum* from its anti-inflammatory effects.

Spleen is a vital organ involved in immune responses. In adjuvant arthritis, spleen serves as the reservoir for the cells and antibody formation which involved in the immune response. Increased in the weight of spleen is associated with the splenomegaly, generalized lymphadenopathy and altered hepatic function.[34] Subplantar administration of FCA significantly increased weight of spleen and decreased the weight of thymus which is in accordance with previous studies of [35]. Decrease in spleen weight and increase in thymus weight might be due to immune-stimulatory effect of *Randia dumetorum* Extract.

ESR is an index of suspension stability of RBC's in plasma. The number and size of RBS is associated with ESR. It also involved in the accelerated formation of endogenous proteins including plasma proteins such as fibrinogen, alpha and beta globulins. ESR is elevated during the inflammation, stress and cell necrosis.[36] In the present investigation treatment with XSEE significantly restores decreased level of RBC along with Hb and elevated level of ESR attributing its anti-inflammatory potential.

In elevated level of the IL-1 inflammatory response in FCA induced arthritis results in increase in granulocyte and macrophages colony stimulating factors which is associated with elevated level of WBC [32,36] which plays a major role in body defense mechanism. XSEE might play important role in inhibition of the release of inflammatory mediator that may decrease WBC level in FCA induced arthritis.

Abrogation of disease progression by abatacept was further supported by the histopathologic analysis of the joints from these animals. Rats that had been prophylactically treated with abatacept at the time of CFA immunization showed no histologic abnormalities, with no evidence of cartilage erosion and bone resorption in their joints in contrast to the diseased control rats that displayed completely destroyed joint architecture. CFA-induced arthritic scores were reduced across all four disease parameters assessed (inflammation, pannus formation, cartilage damage and bone resorption). Consistent with previous studies, the current findings support the role of the CD28 co-stimulation pathway in the development of CFA and the novel mechanism of action of abatacept in preventing disease onset.

CONCLUSION

This study has indicated that *Randia dumetorum* extracts exhibits a potential protective immunomodulary effect by humoral as well as cell mediated immune mechanisms. Analgesic effect of *Randia dumetorum* was observed and also exerted strong anti-inflammatory effects. All these results thus predict that the drug provide pharmacological rationale for the traditional use of the drug against inflammatory disorders such as rheumatoid arthritis.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Principal and management of C U Shah College of Pharmacy and Research, Wadhwan for providing necessary facilities to carry out this work.

Our acknowledgement to Dr. H A Solanki for their technical assistance in the plant collection and identification.

REFERENCES

1. Salerno L, Sorrenti V, Giacomo CD, Romeo G, Siracusa MA. Progress in the development of selective nitric oxide synthase (NOS) inhibitors. Curr Pharm Des, 2002; 8: 177–200.
2. Begum S, Hassan SI, Ali SN, Siddiqui BS. Chemical constituents from the leaves of Psidium guajava. Nat Prod Res, 2004; 18(2): 135–40.
3. Singh S, Majumdar DK. Effect of fixed oil of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. Int J Pharmacol, 1996; 34(3): 218–22.
4. Nadkarni AK. Indian Materia Medica, Mumbai: Popular Prakashan, 1954; p. 1298.
5. Campbell SM. Rheumatoid arthritis: current strategies. Hosp Med, 1988; 34: 29–32.
6. Scott DL, Shipley M, Dawson A, Edwards S, Symmons DP, Woolf AD. The clinical management of rheumatoid arthritis and osteoarthritis: strategies for improving clinical effectiveness. Brit J Rheumatol, 1998; 37: 546–54.
7. Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-bailey D, Croxtall J, et al. Inducible isoforms of cyclooxygenase and nitric oxide synthase in inflammation. PNAS, 1994; 91: 2046–50.
8. Pincus T, Marcum SB, Callahan LF. Long-term drug therapy for rheumatoid arthritis in seven rheumatology private practices. II. Second line drugs and prednisone. J Rheumatol, 1992; 19: 1885–94.
9. Quan LD, Thiele GM, Tian J, Wang D. The development of novel therapies for rheumatoid arthritis. Expert Opin Ther Pat, 2008; 18: 723–38.
10. Lawrence RC, Helmick CG. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum, 1998; 41: 778–99.
11. Payne R. Limitations of NSAIDs for pain management: toxicity or lack of efficacy? J Pain, 2000; 1: 14–8.
12. Simon LS. DMARDs in the treatment of rheumatoid arthritis: current agents and future developments. Int J Clin Pract, 2000; 54: 243–9.
13. Park HJ, Lee JS, Hong MS, Kim CJ, Kim JW, Lim S. The anti-nociceptive and anti-inflammatory effect of Achyranthes japonica. Korean J Orie Med, 2004; 25: 8–14.

14. Halliwell B, Hoult JR, Blake DR. Oxidants inflammation and anti-inflammatory drugs. FASEB J, 1988; 2: 2867–73.
15. Kirtikar KR, Basu BD. Indian Medicinal Plants, Panni office, Bhuwaneswari Ashrama, Bahadurganj, Allahabad, 1991; p.648-652.
16. Agrawal SS, Singh VK. Immuno modulators-A review of studies on Indian medicinal plants and synthetic peptides, Part- 1, Medicinal plants, Proc. Indian Natl Sci Acad B, 1999; 65: 179-204.
17. Subramaniam S, Michael Bokel, Wolfgang K. A hemolytic saponin Randianin from *Randia dumetorum.* Phytochem, 1989; 28: 1544-1546.
18. Dastur JF. In Medicinal Plants of India and Pakistan, Bombay, D. B. Taraporevala Son's & Co. Pvt. Ltd., 1962; p.140.
19. Khandelwal KR. Practical Pharmacognsoy, 10th edition, Nirali Prakashan, Pune, 2003; p.162-65.
20. Pearson CM, Wood FD. Studies on polyarthritis and other lesions induced in rats by injection of mycobacterium adjuvant, I. General clinic and pathological characteristics and some modifying factors. Arth rheum, 1959; 2: 440-459.
21. Van de Berg WB, Joosten LAB, Helsen MMA, van de Loo FAJ. Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. Clin Exp Immunol, 1994; 95: 237–243.
22. Wilkonson PC. Neutrophil adhesion test. In: Vane JR, Ferraria SH (eds). Handbook of Experimental Pharmacology, Vol. 1. Berlin: Springer Verlag, 1978; p.109.
23. Joosten LAB, Helsen MMA, Van de Loo FAJ, van den Berg WB. Anticytokine treatment of established collagen type II arthritis in DBA/1 mice: a comparative study using anti-TNF alpha, anti-IL-1 alpha, beta and IL-1Ra. Arthritis Rheum, 1996; 39: 797–809.
24. Chen Chunxia, Zhang Peng, Pi Huifang, Ruan Hanli, Hu Zehua,Wu Jizhou. Extracts of Arisaema rhizomatum C.E.C. Fischer attenuate inflammatory response on collagen-induced arthritis in BALB/c mice. Journal of Ethnopharmacology, 2011; 133, 573–582.
25. Joosten LAB, Lubberts E, Helsen MMA, van den Berg WB. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. J Immunol, 1997; 159: 4094–4102.

26. Taniguchi K, Kohsaka H, Inoue N, Terada Y, Ito H, Hirokawa K, Miyasaka N. Induction of the p16INK4a senescence gene as a new therapeutic strategy for the treatment of rheumatoid arthritis. Nat Med, 1999; 5: 760-767.
27. Robert H, Shmerling, et al. The Americn Journal of Medicine, 1991; 91: 528-534.
28. Weyand CM. Newinsights into the pathogenesis of rheumatoid arthritis. Rheumatology, 2000; 39: 3–8.
29. Weyand CM. Newinsights into the pathogenesis of rheumatoid arthritis. Rheumatology, 2000; 39: 3–8.
30. Costa B, Colleoni M, Conti S, Parolaro D, Franke C, Trovato AE, et al. Oral antiinflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn- Schmiedeberg Arch Pharmacol, 2004; 369: 294–9.
31. Walz DT, Dimartino MJ, Misher A. Adjuvant-induced arthritis in rats. II. Drug effects on physiologic and biochemical and immunologic parameters. J Pharmacol Exp Ther, 1971; 178: 223–31.
32. Eric GB, Lawrence JL. Rheumatoid arthritis and its therapy, The textbook of therapeutics drug and disease management, Baltimore: Williams and Wilkins company, 1996; p.579–95.
33. Yu Y, Xiong Z, Lv Y, Qian Y, Jiang S, Tian Y. In vivo evaluation of early disease progression by X-ray phase-contrast imaging in the adjuvant-induced arthritic rat. Skeletal Radiol, 2006; 35(3): 156–64.
34. Ismail MF, EL-Maraghy SA, Sadik NAH. Study of the immunomodulatory and antiinflammatory effects of evening primrose oil in adjuvant arthritis. African J Biochem Res, 2008; 2(3): 74–80.
35. Pedernera AM, Guardia T, Calderon CG, Rotelli AE, Rocha NE, Genaro SD, et al. Antiulcerogenic and anti-inflammatory activity of the methanolic extract of Larrea divaricata Cav. in rat. J Ethnopharmacol, 2006; 105(3): 415–20.
36. William JK, Arthritis, allied condition. A textbook of rheumatology, Baltimore, Tokyo: Waverlay Company, 1996; p.1207.