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METHANOLIC FRUITS EXTRACT OF *RANDIA DUMETORUM* SUPPRESSES INFLAMMATION AND CARTILAGE DESTRUCTION IN COLLAGEN-INDUCED ARTHRITIC RAT.

Ritesh G. Patel*[1],
Nimish L. Pathak[1], Jaimik D. Rathod[1], Dr. Laxmanbhai D. Patel[1], Ravi U. Thaker[2].

[1]Department of Pharmacology, C.U.Shah College of Pharmacy and Research, Wadhwan, Gujarat-363030.
[2]Department of Pharmacology, Maliba Pharmacy College, Gopal Vidhyanagar, Bardoli, Surat.

ABSTRACT

Randia dumetorum has multiple applications in traditional medicine because it exhibit Analgesic, Antifertility, Antibacterial, Anti-inflammatory and 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 collagen-induced arthritis (CIA) 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 collagen-induced arthritis (CIA) animal model. CIA was induced in female Wistar rats by intradermal injection of bovine collagen-II in Freund's incomplete adjuvant (IFA). CIA 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 45. The results showed that treatment with RD markedly reduced paw swelling and arthritic index even in the established CIA. Radiologic and histopathologic changes in the arthritic joints were also significantly reduced in the RD-treated versus vehicle-treated rats. Moreover, Rheumatoid factor was significantly reduced in RD 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, collagen-induced arthritis.

Correspondence to Author

Ritesh G. Patel

92, Ramnagar Society, Ankleshwar-393001, Dist: Bharuch, Gujarat, India.

Email: ritesh_patel_pharma@yahoo.com

INTRODUCTION

Rheumatoid arthritis (RA) is a kind of chronic immunological and inflammatory disease. RA progression is associated with an imbalance of Th1/Th2 and overproduction of antigen-specific immunoglobulins [1]. One of the most widely used models for studying RA is collagen-induced arthritis (CIA) in female rat, which shows many features with human rheumatoid arthritis [2]. This destructive inflammatory arthritis, called as CIA, can be induced in mice by the immunization with type II collagen [3]. This evidence and other observations suggest that autoimmunity to type II collagen play a role in the pathogenesis of RA. IgG anti-type II collagen antibodies can be commonly found in both RA and non-RA. IL-10 has been reported to exert a protective effect in CIA at high doses [4]. IL-18 are pro-inflammatory cytokines, and IL-18 is a member of IL-1 cytokine family that was originally identified [5,6]. In addition, IL-18 is a novel cytokine with pleiotropic activities that is critical to the development of Th1 responses in synergy with IL-12 [5]. It has been demonstrated that RA synovial tissues showed high IL-18 mRNA expression and high IL-18 protein synthesis as well as IL-18 receptor expression [7]. Furthermore, IL-18 is at the epicenter of the inflammatory process in CIA mice [8,9].

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 [10]. The methanol extract showed the presence of glycosides, randioside A, mollisidial triterpenoid glycosides and randianin, six saponins-dumetoronins A to F [11]. 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 [12]. 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 [11]. 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 [13].

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.

Induction of CIA and RD treatment:

Arthritis was induced in female Wistar rats using Collagen. Collagen was dissolved overnight at 4°C in 0.1M Acetic acid to prepare concentration 2mg/ml. This solution was added drop wise to an equal volume of chilled incomplete freund's adjuvant (IFA). On day first, animals (Six groups, each containing six animals) receive total 0.5 ml IFA, equally divided in 5 sites. All injections are intradermal, one at the base of each appendages and one in the nape of neck. Seven days post-immunization, the animals receive identical booster injections. In the therapeutic treatment protocol for the established CIA, treatment with *Randia dumetorum*, Dexamethazone, and vehicle

were initiated on the day after the onset of arthritis (day 21) and continued once daily until day 45 of the experiment[14]. Rats were treated orally with different dose of *Randia dumetorum*(RD) (100, 200, 300 mg/kg of body weight), Dexamethazone (1mg/kg) and vehical until day 45 of experiment.

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 scoring, and measurement of bi-hind paw volumes every 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 [15].

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:

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

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 [16].

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 [17].

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[18-21].

RESULTS:

Following the primary and booster injections of CII/IFA, rats developed arthritis from day 21st onwards, and treatment with RD, Dexamethasone (1 mg/kg), and vehicle were initiated on day 21st after the primary immunization and continued until day 45th of the experiment. As shown in Fig. 1,2, even after the onset of arthritis, RD at high doses (100, 200 and 300 mg/kg) did markedly reduce hind paw volume of the arthritic rats in a dose-dependent manner as compared to the vehicle-treated arthritic rats.

As shown in Fig.3,4,5, Arthritic index, Rheumatoid factor & Spleen Index were

significantly decreased in treatment with RD (100 mg/kg, 200 mg/kg and 300mg/kg) and dexamethasone (1 mg/kg) treated animal as compare to disease control treatment.

As shown in Fig.6,7, 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.

As shown in Fig.8, [NC]: Histology of synovial joint of normal control rat with intact morphology of synovium and synovial lining, [DC]: CA induced disease control rat showed plenty of lymphocytic infiltration [↓]in synovial lining with severe inflammation and marked angiogenesis [↕] studied with proliferation of synovial cells [↓↓], [DEXA]: Dexamethasone [5 mg/kg] treated rats showed significant protection with mild lymphocytic infiltration [↓] with no evidence of thickening of synovial lining and angiogenesis, RD 100: RD 100 treated rats showed milder angiogenesis [↕], lymphocytic infiltration [↓] and synovial lining thickening[↓↓]. RD 200: RD 200 treated rats showed milder angiogenesis [↕], synovial lining thickening [↓↓] with no evidence of lymphocytic infiltration. RD 300: RD 300 treated rats showed milder lymphocytic infiltration [↓] and synovial-lining thickening [↓↓] with no evidence of angiogenesis.

Evaluation of the development of arthritis:

Paw Edema:

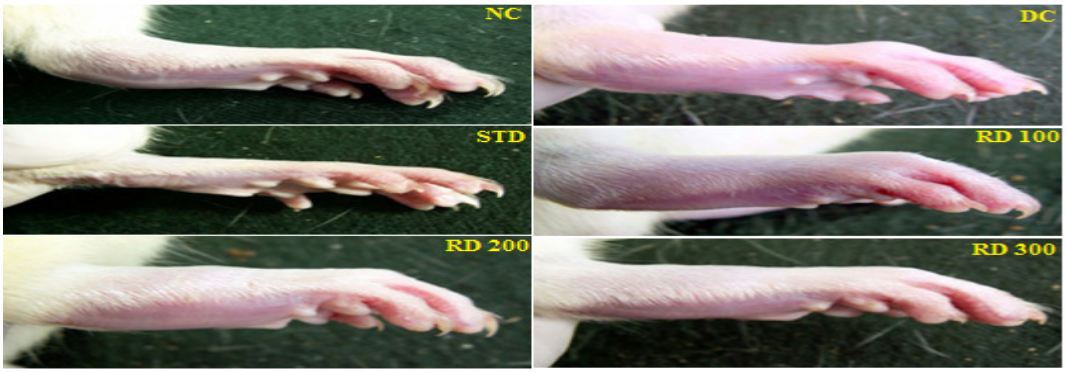

FIG 1 Effect of Methanolic extract of *Randia dumetorum* on Paw Edema.

Paw Volume:

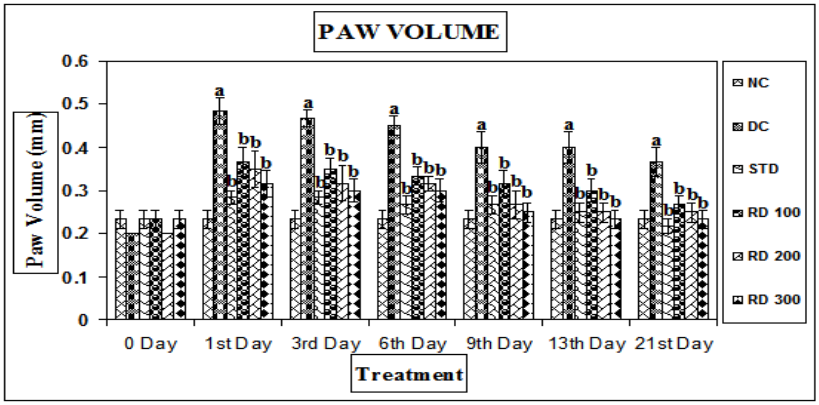

FIG 2 Effect of Methanolic extract of *Randia dumetorum* on Paw Volume.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with disease 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:

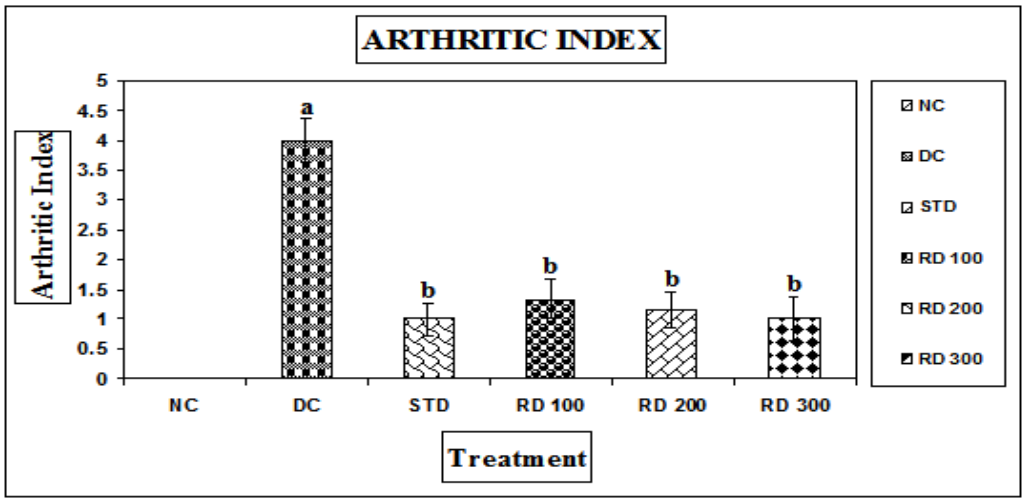

FIG 3 Effect of Methanolic extract of *Randia dumetorum* on Arthritic Index.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with disease 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.

Rhematoid Factor:

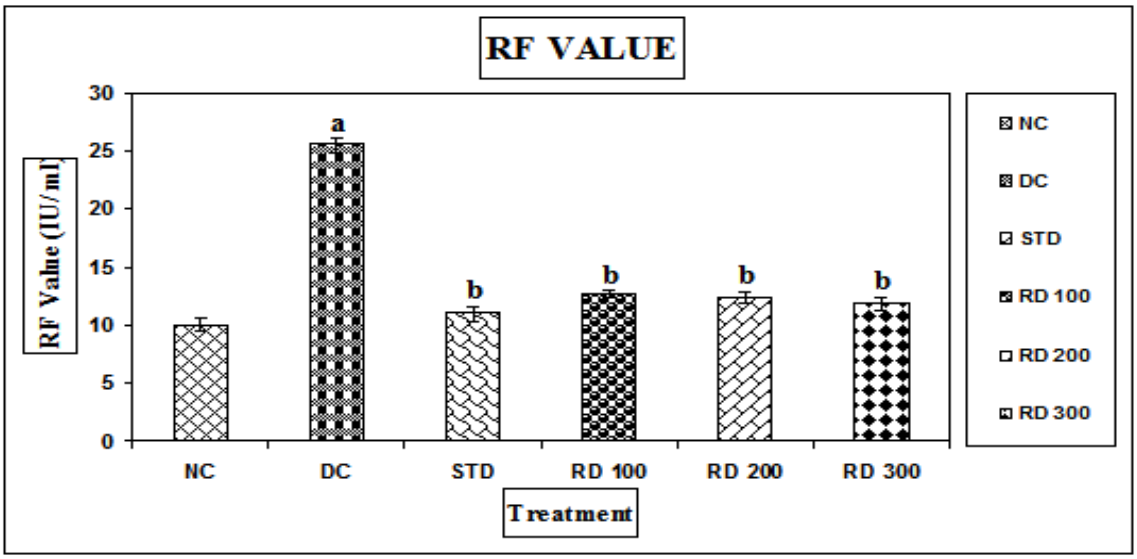

FIG 4 Effect of Methanolic extract of *Randia dumetorum* on RF Value.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with disease 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.

Spleen Index:

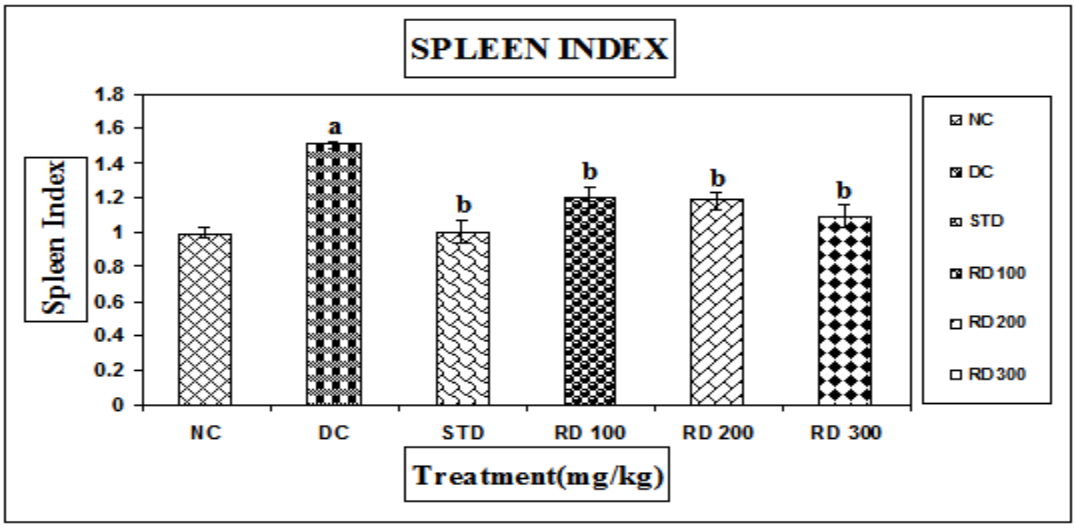

FIG 5 Effect of Methanolic extract of *Randia dumetorum* on Spleen Index.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with disease 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.

Radiology Examination:

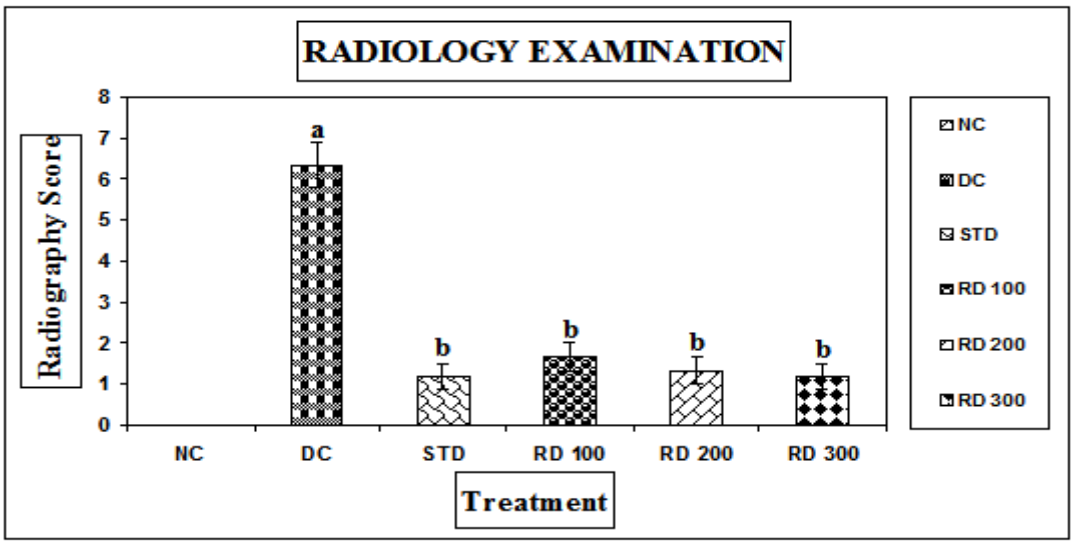

FIG 6 Effect of Methanolic extract of *Randia dumetorum* on Radiology Examination.

Each bar represents the mean ± SEM. Number of animals in each group 6. "**a**" indicate P value<0.001,when compared with disease 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.

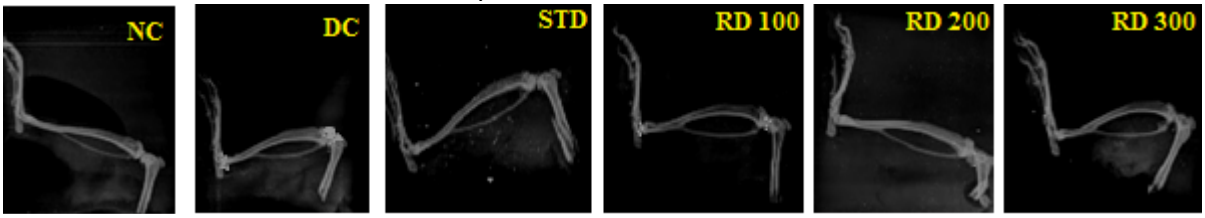

FIG 7 Effect of Methanolic extract of *randia dumetorum* on Radiographs of joints

Histological Examination:

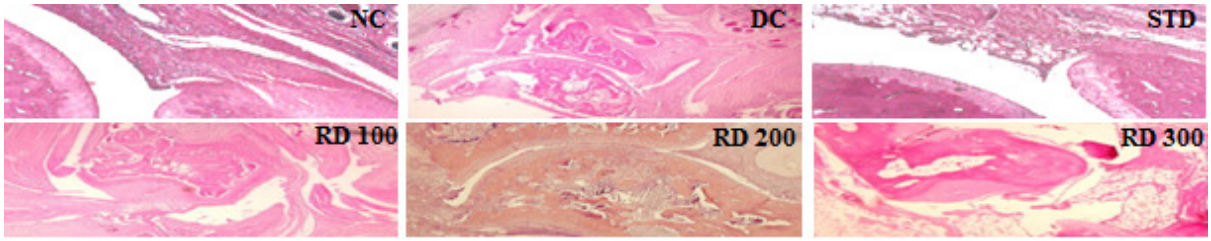

FIG 8 Effect of Methanolic extract of *Randia dumetorum* on histopathology of synovial joint

DISCUSSION:

RA is a complicated refractory autoimmune disease characterized by a number of the inflammatory and destructive events such as joint pain and swelling, synovial hyperplasia, pannus formation, structural damage to cartilage, bone, and ligaments, joint malformation [22]. Collagen II (CII) is a major protein constituent of joint cartilage that is a common target attacked by immune cells when an immunization provokes an autoimmune response. CIA is a well-established in vivo model that has been used in numerous studies to investigate the pathogenesis of RA and for identification of potential therapeutic targets [23]. Recent research evidenced that the main pathological of RA was strongly related with high levels of pre-inflammatory cytokines such as TNF-_, IL-1_, IL-6 and IL-33 in serum [24]. The current study examined the effects of *Randia dumetorum* on disease induction and progression in CIA, a rat model of RA.

In our studies, RD has anti-arthritis efficacy and inhibited various aspects of inflammatory process in CIA: arthritis deterioration, the secretion of pro-inflammatory cytokines and RA factor.

The histopathologic analyses revealed that, in relation to the development of inflammatory and arthritic lesions in the joints, most of the vehicle-treated CIA mice experienced proliferation of synoviocyte, pannus formation, cartilage hyperplasy and/or destruction and even bone erosion. In contrast, treatment with RD not only showed significant reduction of synovial cellular infiltration, joint space narrowing, pannus formation, but also markedly protected the affected joints against cartilage destruction and bone erosion.

In most groups of RD treatment, results showed dose dependent effect. Treatment with RD effectively reduced of paws edema. On the 16th day RD(100mg/kg), RD(200mg/kg), RD(300mg/kg) were firstly appeared reduction of paws and knee joints edema.

The level of RF & Spleen Index was observably depressed in all groups treated with RD, These in vivo results demonstrated that RD was effective on suppressing the development of CIA in rat.

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 collagen 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. Collagen-induced arthritic scores were reduced across all four disease parameters assessed (inflammation, pannus formation, cartilage damage and bone resorption). Consistent with previous studies [25], the current findings support the role of the CD28 co-stimulation pathway in the development of CIA 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.

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