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Research Article

Ameliorating Effect of *Capparis aphylla* Roth. in Adjuvant-induced Arthritis in rats with Reference to Changes in Reactants RF, CRP, Radiological Erosions

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ABSTRACT

Despite abundant anti-arthritic therapies, there always remains an opportunity to identify novel drugs as well as targets. One such emerging drug *Capparis aphylla* Roth. acting on inflammatory cascades. This study was planned to investigate the effect of *C. aphylla* Roth. on inflammatory cascade in rheumatoid arthritis induced in wistar albino rats. The rheumatoid arthritis was induced by injection of 0.2 mL complete Freund's adjuvant. The animals were randomized into six groups receiving either vehicle (control), injection of 0.2 mL complete Freund's adjuvant (model) or treatments (indomethacin 100 mg/kg and methanolic extract of *C. aphylla* Roth. 190, 240, 300 mg/kg) for 21 days. Various hemodynamic parameters, anti-inflammatory parameters, and x-ray and histopathology of synovial joints were carried out. Paw volume, body weight, arthritic index, ESR, RF, CRP, A/G ratio estimated. Treatment with methanolic extract of *C. aphylla* Roth. Significantly prevented the rise in body weight, serum A/G ratio and increased arthritic index, paw volume, ESR, RF, CRP. The normal architecture of synovial joints was preserved in histopathological analysis by methanolic extract of *C. aphylla* Roth. treatment. Furthermore, the prevention of damage in joints carried out by methanolic extract of *C. aphylla* Roth. Altogether, methanolic extract of *C. aphylla* Roth. prevented architecture and functions of joints by augmenting the inflammation in complete Freund's adjuvant rats. Such promising effects are attributed to inflammation by novel herbal drug *C. aphylla* Roth.

INTRODUCTION

Since the precise order of many mechanisms in disease origin and propagation is yet unknown for rheumatoid arthritis (RA), it is challenging to develop a unified pathogenesis hypothesis. However, generalizations are made that minimize the significance of the subsequent immunological and non-immunological inflammation caused by arthritic changes (rheumatic joint destruction) RA.^[1] The rheumatoid joint's five main causes of inflammation and destruction, intracellular signaling and proliferation, adhesion, inflammation, matrix degradation, and angiogenesis, lead to a vicious cycle that continues over time. The cause of the prevalent chronic inflammatory

autoimmune disease known as RA is uncertain. It results in irreversible functional and anatomical joint impairment and affects less than 1% of the population. Modern rheumatology's focus is early diagnosis using new diagnostic technologies because it is crucial for early therapy with disease-modifying medications (DMARD).^[2] In RA's inflammatory articular process that leads to synovial proliferation and cartilage degradation, cytokines play a key role.

Acute phase response (like CRP), aberrant immunoglobulin levels, and elevated rheumatoid factors (RFs) are also thought to be modifying events during inflammatory and immunogenic reactions in addition to proinflammatory

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cytokines.^[1] The sole immunological RA marker included in the categorization criteria is RF, which has been recognized for over 50 years. About 80% of sera from RA patients include RF.^[2] In addition to the outcome metrics, radiological erosion has historically served as the “gold standard” for determining the severity of anatomical alterations in RA.^[1] A typical aspect of many inflammatory arthritis is bone loss. Osteoblast activity and number are increased as a result of an inflammatory reaction, which causes bone degradation. One of the key indicators for the diagnosis of RA is the presence of bone erosions and peri-articular osteopenia.

A systemic inflammatory condition with alterations to the bone and cartilage resembling those seen in RA is shown by the full Freund's adjuvant-induced arthritis model. The pathophysiological similarities between RA and adjuvant arthritis in humans include bone resorption, cellular invasion of the joint area, and joint swelling. When adjuvant is injected into the foot pad, severe arthritis is created, which leads to strong bone loss. It is a distinguishing hallmark of RA that the synovium, commonly called the “pannus,” lines the joint capsule with cartilage and bone and is the primary source of permanent tissue destruction.^[3] A further distinction was made between outcome measurements, such as death or the advancement of radiological erosions, and process measures, such as joint scores (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels). Acute changes are reflected in the former, whereas cumulative harm is reflected in the latter.^[1]

For rheumatoid arthritis, sciatica, enlarged cervical glands, swellings, skin eruptions, and ringworm, *Capparis aphylla* Roth. has been used traditionally as an anti-inflammatory agent.^[4] Second, the root bark of *C. aphylla* Roth. is rich in spermidine alkaloids, which are used to treat inflammation, asthma, and gout.^[4] It is believed that the various components of *C. aphylla* Roth. have analgesic, diaphoretic, alexiteric, laxative, anthelmintic, antibacterial, antifungal, and antiviral properties and are effective against cough, asthma, ulcers, boils, vomiting, piles, and all inflammations.^[5] In the conventional medical system, it has been demonstrated that the bark is effective in treating inflammation, coughing, and asthma; the roots are effective in treating fever; and the buds are effective in treating boils.^[6] Sundaram MS *et al.* ameliorated effectiveness of Guggulipid in adjuvant induced arthritis by suppressing inflammation. According to Sahdeo Prasad, arthritis is treated using ayurvedic botanicals that reduce inflammation. A total of 250 species of woody climbers, trees, and shrubs make up the genus *Capparis*.^[6] The plant, a member of the Capparidaceae family, is also called caper berry, karira, kerada, karer, or kair. The plant typically thrives in wastelands and other dry, exposed habitats, frequently on slopes. It can be found in the deserts, particularly in Rajputana, Punjab, and Sind. It can also be found in the wild in the Western Ghats, Rajasthan, and

Gujarat. The plant can grow up to 6 meters tall (occasionally 10 meters), with a clear bole of 2.4 meters.^[7] It is a big, climbing, thorny shrub that is heavily branched and spinous.

Consequently, the present study has been undertaken to illustrate the beneficial outcome of the drug kalpa amruthaa (KA) in adjuvant-induced arthritic rat model in reducing the pathological lesions caused by the proinflammatory cytokines via analyzing the effect of KA on the protein expressions of TNF- α and IL-1 β , on the levels of acute phase proteins, immunoglobulins and on the radiological, histopathological and electron microscopical changes.^[1] Most of the time, DMARDs are used to treat arthritis, however, it has been recognized that they can also contribute to and speed up atherosclerosis, which increases the stress on the cardiovascular system. They also impact vascular healing processes and endothelial damage. Further study is heavily on the fundamental cause in order to reduce these adverse effects. This study offers crucial support for the use of plants to reduce inflammation at its source in the treatment of arthritis.

MATERIALS AND METHODS

Chemicals and Reagents

The dandruff's reagent, methanol, alumina oxide, ethyl acetate, Na azide, Na citrate, succinic acid, bromocresol, brij-95, formalin, formic acid, Freund's adjuvant was obtained from Astron chemical, Ahmedabad, Gujarat, India. The total protein kit, RF latex kit, CRP kit were obtained from Coral Clinical System, Gujarat, India.

Collection and Authentication of the Plant Material

Roots of *C. aphylla* Roth. was obtained from the herbal farm at Amreli. The plant was authenticated by Dr. Geetha K.A. (senior scientist) at Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India.

Experimental Animals

Female wistar albino rats weighing 200–250 g were purchased from the Zydus Research Center Ahmedabad. The animals were fed *ad libitum* and housed in a room with a controlled ambient temperature ($22 \pm 2^\circ\text{C}$), humidity ($50 \pm 10\%$), and a 12-h light/dark cycle. Animals were acclimated to the housing conditions and handled for 3–4 days before experiments. All experiments were performed between 08:00 AM and 4:00 PM. All experimental procedures were conducted according to the OECD Guidelines for the Care and Use of Laboratory Animals. The Ethical Committee on Animal Care approved the experimental protocols and approval number is APC/2011-IAEC/1116.

Preparation of Drug Solution

Accurately weighed quantity of dried extract was suspended in distilled water to prepare the appropriate stock solution of the drug i.e., 190, 240, 300 mg/kg.

Indomethacin 100 mg/kg was also suspended in distilled water. The doses were administered by selecting the appropriate concentration of the stock solution.

Preparation of Extract of *C. aphylla* Roth^[8]

In 3.2 kg root bark of *C. aphylla* were percolated three times with ethyl alcohol at room temperature for 2 to 3 weeks. The solvent from the combined yellowish-brown percolate was removed under reduced pressure. 38 g of dark brown viscous residue was taken up in 1N hydrochloric acid and taken out exhaustively with ethyl acetate to remove neutral substances. The aqueous acidic solution was basified with ammonia, yielding yellow precipitates. These yellow precipitates were then extracted with chloroform, which gave the alkaloid concentrate on evaporation. The column chromatography of the chloroform extract was carried out in neutral alumina oxide and eluted with a mixture of ethyl acetate: methanol (4:1), thus isolating the yellow colored. The yellow compound so obtained contained traces of impurities.

Induction of Complete Freund's Adjuvant Arthritis

Arthritis was induced in group II- VI animals on day one by injection of 0.2 mL complete Freund's in sub plantar region of the left hind paw. group III (Std), group IV (test drug 190 mg/kg), group V (test drug 240 mg/kg), Group VI (test drug 300 mg/kg). On 7th, 14th, 21st days the following parameters was evaluated.

Experimental Protocols

Rats were divided into 6 equal groups (6 rats for each). Group 1 normal control group: rats received pure drinking water and regular food; Group 2 model control group: Freund's adjuvant; Group 3 Standard group: CFA+ Indomethacin 100 mg/kg; Group 4 test group: CFA+MECA 190 mg/kg; Group 5 test group: CFA+ MECA 240 mg/kg; Group 6 test dose: CFA+ MECA 300 mg/kg. Animals were treated with drugs for 21 days.

On 7th, 14th, 21st days, body weight, arthritic index, paw edema, serum rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), serum C - reactive protein (CRP), sérum A/G ratio, X-ray, histopathology of synovial joints parameters was evaluated.

Histopathological Analysis^[1,3]

The proximal interphalangeal joint of the hind paw of the rats were removed and separated from the surrounding tissues and weighed. The joints fixed in 10% formalin were decalcified, sectioned and finally stained with hematoxylin and eosin to examine the histopathological changes during the experimental period in all the groups under light microscope. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage/ subchondral bone); 3, severe change (pannus invasion

into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels). Histopathological changes in the knee joints were scored in the femur region on 5 semi serial sections of the joint, spaced 70 µm apart. Scoring was performed on decoded slides by two observers, as described earlier.

Radiological Analysis^[1,3]

Before sacrificing the animals, X-rays were taken at the joints of the animals' hind paw to evaluate the bone damage. Radiographs were taken using X-ray apparatus and industrial X-ray film. The X-ray apparatus was operated at 220V with a 40V peak, 0.2 s exposure time, and a 60 cm tube-to-film distance for anterior-posterior projection. 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 and abnormalities were graded as follows:

- Periosteal reaction, 0-3 (none, slight, moderate, marked);
- Erosions, 0-3 (none, few, many small, many large);
- Joint space narrowing, 0-3 (none, minimal, moderate, marked);
- Joint space destruction, 0-3 (none, minimal, extensive, ankylosis). Bone destruction was scored on the patella as described previously.

Statistical Analysis

Significance of the difference between mean values was determined by one-way variance analysis (ANOVA) followed by the Tukey's test for multiple comparisons using Statistical Package for Social Sciences (SPSS) computer package. Significant difference between control and treatment groups was assigned at $p < 0.05$.

RESULTS

Body Weight Change

The body weight of the control rats progressively increased while the gain in body weight of arthritic rat was retarded significantly. Drug treated group showed progressive increase in body weight (Fig. 1).

Arthritic Index

In this study, the incidence of inflammation was 100% on administration of Freund's adjuvant (i.e all the rats responded with an arthritic scored >1), if the rats were not treated with anti-inflammatory agent. There was a difference in the severity of inflammation in treated group and this could be attributed to the action of standard or MECA (Fig. 2).

Paw Volume

An intraplanar injection of CFA into the left hind paw produced a more marked inflammatory response in the



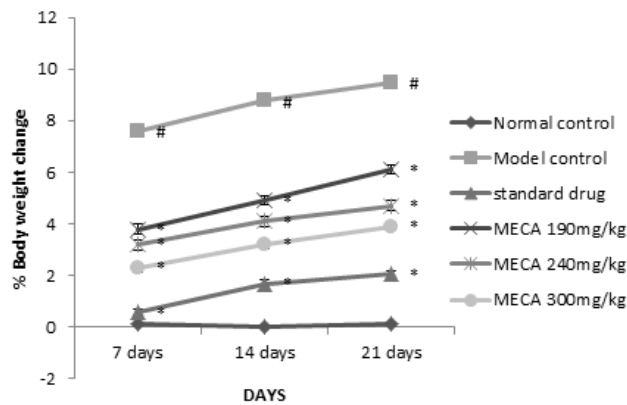


Fig. 1: Effect of MECA on body weight change

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

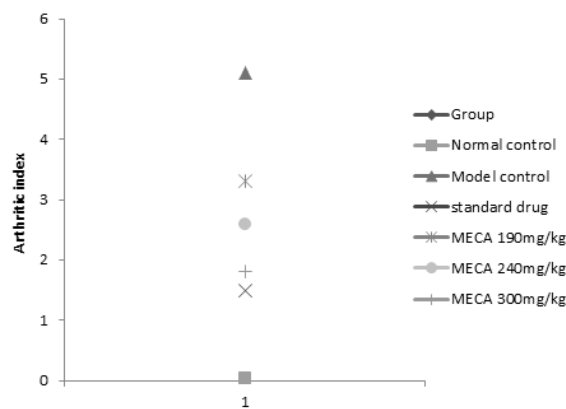


Fig. 2: Effect of MECA on Arthritic Index (AI)

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

model control animals than the normal control. Seven days after CFA injection, the edema volume was significantly higher in model control animals than in normal control animals (Fig. 3).

Erythrocyte Sedimentation Rate

The indomethacin, MECA 190 mg/kg, MECA 240 mg/kg and MECA 300 mg/kg treated ESR was significantly lower compared to model control group (Fig. 4).

Serum Rheumatoid Factor

A significant reduction in RF was observed in indomethacin, MECA 190 mg/kg, MECA 240 mg/kg and MECA 300 mg/kg treated group as compared to model control group (Fig. 5).

Serum C-Reactive Protein

A significant reduction in CRP was observed in indomethacin, MECA 190 mg/kg, MECA 240 mg/kg and MECA 300 mg/kg treated group as compared to a model control group (Fig. 6).

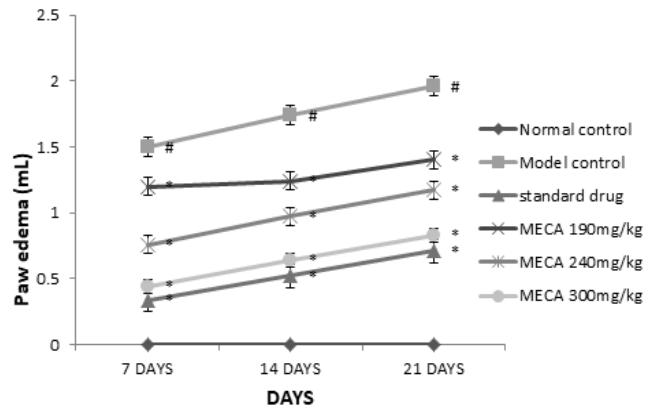


Fig. 3: Effect of MECA on paw volume

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

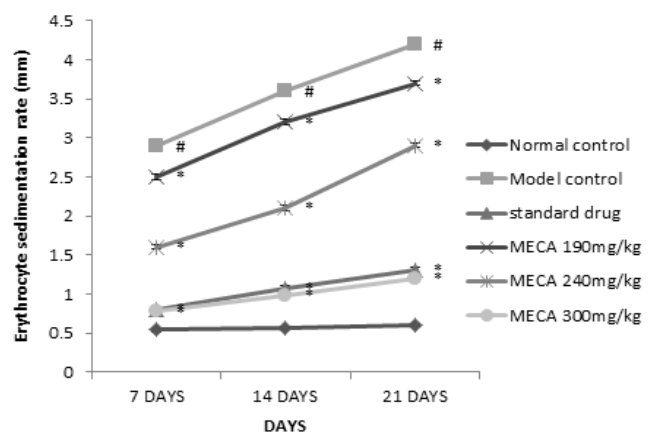


Fig. 4: Effect of MECA on erythrocyte sedimentation rate (ESR)

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

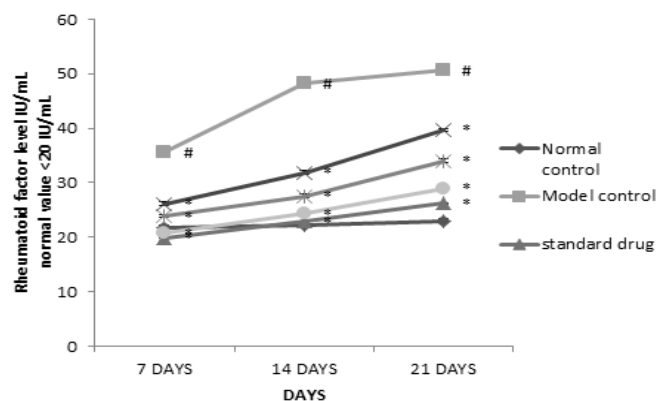


Fig. 5: Effect of MECA on serum Rheumatoid Factor (RF)

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

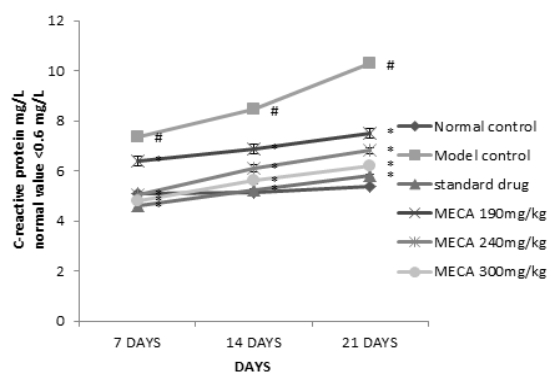


Fig. 6: Effect of MECA on serum C-Reactive Protein (CRP)

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

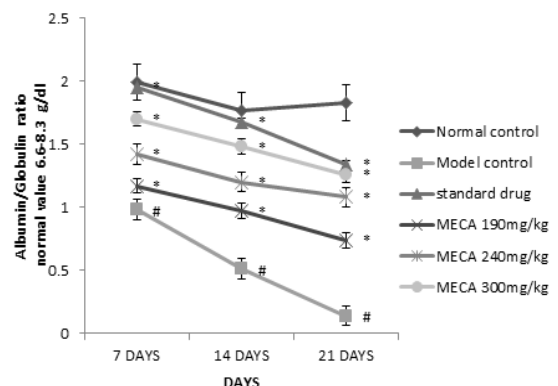


Fig. 7: Effect of MECA on serum albumin globulin ratio (A/G ratio)

Values are expressed as mean $\pm$ SEM. N=6. Values are statistically evaluated using ANOVA analysis followed by Dunnett's test. Significant values were compared with #p < 0.05 normal control Vs model control & *p < 0.05 model control Vs all MECA groups

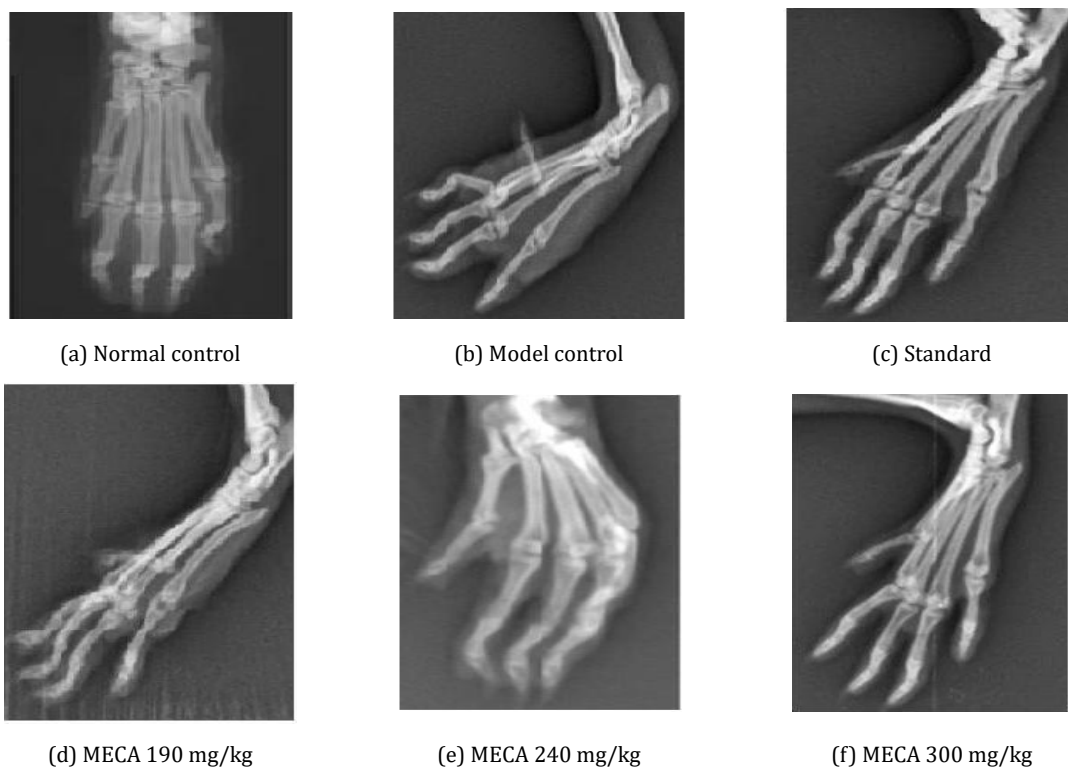


Fig. 8: Effect of MECA on radiological changes of control and experimental animals

Serum Albumin Globulin ratio (A/G ratio)

There was a significant decrease in total protein albumin level, A/G ratio but a significant increase in the globulin level by indomethacin in CFA inflammatory rats. The above change was brought back to near-normal levels by MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg (Fig. 7).

Effect of MECA on Radiological Changes of Control and Experimental Animals

Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis. Freund's

Complete Adjuvant treated 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 pseudo widening of all joint spaces and bending of phalangeal joints. In contrast, in rats treated with MECA 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.



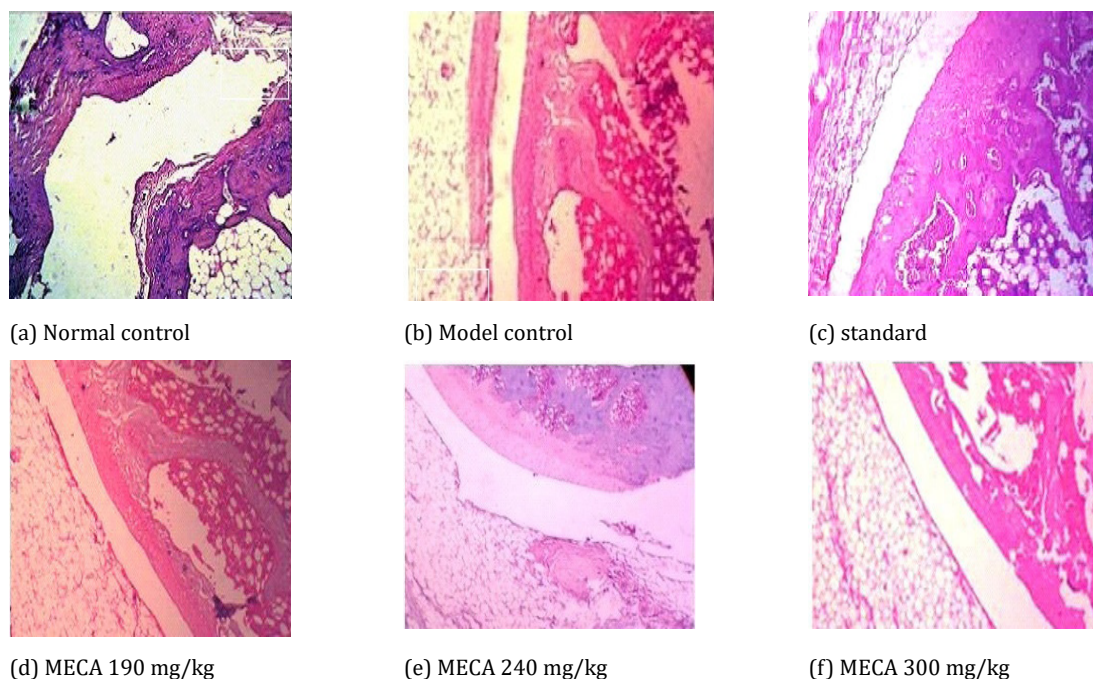


Fig. 9: Effect of MECA on histopathological changes of control and experimental animals

The drug control rats showed the normal architecture of phalangeal joints as that of control rats (Fig. 8).

Effect of MECA on Histopathological Changes of Control and Experimental Animals

As shown in Fig a [NC]: Histology of synovial joint of normal control rat with intact morphology of synovium and synovial lining Fig b [MC]: FA induced disease control rat showed plenty of lymphocytic infiltration [↓] in synovial lining with severe inflammation and marked angiogenesis [↑] studied with proliferation of synovial cells [↓↓] Fig c [SD]: indomethacin 100 mg/kg treated rats showed significant protection with mild lymphocytic infiltration [↓] with no evidence of thickening of synovial lining and angiogenesis Fig d MECA 190 mg/kg: MECA 190 mg/kg treated rats showed milder angiogenesis [↑], lymphocytic infiltration [↓] and synovial lining thickening [↓↓]. Fig e MECA 240 mg/kg: MECA 240 mg/kg treated rats showed milder angiogenesis [↑], synovial lining thickening [↓↓] with no evidence of lymphocytic infiltration. Fig f MECA 300 mg/kg: MECA 300 mg/kg treated rats showed milder lymphocytic infiltration [↓] and synovial-lining thickening [↓↓] with no evidence of angiogenesis (Fig. 9).

DISCUSSION

RA is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant-induced arthritic model of chronic inflammation is considered as the best available experimental model of RA.^[3]

Inflammation (Latin, *inflammō*, "I ignite, set alight") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or

irritants.^[1] Inflammation is evaluated by various experimental animal models. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process e.g. chronic inflammation is challenged by complete Freund's adjuvant induced inflammation. Conventional medicine, including treatment with steroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and biological agents such as tumor necrosis factor alpha (TNF- α) and interleukin-1beta (IL-1 β) antagonists^[9-12] has shown only limited success against all forms of inflammatory disorders.^[13] Herbal products are receiving increasing public interest, and herbal treatment is now the most popular complementary and alternative medicine therapy. NSAIDs and analgesics do not alter the course of RA or prevent joint destruction; hence, they cannot be used as the only treatment.^[6] Serious adverse effects of long-term oral glucocorticoids are well known and these drugs must be used carefully.^[6] Herbal medicines are very cheap in comparison to the conventional form of medication. Herbal medicines can be consumed without any prescription and can be found very easily from a local drug store. Herbal medicines are known to be more productive in comparison to other forms of medication in curing certain conditions. They are known to be all natural unless mixed with other chemical components. One of the greatest benefits associated with herbal medicine is the nonexistence of side effects. The herbal treatment is now the most popular complementary and alternative medicine (CAM) therapy. The result presented here demonstrates the pharmacological effect of the drug *C. aphylla* Roth. utilizing in anti-inflammatory action. Experimental models of inflammation utilizing rats, have been used

extensively in studying the inflammation in the pathogenesis of inflammatory disease.^[14] Immune system activation during inflammation leads to increased circulating levels of proinflammatory cytokines.^[15, 16] In present study we were using model Freund's adjuvant induced inflammation in rats. Freund's adjuvant-induced inflammation in rats shares many features with human inflammation including joint swelling, pannus formation, joint destruction and bone erosion and is widely used to evaluate anti-inflammatory and immunosuppressive / immunomodulatory drug properties.^[17] During the developmental course of disease, there is initially an acute peri-articular inflammation characterized by synovial mononuclear cell infiltration. This is followed by chronic inflammation involving synovial hyperplasia and destruction of peri-articular bone and cartilage like that seen in human inflammation, particularly RA. Severe arthritis eventually leads to bony ankylosis and deformities of the paws. In accordance complete Freund's adjuvant (CFA) administration is also known to produce both primary and secondary lesions with majority of consequent pathological changes like that observed in inflammation. Similar observation was made in the present study where by injection of CFA 0.2 mL in the left hind paw induced inflammation as a primary lesion within 3 to 5 days. After a delay of 11 to 14 days, the secondary lesion also causes raised edema in the non-injected paw. Secondary there was an increase in arthritic index measuring presence of nodules in nose and tail or slight inflammation in paw, moderate inflammation in hind paw, marked inflammation in hind paw. These observations suggested that CFA administration produced inflammation in experimental animals. Treatment with methanolic extract of *C. aphylla* Roth. significantly ameliorate the change in primary and secondary lesions as well as arthritic index induced by CFA suggest its protective role against AA. As the disease progress numerous parameters of physical wellbeing are affected. Changes in body weight is a useful index to assess the course of the disease and the response to therapy of anti-inflammatory drugs inquest.^[18] The reduction in body weight observed in untreated arthritic condition may be due to the impaired absorption of the intestine. Earlier findings suggest that absorption of ¹⁴C glucose and ¹⁴C leucine in rat intestine was reduced in the case of inflamed rats. it is well known that inflammation leads to body mass loss, probably due to inflammatory cytokines, pain, loss of appetite, increased energy expenditure and enhanced protein catabolism.^[19] Similar finding was observed in adjuvant arthritic condition.^[20] Finding of the current study were in accordance to the above facts, CFA administration caused decreased in body weight as compared to normal animals. The increase in body weight during MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg treatment reveals the restoration of absorption capacity of the intestine in the treated group due to

reduction of inflammation. Among test groups significantly lower ESR level got by MECA 300 mg/kg ($3.8\% \pm 0.03^*$; $4.9\% \pm 0.06^*$; $6.1\% \pm 0.04^*$). ESR is an indirect measurement of acute phase response for determining the disease activity in inflammation.^[21] Although CRP is a better marker for inflammation and though ESR is influenced by several factors such as the plasma concentration of fibrinogen, immunoglobulins, RF and Hb, the increased level of ESR in inflammatory rats adds information reflecting the chronicity and severity of the disease better than CRP.^[22] Hence, a combination of the tests might be worthwhile. The erythrocyte sedimentation rate also called a sedimentation rate, sed rate or biernacki reaction, is the rate at which red blood cells precipitate in a period of 1 hour. It is common hematology test which is a nonspecific measure of inflammation. The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red blood cells from stacks called 'rouleaux' which settle faster. Rouleaux formation can also occur in association with some lymphoproliferative disorders in which one or more immunoglobulins are secreted in high amounts. Thus, the ESR is increased by any cause or focus of inflammation. The basal ESR is slightly higher in females than males. In line with this notion, ESR in model control animal receiving CFA showed higher ESR value than normal control animals estimated on 7th, 14th and 21st days. MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg treated group significantly lowered ESR as compared to model control corresponding to 7 days, 14 days, 21 days. Among test groups, significantly lower ESR level got by MECA 300 mg/kg ($4.8 \pm 0.06^*$; $5.6 \pm 0.05^*$; $6.2 \pm 0.15^*$ mg/L in 7; 14; 21 days). RF is the one immunological marker of inflammation included in the classification criteria and has been known for over 50 years. Rheumatoid factor is antibody against organism and form a complex which is increased due to inflammation. RF factor is observed positively in 80% of RA patients and is also increased in diffuse collaged disease.^[23] It was demonstrated in several studies to be present in the sera of patients with rheumatic and non-rheumatic diseases and even of healthy persons.^[24] Treatment with MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg showed a significant decrease in RF value compared to model control throughout the study of 7, 14 and 21 days. MECA 300 mg/kg more significantly decrease in RF value ($20.73 \pm 0.29^*$; $24.28 \pm 0.27^*$; $28.95 \pm 0.24^*$ IU/mL in 7; 14; 21 days). C-reactive protein is a member of the class of acute phase reactants synthesized by hepatocytes in response to proinflammatory cytokines in particular IL-6. and is used mainly as a marker of inflammation. The value of the acute phase reactant CRP as an indicator of inflammation



activity and progression is well recognized.^[25] CRP factor is a diagnostic index of bacterial infection, chronic inflammation. C-reactive protein increased in inflammation & binds on phosphocholine present on dead cells due to inflammation. Measuring and charting C-reactive protein values can also prove useful in determining disease progress or the effectiveness of treatments as its levels rise dramatically during inflammatory processes.^[26] The level of CRP is significantly reduced in MECA treated groups as well as in indomethacin treated groups. The increased level of CRP in inflammatory rats of present study supports the earlier reports demonstrating an abnormal elevation of plasma CRP in rats and patients that might be primarily due to the increased activation of proinflammatory cytokines such as IL-1 and TNF- α in combination with IL-6^[27, 28] and moreover, the promoter genes for CRP are highly sensitive and greatly potentiate their induction. The increased level of CRP in turn activates the vascular epithelium, accelerating the acute phase response in the blood. Besides that, the increased CRP level might enhance the monocyte activation in inflammatory rats, since binding sites for CRP have been identified in monocytes^[29, 30] and CRP also increases the mRNA expression of IL-1 and TNF- α by human macrophages,^[31] thereby augmenting the inflammatory response. The interpretation in turn supports the present findings showing increased level of these cytokines in inflammatory conditions. In the present study the level of CRP is significantly reduced in MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg treated groups, and in indomethacin treated groups compared to model control group. Among test groups significantly lower CRP level got by MECA 300 mg/kg ($4.8 \pm 0.06^*$; $5.6 \pm 0.05^*$; $6.2 \pm 0.15^*$ mg/L in 7; 14; 21 days). In inflammation changes in plasma protein level with an increase in the globulin fraction and decrease in albumin fraction were well documented.^[32] These biochemical abnormalities result from a more basic liver malfunction.^[33] Decreased serum albumin is due to arthritis & globulin is increased disproportionately to albumin, decreasing the albumin/globulin ratio. General reduction of liver protein synthesis can be assessed by measuring albumin levels, because levels of this protein are lowered during inflammation and further, it was also reported that albumin synthesis was reduced by IL-1.^[34] Also, the mediators released such as histamine, bradykinin and prostaglandins during inflammation increase the permeability of vascular tissues to albumin, leading to reduced serum levels^[35] and.^[36] The decrease in total serum protein (25.50%) and albumin levels observed in inflammatory rats falls in line with the previous reports of 117 and 118 Suggesting the induction of inflammation by CFA. Treatment with MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg significantly prevent the decline in serum albumin level and rise in serum globulin serum level induced by CFA ($4.8 \pm 0.09^*$;

$5.6 \pm 0.14^*$; $6.2 \pm 0.11^*$). The hallmark of inflammation is the X-ray appearance of inflammatory joints commonly referred to as diminished joint space. In inflammatory rats, erosion representing bony destruction was evident on bone unprotected by cartilage, since they are exposed directly to cytokines such as TNF- α and IL-1, which stimulate the chondrocytes to produce proteolytic enzymes such as collagenases, glycohydrolases and neutral proteases degrading the cartilage. As a result, the pannus invades the joint and subchondral bones and eventually the joint is destroyed and undergoes fibrous fusion or ankylosis.^[37-42]

In light of the above result, it might be concluded that the profound beneficial effect of MECA contributes to abate the disease process in inflammation. This effect might be attributed to phyto, like spermicide alkaloid, sitosterol, and carbohydrate. Chemical test and TLC study for identification of spermicine alkaloid show presence of spermicide alkaloid having anti-inflammatory activity. In the present study three test doses MECA 190 mg/kg, MECA 240 mg/kg, MECA 300 mg/kg for the study.

CONCLUSION

In the light of the above results, it might be concluded that the drug MECA exhibited a potent antiarthritic effect by reducing the pathological lesions via down regulating the levels of proinflammatory cytokines thereby reducing the levels of acute phase proteins and also via its enhanced immunomodulatory property. The improved effect of MECA might be attributed to the combined interactions of the spermidine alkaloids like caparicine.

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