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Research Article

Isolation of Chemical Constituents from *Prosopis juliflora* bark and Anti-inflammatory activity of Its Methanolic Extracts

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ABSTRACT

The methanolic extracts of *Prosopis juliflora* bark used for the evaluation of anti-inflammatory activity and isolation of constituents. For the evaluation of anti-inflammatory activity carrageenan and histamine induced paw oedema served as acute models and formation granulation tissues by cotton pellets served as a chronic model in rats. The effect was compared with diclofenac sodium, used as standard drug. The methanolic extract of *Prosopis juliflora* bark (MEPJ) 100, 200 and 400 mg/ kg (p.o.) exhibited significant anti-inflammatory activity in acute and chronic inflammatory models. At the dose level 400 mg/ kg (p.o.) showed maximum inhibition of 55.32% in carrageenan induced rat paw oedema while the standard diclofenac inhibited it by 61.33 % after 3 h of carrageenan injection. All the doses of MEPJ showed dose dependent inhibition against histamine and serotonin induced rat paw oedema as compared with control animals. Furthermore, the same dose levels successfully reduced formation granulation tissues by cotton pellets in rats. Alkaloids, 3''''-Oxo-juliprosopine and Secojuliprosopinal were isolated and characterized.

Key words: *Prosopis juliflora* bark; isolation; anti-inflammatory; carrageenan; cotton pellets induced granuloma

INTRODUCTION

Inflammation is a natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganism, toxic chemical substance and physical damage to tissue. Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. The inflammatory process is invariably characterized by a production of prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor (PAF) and by a release of chemicals from tissues and migrating cells.[1)] The continued search for potential effective and safe anti-inflammatory agents cannot be over-emphasized. This is important, in view of the roles played by the inflammatory process and inflammatory mediators in diseases such as asthma, rheumatoid arthritis, cancer and neurodegenerative disorders. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory and toxicity studies of methanolic extract of p*rosopis juliflora* (MEPJ) in standard animal models.

The plant *prosopis juliflora* (Sw.) DC. belonging to the family of Leguminosaeia, which is widespread in India, Saudi Arabia, and in United States. Leaves of this plant are used for the preparation of bread, biscuits, sweeties, syrups and liquors.[2)] Leaf extracts of *Prosopis juliflora* possess anti-diabetic, anti-asthmatic, anti-allergic, anti-inflammatory activity and antifungal activity.[3-9)] Parts of the plant useful in intoxication of some poison.[10-12)] Previous phytochemical screening of this plant showed that the presence of piperidine alkaloids.[13,14)] The phenolic compounds like flavonol glycosides and hydroxycinnamic acid from *Prosopis juliflora* pollen were reported to posses antioxidant with high free radical scavenging activity.[15,16,17)] Recently, L-tryptophan, syringin, juliflorine and (-)-laricirenol were isolated from mesquite.[1,18,19)] In this study, we reported the isolation and identification of some alkaloids from the methonolic extract of p*rosopis juliflora* bark and its anti-inflammatory activity.

METRIALS AND METHOD

Plant Material The plant *prosopis juliflora* were collected in the month of December 2006 from Komarapalayam, Namakkal District, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Coimbatore, TamilNadu, India and the Voucher Specimen (BSI/SC/5/23/06-07/TECH.230) was retained in our laboratory for future reference. The Bark of the plant materials were dried under shade and then powdered with a mechanical grinder. The powder was passed through Sieve No 40 and stored in an airtight container for further use.

Preparation of Extract The dried powdered plant material was extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 7.75 %). The methanolic extract was concentrated to dryness *in vaccuo* at

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35°C. Active constituents from the dried extracts were separated by column chromatography with different solvent ratio.

Preliminary Chemical Tests The extract was subjected to preliminary screening, for various active phytochemical constituents such as alkaloids, carbohydrates, steroids, protein, tannins, phenols, flavonoids, gum and mucilage, glycosides, saponins and terpins.[20)]

Vehicle The extract at the different doses of 100,200 and 400 mg/kg was suspended in aqueous Tween 80 solution (2%) and Diclofenac (12.5mg/kg) (Torrent,Bombay) in saline were used for the present study.

Isolation of Alkaloids Fresh barks (1kg fresh wt) of *Prosopis juliflora* (Sw.) DC. were soaked in MeOH for 2 weeks at room temp with the extract filtered and concentrated to dryness *in vaccuo* at 35°C, with the concentrate (20 g) was then partitioned between water and ethyl acetate with the aqueous layer was basified with ammonium hydroxide (pH 9) and extracted repeatedly with chloroform. The combined chloroform layers were concentrated to dryness *in vacuo* at 35°C with the concentrate (5 g) separated by column chromatography into various fractions of MeOH- H_2O-TFA (trifluoroacetic acid) (80: 20: 0.1, v/v), MeOH-TFA (100: 0.1, v/v) and CH_3Cl-TFA (100: 0.1, v/v), respectively. The MeOH-H_2O-TFA fraction was concentrated to dryness *in vacuo* at 35 °C. The combined fractions were identified by TLC and recrystallized with methanol.

Animals All the experiments were carried out using male, Swiss Albino mice (25-30 g) and Wistar rats (150-200 g) were obtained from animal house, IRT-Perundurai Medical College, Erode, Tamil Nadu, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Register Number: 688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Acute Oral Toxicity Studies Acute oral toxicity studies were performed according to OECD-423 guidelines (acute toxic class method).[21)] Swiss mice (n =5) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The MEPJ (suspended with 0.5%, w/v, Carboxy Methyl Cellulose) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 50, 300 and 2000 mg/kg.

Carrageenan-Induced Paw Oedema The rats were divided into 5 groups (n = 6). The extract and the standard used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 hours before the experiment. On the day of the experiment they were assigned to 5 groups of six animals each. They were marked and numbered for identification. Paw oedema was induced by subplantar injection into the rat right hind paw of 0.1 ml sterile saline containing 1% carrageenan (control group). A group of rats were treated with MEPJ and standard drugs were administered orally concomitantly with carrageenan injection. Control group of animals received the same volume of vehicle instead of the tested agents. The volume of the paw was measured by a plethysmometer immediately after the injection as previously described.[3-4)] The increase in paw volume was taken as oedema volume. The percentage of inhibition of inflammation was calculated for comparison. The ratio of the anti-inflammatory effect of MEPJ was calculated by the following equation: anti-inflammatory activity (%) = (1-D/C) x 100, where D represents the percentage difference in paw volume after MEPJ was administered to the rats, and C represents the percentage difference of volume in the control groups.

Histamine Induced Inflammation The anti-inflammatory activity of the extract was measured with phlogistic agent of histamine, which act as mediator of inflammation. The paw oedema was induced in rats by sub plantar injection of freshly prepared histamine (0.1 ml of 0.1% w/v) solutions respectively and the paw oedema was measured as mentioned earlier.[22)]

Cotton Pellet Granuloma Model In cotton pellet the animals were divided into five groups as described in the carrageenan induced paw oedema model. The of Penn *et al* with slight modification was used.[23)] The animals were anaesthetized with pentobarbitone (30 mg/kg, s.c.). The back skin was shaved and disinfected with 70 % ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with Diclofenac (12.5 mg/kg, p.o.) and methanol extract of *prosopis juliflora* for 7 days. Then, the pellets were dissected out and dried until the weight remains constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

Statistical Analysis All the results were expressed as mean ± standard error (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett' t-test. $p < 0.05$ were considered as statistically significant.

ISSN: 0974-6943

RESULT

The methanolic extract of *Prosopis juliflora* was evaluated for anti-inflammatory activity in standard animal models and the results were summarized in Figures 1-3.

Preliminary Chemical Tests The extract showed positive test for carbohydrates and glycosides, alkaloids, saponins, steroids, and tannins.

Isolation of Alkaloids The first isolated compound is pale yellow gum, is an alkaloids (proved by alkaloidal chemical test) with specific optical rotation of $[\alpha]^{28}_{D}$ +5.1(c1.0, MeOH), IR λmax (KBr) cm^{-1} : 3423, 2926, 2856, 1681, 1458, 1201, 1137, 1011, 801 and the ^{1}HNMR shows δH:3.15-3.23(m), 3.79 (br,s), 1.66-1.75 (m), 1.50-1.61 (m), 2.96-3.04 (m), 3.15-3.12 (m), 3.81 (br.s), 1.66-1.90 (m), 1.50-1.61 (m), 2.96-3.04 (m), 1.86-1.94, 9.28 (s), 6.53 (t,J=77Hz), 2.34 (dt,J=7.3,7.3 Hz). Second isolated compound is also an alkaloid (proved by alkaloidal chemical test) which like a yellow gummy mass with the specific optical rotation of $[\alpha]^{28}_{D}$ +4.0 (c 1.0, MeOH), IR í max (KBr) cm^{-1} : 3420, 2928, 2855, 1680, 1456, 1200, 1136, 837, 722 and the ^{1}HNMR showsλH:3.19-3.25 (m), 3.83 (br,s), 1.77-1.80 (m), 1.55-1.65 (m), 3.00-3.08 (m), 1.91-1.99 (m), 2.05 (t,J=73Hz), 1.91-1.97 (m), 2.38 (m) h-ax: 3.40 (d,J=18.3Hz), %.46 (S). The above spectral data were matches with already identified compound from the plant of *Prosopis juliflora* and they were confirmed as 3-Oxo-juliprosopine (Compound. 1) and Secojuliprosopinal (Compound.2). However, due to paucity of isolated compounds we are undertaken the methanolic extract for present study.

Carrageenan-Induced Paw Oedema The anti-inflammatory effect of the MEPJ and Diclofenac on the carrageenan –induced hind paw oedema mode is shown in Fig. 1. Standard drug Diclofenac (12.5 mg/kg, p.o.) produced a significant reduction in paw oedema volume (61.33%). Treatment with MEPJ reduced the carrageenan induced paw oedema volume in a dose dependant manner. MEPJ showed maximum (p<0.001) inhibition of 55.32 % at the doses of 400 mg/kg, p.o.., after 3 h of treatment of carrageenan induced paw oedema. Similarly, the inhibition were 39.07 % (p<0.001) at the dose of 100 mg/kg, p.o. and 44.90 % (p<0.001) at the dose of 200 mg/kg, p.o. in pretreated rats.

Histamine Induced Inflammation Fig. 2 shows the effect of histamine induced paw oedema in rats. The maximal paw oedema volume was observed at 1h of after subplantar injection of histamine in the vehicle treated groups. The percentage of reduction was found 35.42, 45.01 and 53.87 % at 100, 200 and 400 mg/kg, p.o., of MEPJ, respectively. Whereas, Diclofenac (12.5 mg/kg, p.o.) showed 59.80 % of inhibition in histamine induced paw oedema.

Cotton Pellet Granuloma Model The effect of MEPJ on cotton pellet induced granuloma in rats shown in Fig.3. In this, the mean weight of the cotton pellets was determined. Treatment with the MEPJ significantly decreased the granuloma weight 28.48, 41.13 and 52.32 at the dose levels 100, 200 and 400 mg/kg, p.o., respectively. Similarly 56.32% decreased was found in Diclofenac (12.5 mg/kg, p.o.,) treated rats.

DISCUSSION

Carrageenan-induced local inflammation is commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID). It appears that the onset of the carrageenan local inflammation has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radical, as well as to the release of other neutrophil-derived mediators.[18] In particular, the initial phase of inflammation (oedema, 0–1 h) which is not inhibited by NSAID such as Diclofenac or aspirin, has been attributed to the release of histamine, 5-hydroxytryptamine and bradykinin, followed by a late phase (1–6 h) mainly sustained by prostaglandin release and more recently has been attributed to the induction of inducible cyclo-oxygenase (COX-2) in the tissue.[19]

The potential of the MEPJ for its anti-inflammatory effect and short-term toxicity was investigated. The effect of MEPJ at the dose of 100, 200 and 400 mg/kg showed significant anti-inflammatory activity. Significant anti-inflammatory activity was observed for MEPJ in carrageenan-induced oedema and also the chronic model.

The present study investigates that the anti-inflammatory activity of methanolic extract of *prosopis juliflora*. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation and is believe to be bi-phasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome.[24] It has been reported that the second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-edematous effect of natural products.[25-26]. Prostaglandins play a major role in the development of second phase of reaction that is measured at 3 hours time. These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain.[27] It is also the prostaglandin amplifies the pain mechanism and enhances vascular permeability whilst the leukotrienes contract smooth muscle blood vessels. And it leads to enhance the vascular permeability and mediate pro inflammatory and allergic response.[28-29] Diclofenac is a potent inhibitor of prostaglandin formation and thereby reduces inflammation and arthritic pain. Furthermore, diclofenac has been reported to reduce chemotaxis oxygen derived free radical generation and neutral production.[30] Based on these reports, it can be inferred that the inhibitory effect of the extract of *Prosopis juliflora* on carrageenan-induced inflammation in rats may be due to inhibition of the mediators responsible for inflammation.

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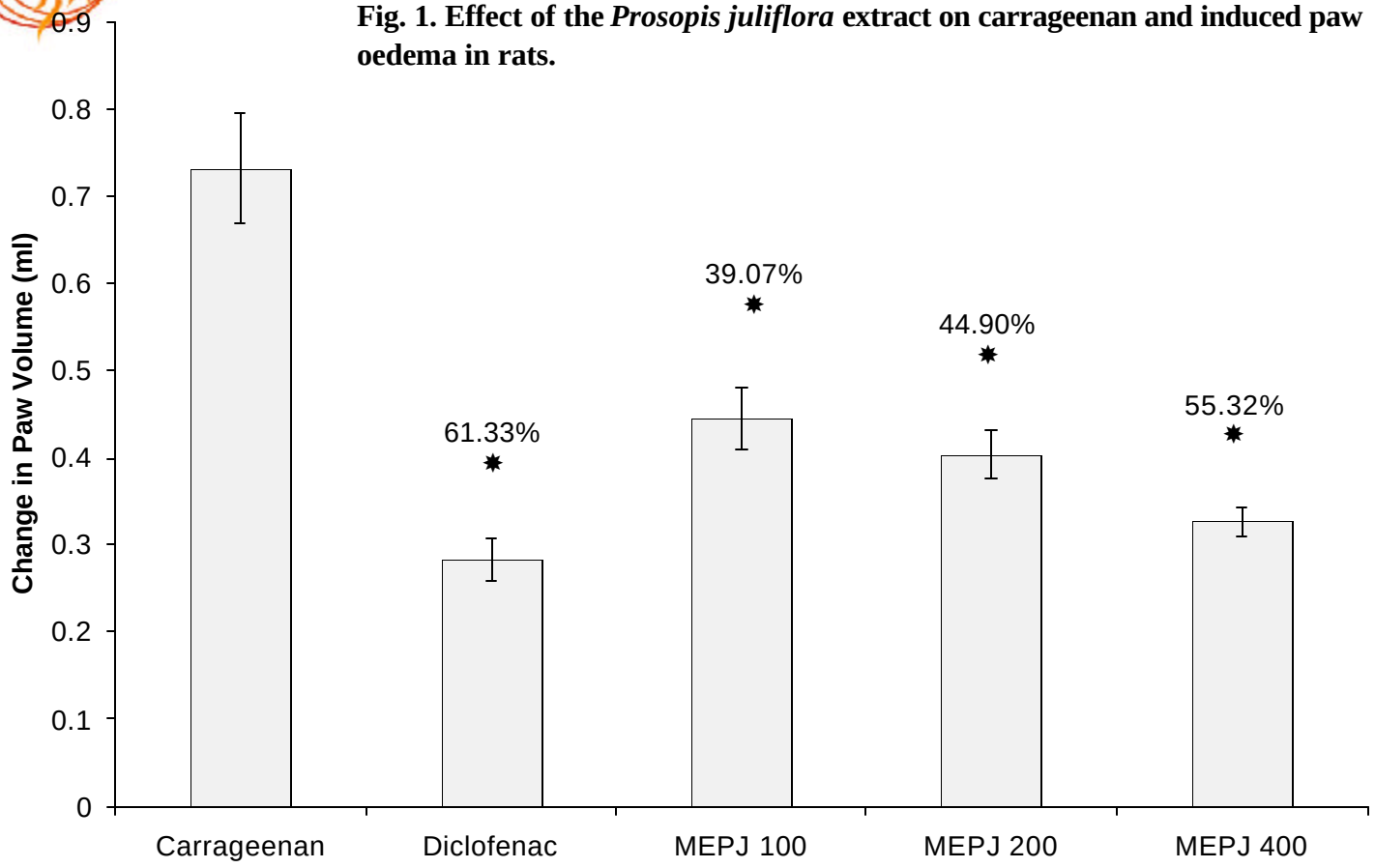
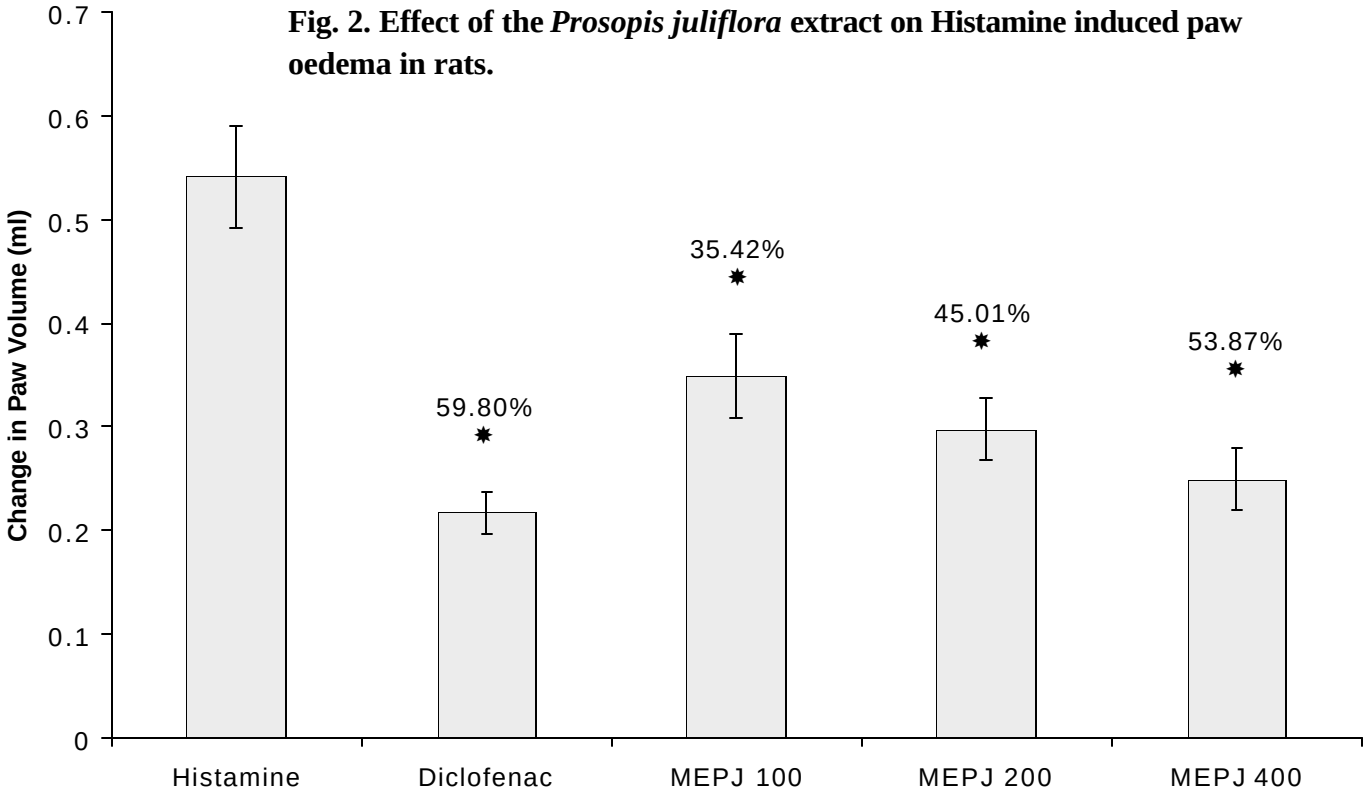
Fig. 1. Effect of the *Prosopis juliflora* extract on carrageenan and induced paw oedema in rats.



Values are mean ± SEM (n = 6). ✸p < 0.001 compared with control.

Fig. 2. Effect of the *Prosopis juliflora* extract on Histamine induced paw oedema in rats.



Values are mean ± SEM (n = 6). ✸p < 0.001 compared with control.

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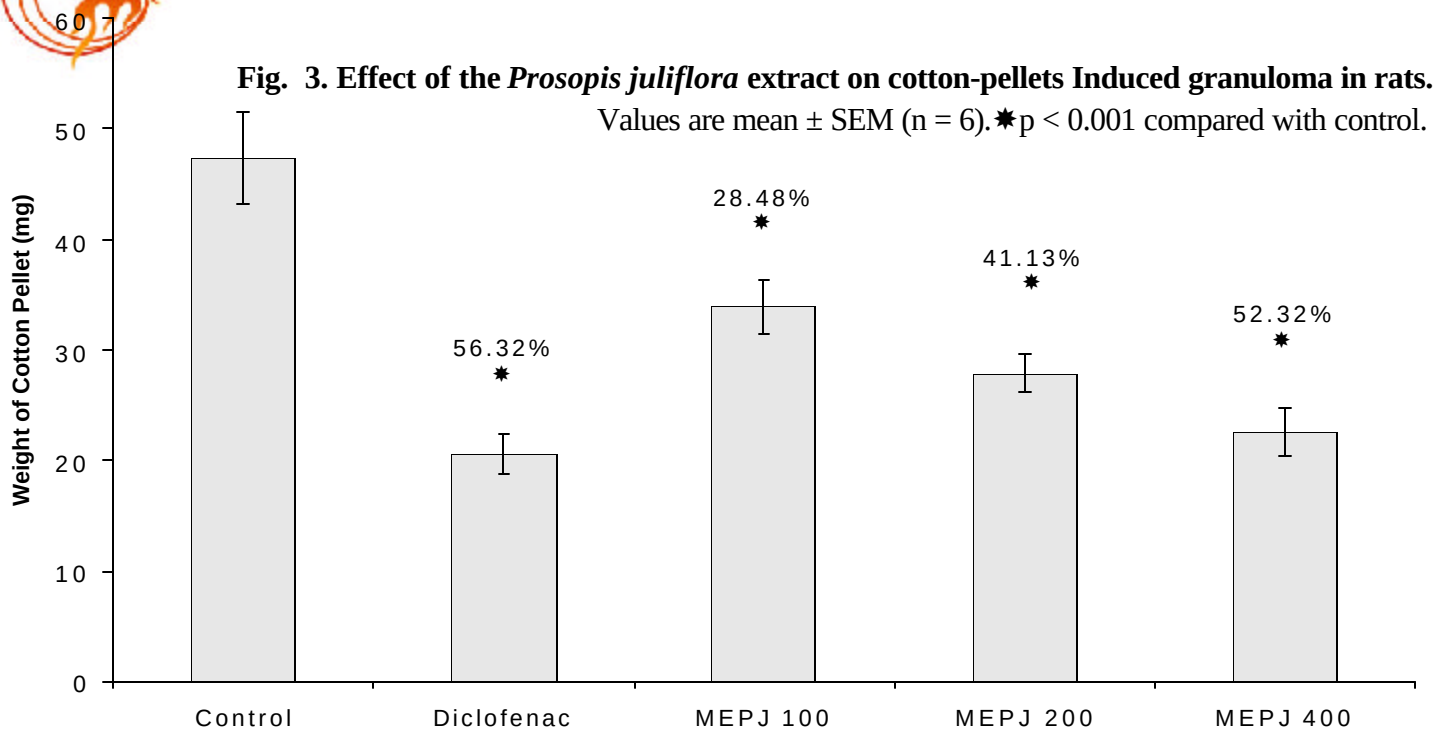
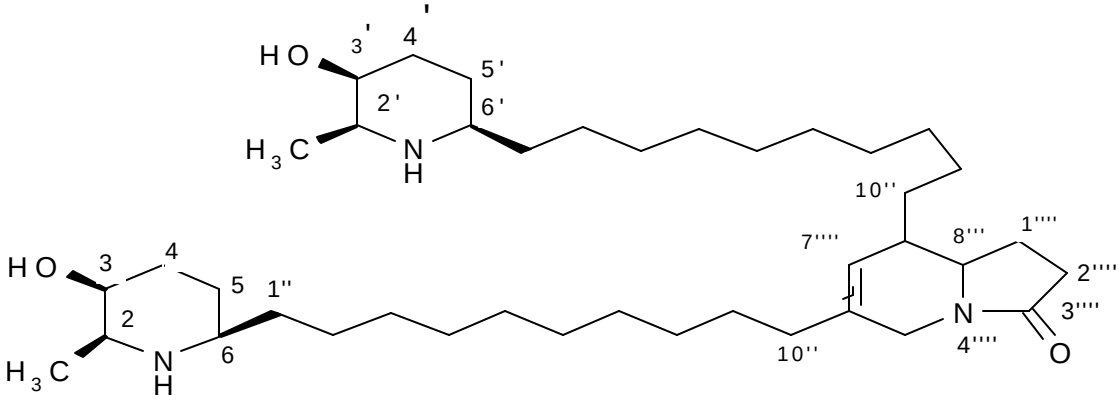
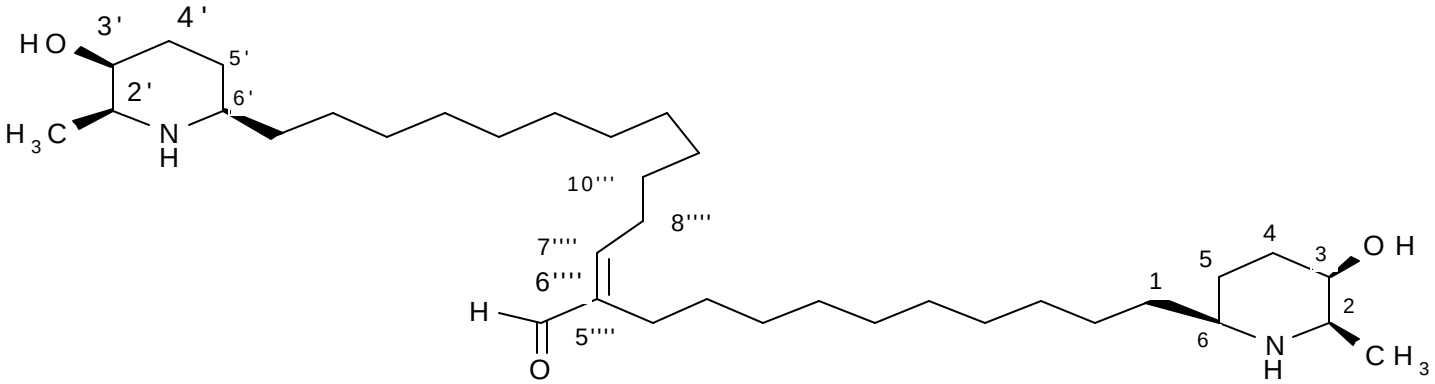
Fig. 3. Effect of the *Prosopis juliflora* extract on cotton-pellets Induced granuloma in rats.

Values are mean ± SEM (n = 6). ✸p < 0.001 compared with control.





Structure 1:3''''-Oxo-juliprosopine



Structure 2: Secojuliprosopinal

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Histamine is one of the important inflammation mediators and it is potent vasodilators substance and increase the vascular permeability.[31,32] The study showed that the dose of MEPJ effectively suppressed the oedema produced by the histamine, which is indicates that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators *viz*. histamine, serotonin and prostaglandin might be involved in the inflammation. So, it may suggest that its anti-inflammatory activity is possible packed by its anti-histaminic activity. Chronic inflammation is a reaction arising when the acute response is in sufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophills and exudation. The cotton pellet granuloma widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellets correlates with the amount of granulumatous tissues.[33-34] Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides.[31,32] This result indicates the efficacy of the extract, which possesses anti-inflammatory activity.From the data of the results of present study indicating that, the extract exhibited significant anti-inflammatory activity. Thus this study substantiates the use of this plant as an anti-inflammation folklore medicine. The present screening permits to support, in part, the attributed properties of the evaluated species, often reported to treat ailments in which inflammatory processes could be involved. Moreover, MEPJ was subjected to preliminary phytochemical investigation which showed the presence of alkaloids, tannins, Saponins, steroids, glycosides and carbohydrates etc. Hence the same extract was further used for the isolation of its active constituents by column chromatography and isolated two alkaloids which were identified as 3??-oxo-juliprosopine and secojuliprosopinol.

Current chemical and pharmacological research is being carried out on the active extracts of *Prosopis juliflora* which may be a potential source of natural products with anti-inflammatory activity.

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