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ORIGINAL ARTICLE

Anti-arthritic activity of *Barleria prionitis* Linn. leaves in acute and chronic models in Sprague Dawley rats

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KEYWORDS

Arthritis;
Ipsilateral;
Contralateral;
Formaldehyde;
Freund's Complete Adjuvant

Abstract *Aim:* The present investigation was designed to evaluate anti-arthritic potential of ethyl acetate fractions of chloroform extract from leaves of *Barleria prionitis*.

Materials and methods: *Barleria prionitis* L. leaves were defatted by petroleum ether and then successive extraction was done with chloroform and methanol by the hot Soxhlet extraction method. Chloroform extract was further fractionated with solvent ethyl acetate to obtain EABP. This fraction was evaluated at two doses 125 and 250 mg/kg, against formaldehyde-induced acute non immunological and Freund's Complete Adjuvant-induced chronic immunological arthritis in rats. Arthritis assessment, paw volume, body weight, motor incoordination and nociceptive threshold were measured. Haematological assessments of red and white blood cells, erythrocyte sedimentation rate, as well as histopathological studies were also done on day 21, after animals were sacrificed.

Results: Dose dependent and significant inhibition of oedema was observed in both acute as well as chronic models. The extract at dose 250 mg/kg showed most potent and significant ($P \leqslant 0.05$–0.01) paw oedema inhibition which is supported by the results of body weight, biochemical parameters, motor incoordination and nociceptive threshold in Freund's Complete Adjuvant-induced arthritis model.

Treatment with EABP also decreased the histopathological alterations induced by Freund's Complete Adjuvant.

Conclusion: In the present investigation, extract protects synovial membrane by improving the health status through haematinic parameters and exhibits promising anti-arthritic activity. This finding thus supports the traditional use of *Barleria prionitis* for rheumatism. However, further studies are needed to carry out the isolation of active constituents of the fraction responsible for the activity.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune multisystem disease characterized by pain, synovial membrane inflammation, peripheral joint inflammation, morning stiffness, destruction of articular tissues and restricted joint movement.[1–3] The pathology of RA is complex and aetiology underlying RA remains unknown. It is clear that destructive changes in the cartilage and bone, and bony outgrowths restricting mobility of the joint occur in the patients.[4] Arthritis can cause severe disability and ultimately affects a person's ability to carry out everyday tasks, restricts the quality of life and causes

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premature death.[5] Any part of the body can become inflamed or painful from arthritis. It is one of the most common inflammatory disorders affecting approximately 0.5–1.0% of global adult population, with females being affected three times more than males.[6–8]

Treatment of RA has moved from conventional strategies such as non-steroidal anti-inflammatory drugs (NSAIDS), corticosteroids, immunosuppressants, disease modifying anti-rheumatic drugs (DMARDSs) to newer biological agents such as TNF-α and monoclonal antibodies.[9–11] Despite the progress made in the treatment of disease, these treatments fail to produce long term benefits and produce serious adverse effects such as gastrointestinal ulcer, renal morbidity, cardiovascular complications, haematological toxicity and nephrotoxicity, which limit their utility in the treatment of the disease.[12,13] Besides their side effects the current treatment is also of high cost, so patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief. Thus complementary and alternative medicines may meet the requirement of large number of patients suffering from this disease.[14,15]

Barleria prionitis L. (Family Acanthaceae) is a medicinal plant found throughout South Africa, India, Sri-Lanka, and tropical Asia. It is commonly known as Vajradanti.[16,17] In traditional system of medicines, almost every part of the plant is used for the treatment of various diseases like toothache, fever, inflammation, gastrointestinal disorders, expectorant, boils, glandular swellings, catarrhal affections, ulcers, tonic, diuretic, itching of leprosy ulcers, lacerated soles of feet in rainy season and the oil extract of plant is recommended to arrest the greying of hairs.[18–21]

Phytochemical studies of *B. prionitis* revealed the presence of glycosides, steroids, tannins and flavonoids.[22] Iridoid glycosides, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetyl barlerin, 7-methoxydiderroside and lupulinoside have been isolated from aerial parts.[23] Several reports including ours suggested that plant is having antifungal,[19,24] antiviral,[25] anti-fertility,[26] antioxidant,[24,27,28] antidiabetic[29] and gastroprotective[30] activities.

Hence we attempted to investigate the anti-arthritic effect of ethyl acetate fraction of chloroform extract of *B. prionitis* in rodent formaldehyde and adjuvant induced arthritis models to prove its importance in the treatment of rheumatism.

2. Materials and methods

2.1. Plant collection

The leaves of *B. prionitis* were collected from Ashoka Nursery, Gharunda, Karnal, Haryana, India in March, 2011. Authentication of leaves was carried out by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum (RHMD), New Delhi. A voucher specimen of the plant (Ref. No. NISCAIR/RHMD/CONSULT/-2010-11/1497/95) has been preserved there for future references.

2.2. Preparation of extracts

The leaves were thoroughly washed under running tap water so as to remove any type of contamination. Then washed leaves were air dried in the shade, powdered in grinder and passed through sieve of mesh size no-40. The dried powder was first defatted by petroleum ether and then successive extraction was done with chloroform and methanol by the hot Soxhlet extraction method. The chloroform extract was concentrated in a rotary evaporator (Heidolph Instrument, Laborota 4000, Germany) under reduced pressure. The dried crude chloroform extract of *B. prionitis* (BPC) was collected and preserved in an airtight glass container at 4 °C–8 °C.

2.3. Bioguided fractionation

Initially, 200 g of BPC was partition-fractionated with 1:1 (v/v, volume ratio) of *n*-hexane and ethanol (50%), the mixture was shaken vigorously and kept for about 30 min to make the two layers separate. The upper layer consisting of *n*-hexane was removed and concentrated in a rotary evaporator to obtain hexane fraction (HBP). The same procedure was repeated with the bottom layer by using equivalent volume of ethyl acetate to get ethyl acetate fraction (EABP) and residual marc (RBP). These were further used in the studies based on chemical constituents.

2.4. Preliminary phytochemical screening

Phytochemical analysis was performed using standard procedures to identify chemical constituents as described by Khandelwal.[31] Precoated thin-layer chromatography plates of silica gel 60 $_{F254+366}$ with support of aluminium sheets 0.1 mm thick and 10 cm × 10 cm were used for the preliminary chromatographic characterization of fraction. EABP fraction (10 mg) was dissolved in methanol (mL). The mobile phase used was chloroform:methanol = 7:3. The plates were sprayed with natural product reagent and polyethylene glycol (NP/PEG) for detecting flavonoids. Phenolic compounds are detected after exposing the plates to ammonia vapours and immediate observation of fluorescent spots under UV light. Anisaldehyde in sulphuric acid was used as detecting agent for steroids.

2.5. Analysis of extract

Stigmasterol and extract were accurately weighed, and dissolved in methanol to produce a solution containing 1.90 mg/25 ml. The sample was run for 20.00 min, and volume of 5.00 µl was injected into the HPLC instrument. HPLC analysis was performed on an Hpersil C 18 150x 4.6 mm. (DRDC/AD/36) column with a UV detector. The mobile phase consisted of Methanol:Acetonitrile:Isopropyl alcohol: 40:20:20. The flow rate was 1.0 mL/min and the detection wavelength of the UV detector was set at 228 nm. The column temperature was set at 30 °C.

2.6. Drugs and chemicals

Formaldehyde (S.d. Fine Chemicals Ltd., Mumbai, India), FCA (Difco Laboratories, USA), Diclofenac (Symed Pharmaceutical Pvt. Ltd., Hyderabad) were used. Solvents used for extraction and chemicals used for phytochemical analysis were of analytical grade procured from approved organization.

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2.7. Dose and route of administration

The dosages of EABP used in this study were 125 and 250 mg/kg per day, which are based on our previous study.[30] Fresh drug solutions were prepared in Tween 80, (2% v/v) at the time of administration and were administered Per Oral (p.o.) so as to avoid any additional stress to the animals.

2.8. Preparation of reference drug

The reference anti-inflammatory drug diclofenac was dissolved in normal saline for the study. The drug solution was freshly prepared and administered orally at dose 4 mg/kg in volumes not exceeding 10 mL/kg.

2.9. Animals

Healthy Sprague Dawley rats (200–300 g) of either sex purchased from a disease free animal house of National Institute of Pharmaceutical Sciences and Education Research, Mohali, Punjab (India), were housed in the animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India). The animals were fed with commercially available feed and were maintained under standard conditions of temperature (25 °C ± 5 °C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. They were transferred to the laboratory twelve hours prior to the experiments and given only water ad libitum. In all the experiments, the animals were kept in cages with raised floors of wide mesh, to prevent coprophagy. The animals were housed and cared for in accordance with the Federal Government Legislation on animal care. Protocols for the study were approved by the Institutional Animals Ethical Committee (IAEC) for Animal Care (Register Number: 562/GO/02/a/CPCSEA) and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

2.10. Experimental procedure

2.10.1. Acute non-immunological formaldehyde-induced arthritis in rats

Sprague-Dawley rats were divided into five groups of six animals each. Baseline recording of the paw volume was made by using plethysmometer (Ugo Basile 7140, Italy).[32] Group I (vehicle control) received Tween 80, (2% v/v, orally), group II (negative control) received formaldehyde, group III and IV received EABP (125 and 250 mg kg^{-1} body weight/rat/day, orally), respectively and animals of group V received diclofenac sodium (4 mg kg^{-1} body weight/rat/day, orally) for 10 days. On day 1, 30 min after the drug administration, acute non-immunological arthritis was induced by sub plantar injection of 0.1 ml formaldehyde (2% v/v) into the right hind paw of all the animals except group I animals and repeated on day 3. Arthritis was assessed by measuring the mean increase in paw volume over a period of 10 days.

2.10.2. Chronic immunological FCA-induced arthritis in rats

2.10.2.1. Induction of arthritis. Adjuvant arthritis was induced in rats according to the method described by Newbould, (1963) with slight modification.[33] In this process, the initial hind paw volumes (both left and right) of the experimental animals were measured by water displacement plethysmography. Following anaesthesia with ketamine (120 mg/kg), 0.1 ml of FCA (a suspension of heat killed *Mycobacterium tuberculosis* in mineral oil) was then injected into the subplantar tissue of the right posterior paw. The volumes of the injected (ipsilateral), primary response; and un-injected (contralateral), secondary response; were measured on alternate days from 2 to 21 days after the adjuvant injection. Unilateral inflammatory oedema of the ipsilateral paw peaking at around days 6–8 was indicative of successful induction of adjuvant arthritis.

2.10.2.2. Experimental procedure. Grouping of animals is same as described in formaldehyde induced non-immunological arthritic model.

Group I: vehicle control (Tween-80 (2% v/v, orally).
Group II: FCA control (Tween 80 (2% v/v, orally).
Group III: EABP (125 mg kg^{-1} body weight/rat/day, orally).
Group IV: EABP (250 mg kg^{-1} body weight/rat/day, orally).
Group V: diclofenac sodium (4 mg kg^{-1} body weight/rat/day, orally)

Thirty minutes after administration of vehicle/drug, arthritis was induced by sub plantar injection of FCA. This was designated as day 0. After immunization with FCA, all groups were maintained on vehicle/drug treatment for 20 more days.[34] Anti-arthritic activity of active fraction was evaluated on paw volume, arthritic score, pain withdrawal latency and fall off time on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and day 21. Moreover body weights of animals were monitored regularly during the course of the experiment. On day 21, blood was withdrawn by retro-orbital puncture for assessment of haematological parameters and animals were sacrificed under light ether anaesthesia to study histopathology of joints.

2.11. Parameter assessment

2.11.1. Arthritic score

Photographs of the arthritic rats were taken on the 21st day with a digital 10-megapixel (Panasonic DMC-FS42) camera. Morphological feature of arthritis was monitored by the same person for all rats on the 21st day according to the extent of erythema and oedema of the joints, using the criteria as follows: normal paw = 0, mild swelling and erythema of digits = 1, moderate swelling and erythema of digits = 2, severe swelling and erythema of digits = 3, gross deformity and inability to use limbs = 4.[35] The arthritic score for each rat on day 0 was determined to be 0. The scores for each paw were then added to get the total arthritic score. Thus, the maximum possible score for each animal was 16.

2.11.2. Paw volume

The paw volume of both hind paws was measured just before FCA injection on day 0 and on alternate days after 2–21 days using a plethysmometer.

2.11.3. Anti-nociceptive activity

The apparatus consists of a hot plate on which the rats were placed for testing (Eddy's Hot Plate Method).[36] Pain threshold was determined by the latency for nociceptive response (withdrawal of any paw) with a maximum cut-off time 15 sec for all groups.

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2.11.4. Motor incoordination test

Motor incoordination was evaluated by Rota-rod apparatus as described earlier.[37] Rats were placed on the rotating rod of device for 1 min. The time taken for the falling of rats from the roller, during the period of 1 min was recorded.

2.11.5. Measurement of organ weight

Rats were sacrificed with ether on the 21st day, the spleen and thymus were removed, and weight of the organs was recorded and corrected for 100 g body weight.

2.11.6. Haematological assessment

On the 21st day, blood was withdrawn from each animal through the retro orbital plexus into a test tube containing anticoagulant (5% EDTA) and haematological parameters were determined. The red blood cells (RBC) were determined by the method of Huxtable,[38] white blood cell count (WBC) was determined using Raghuramulu.[39] Haemoglobin concentration was estimated by the cyan-methemoglobin method of Drabkin and Austin.[40] Erythrocyte sedimentation rate (ESR) was determined using the Wintrobe method.[41]

2.12. Histological analysis

After sacrifice on the 21st day, ankle joints were removed from the hind paw, weighed and fixed for 24 h in 10% formalin. After decalcification in 5% formic acid, processed for paraffin embedding, tissue sections (5 μm thick) were stained with haematoxylin and eosin. An experienced pathologist (Dr. Neeraj Mittal), unaware of the different drug treatment evaluated the slides under light microscope for the presence of hyperplasia of synovium, inflammatory cells, fibrosis and destruction of joint space.

2.13. Statistical analysis

The results were expressed as mean ± SEM. Statistical comparison was made between the drug-treated group and arthritic-control group. Statistical difference between two means was determined by one-way ANOVA followed by Dunnett's multiple comparison test using In-Stat 3 statistical computer software. Only those mean values showing statistical difference $P < 0.01$ or $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Preliminary phytochemical screening

Chromatographic study of ethyl acetate fraction of chloroform extract of leaves showed the presence of terpenoids, alkaloids, flavonoids, saponins and phenols. Further, HPTLC and HPLC studies confirmed the presence of stigmasterol (0.0465% w/w) in the EABP (Fig. 1).

3.2. Formaldehyde induced paw oedema

Fig. 2 summarizes the effect of EABP (125 and 250 mg/kg) and diclofenac on 2% formaldehyde induced arthritis. EABP significantly suppressed the joint swelling when compared with arthritic control between day 4 and day 10 post formaldehyde treatments. Even though both the doses of extract exhibited significant inhibition, dose 250 mg/kg of extract showed activity comparable with standard from day 8 to day 10.

3.3. FCA induced arthritis

Subplantar administration of FCA in the rat paw resulted in the progressive increase in the volume of the ipsilateral (injected) paw as well as contralateral (non-injected) paw. The difference in the volume of ipsilateral and contralateral paw between FCA and drug treated rats was statistically significant in a dose dependent manner. Biphasic response was observed, consisting of acute and polyarthritis phases. In the acute phase of disease erythema of one or more ankle joints was seen followed by involvement of the metatarsal and inter-phalangeal joint (Fig. 3).

3.3.1. Effect of EABP on arthritic index

All the animals administered with FCA results in increased arthritic score in one or more hind paws, which was a biphasic response. The adjuvant ipsilateral paw joints started to show swelling and rigidity around 8–10 days in the arthritis control group, and maximum level of arthritic score was observed on day 18. As shown in Fig. 4 rats treated with 125 and 250 mg/kg of extract showed a significant ($P < 0.01$) and dose dependent decrease in arthritic score from day 10 onward till the end of the study, i.e. day 21 as compared to FCA control animals. Although both the doses showed a significant decrease in the arthritic score, at 250 mg/kg, EABP showed suppressive effect comparable to standard drug.

3.3.2. Effect of EABP on body weight

There was less body weight gain of FCA control rats as compared to vehicle control rats, due to generation of immune response (Fig. 5). In EABP (125 and 250 mg/kg) and diclofenac treated arthritic rats, significant weight gain was observed as compared to disease control animals.

3.3.3. Effect of EABP on paw volume

Immunization with subplantar administration of FCA produced an increase in volume of both the injected as well as non-injected paws in all the FCA treated rats compared to vehicle control. There was maximum paw volume on day 8, after that a slight decrease in paw volume was observed from day 8 to day 12 in FCA treated rats. After 12th day a progressive increase in paw volume was seen till the end of study. On treatment with EABP (125 and 250 mg/kg) a significant ($P < 0.01$) and dose dependent decrease in the primary as well as secondary lesions was seen after 10 days of study. Effect observed with 250 mg/kg was comparable with standard drug diclofenac (Fig. 6).

3.3.4. Effect of EABP on nociceptive threshold

There was consistent decrease in paw withdrawal threshold observed in FCA treated rats compared to vehicle control animals and pain threshold was observed to be lowest on day 8. Administration of 250 mg/kg dose of EABP and diclofenac showed the decreased withdrawal latency from day 2 to day

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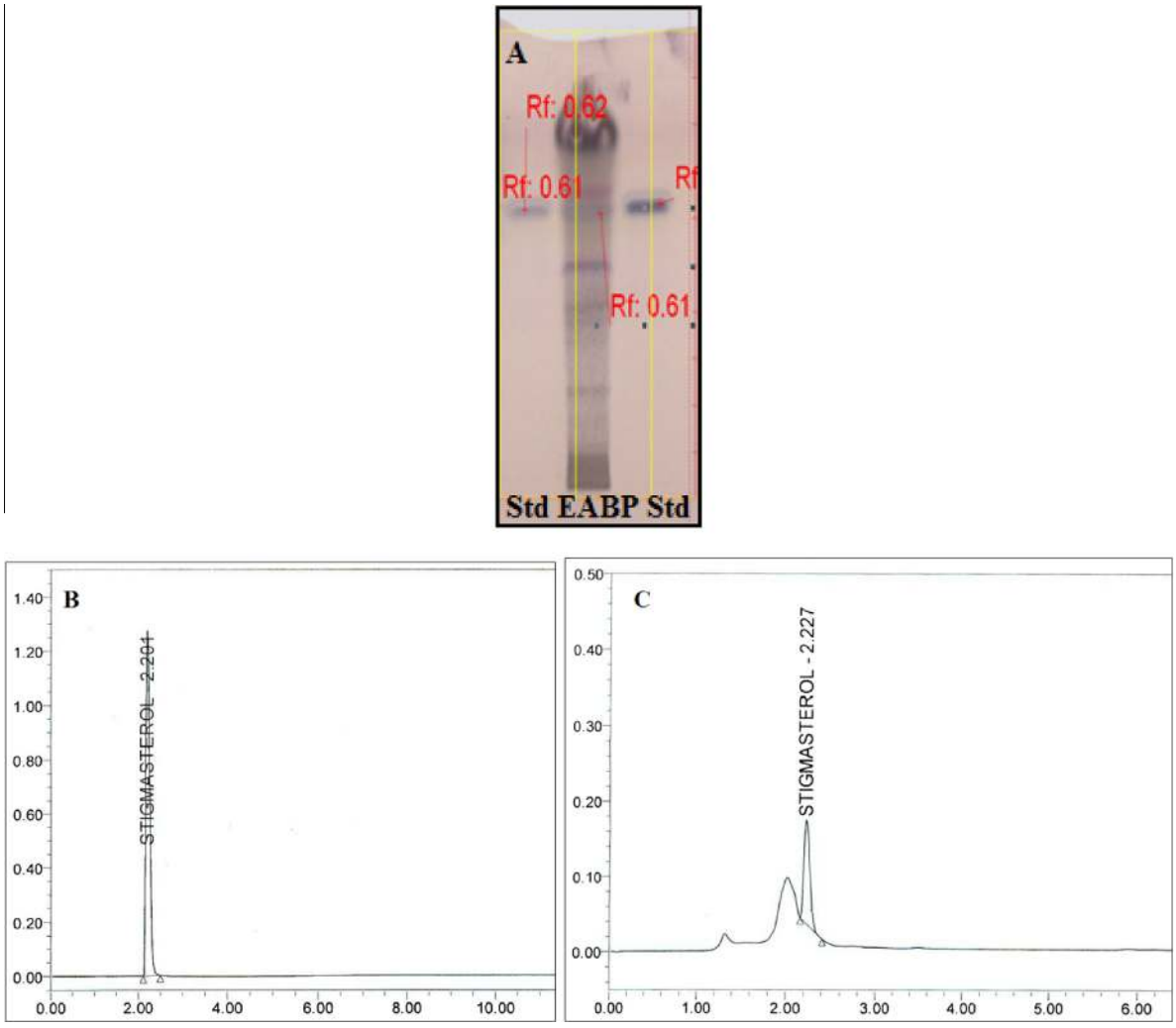

Figure 1 HPTLC (A) and HPLC fingerprinting of Stigmasterol (B) and EABP (C) showing the presence of stigmasterol (0.0465% w/w) in the extract.

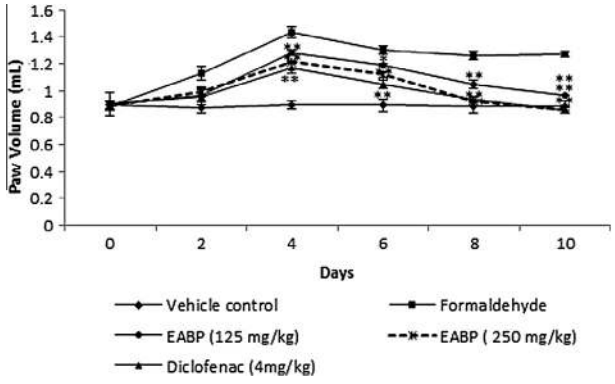

Figure 2 Effect of EABP (125 and 250 mg/kg) on formaldehyde-induced paw oedema. Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction in paw volume was analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the formaldehyde control group.

8, after that there was significant improvement in the paw withdrawal threshold from day 10 as compared to the FCA treated control group. Little improvement was observed in EABP (125 mg/kg) but that was not significant compared to FCA control (Fig. 7A).

3.3.5. *Effect of EABP on fall off time*

Mean fall off time in rota rod test was determined for the assessment of motor in-coordination. Sub plantar administration of FCA results in the decrease in fall off time in the FCA treated group as compared to the vehicle control group. Rats treated with EABP (125 and 250 mg/kg) and diclofenac (4 mg/kg) significantly increased ($P < 0.05$) fall off time from day 2 till day 21 as compared to the FCA control group (Fig. 7B).

3.3.6. *Effect of EABP on organ weight*

There was a decrease in thymus weight whereas the mean spleen weight was increased in the FCA treated rats as compared to the vehicle control group (Table 1). Rise in spleen weight was significantly ($P < 0.01$) inhibited in rats treated with EABP (125 and 250 mg/kg) and diclofenac (4 mg/kg) as

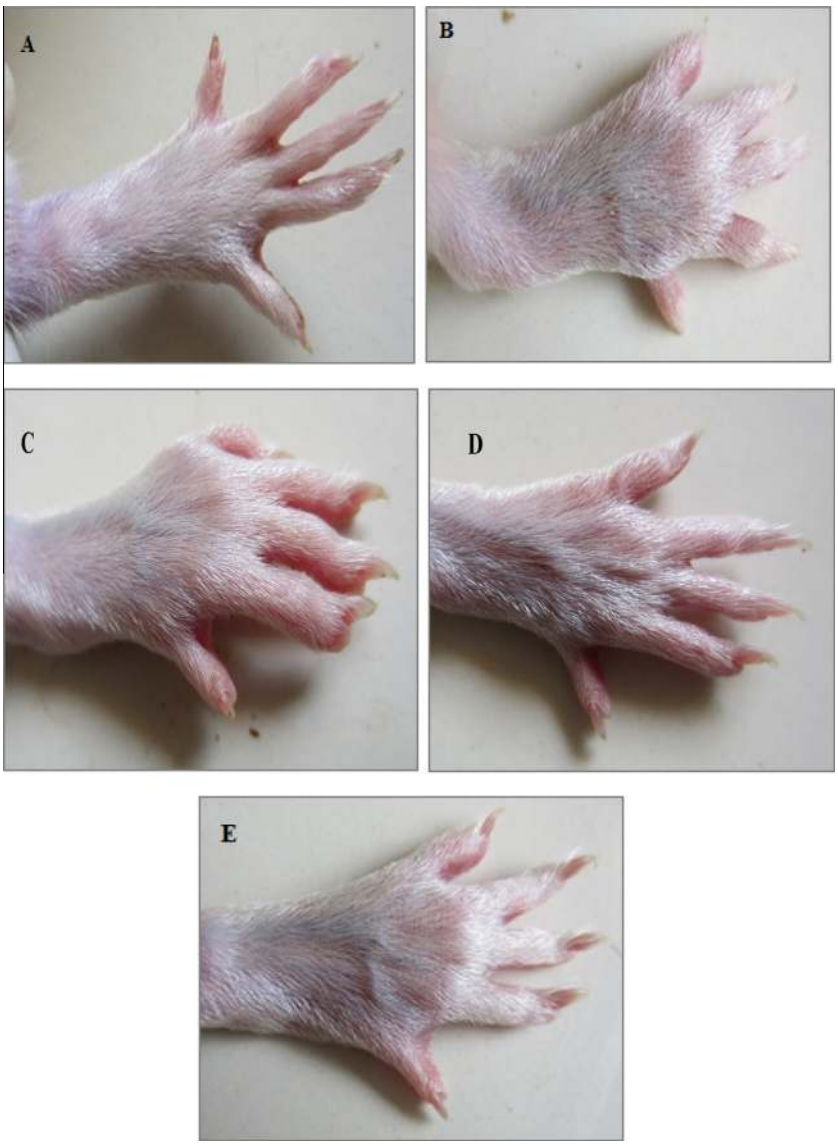

Figure 3 Morphological representations of the rat paw after subplantar administration of FCA. (A) vehicle control (B) FCA treated rats (C) EABP (125 mg/kg) (D) EABP (250 mg/kg) (E) diclofenac (4 mg/kg) treated rats.

compared to FCA treated rats. Only treatment with dose 250 mg/kg of EABP and diclofenac attenuated the decreased weight of the thymus, significantly ($P < 0.01$).

3.3.7. Effect of EABP on haematological parameters

There was a non-significant difference ($P > 0.05$) in W.B.C and R.B.C levels in EABP (125 and 250 mg/kg) and diclofenac (4 mg/kg) treated rats when compared to FCA arthritic animals (Table 2). Increased ESR level in FCA treated rats was observed as compared to vehicle treated rats. Treatment with EABP (125 and 250 mg/kg) as well as diclofenac (4 mg/kg) significantly ($P < 0.01$) attenuated this increased level of ESR as compared to FCA control rats. Furthermore in the diclofenac treated group only, significant ($P < 0.01$) improvement in haemoglobin level was observed.

3.3.8. Effect of EABP on histology of joints

Histopathological studies of the tibiotarsal joints show destructive lesions in connective tissue, vascularity into joint space, and granuloma formation in the FCA treated animals (Fig. 8). There was present normal connective tissue structure with the absence of necrosis in the tibiotarsal joint of the vehicle control group. Diclofenac treatment showed normal connective tissue of tibiotarsal joint with the presence of lesser oedema and absence of necrosis. EABP treated rats produced knee joint protection compared to arthritic rats by reducing the inflammation and necrosis. Rats treated with EABP (250 mg/kg) showed mild necrosis with oedema but granuloma was absent in tibiotarsal joint. Dose 125 mg/kg of EABP showed granuloma formation along with oedema and necrosis with few inflammatory cells.

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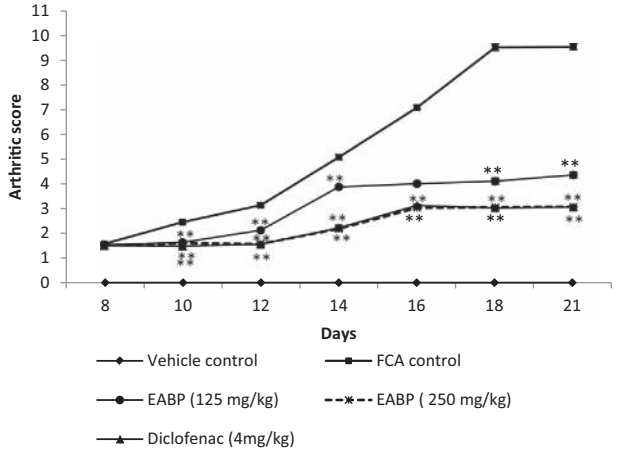

Figure 4 Effects of EABP (125 and 250 mg/kg) on arthritic score in FCA model. Values are plotted as the mean ± SEM, $n = 6$ in each group; Decreased arthritis score was analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to FCA control.

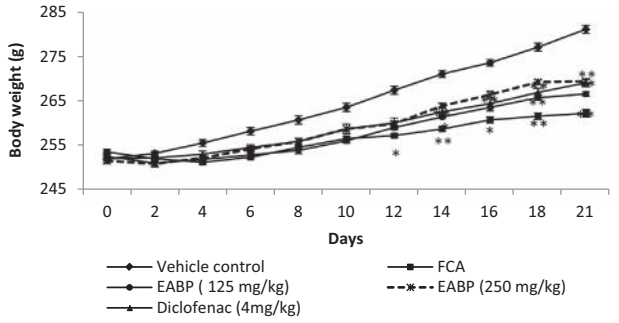

Figure 5 Effects of EABP (125 and 250 mg/kg) on body weight in FCA model. Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction in body weight was analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to FCA control.

5. Discussion

Rheumatoid arthritis is a chronic, inflammatory, autoimmune disorder affecting about 1% of adults worldwide.[6,42] In recent years, natural products are becoming an important area of interest for the development of new therapeutic entities due to their higher safety and lower cost. The present investigation examined the anti-arthritic activity of ethyl acetate fraction of chloroform extract of *B. prionitis*. Dosage selection was based on the previous research conducted by our research group.[30] We investigated the activity of fraction on rheumatoid arthritis by firstly evaluating against formaldehyde induced paw oedema, which is a very common and simple method for screening of anti-arthritic potential. Formaldehyde induced joint oedema inhibition has been commonly used as an experimental animal model for evaluation of anti-arthritic activity.[43] Biphasic pain, i.e. early neurogenic followed by tissue mediated response and localized inflammation is produced by

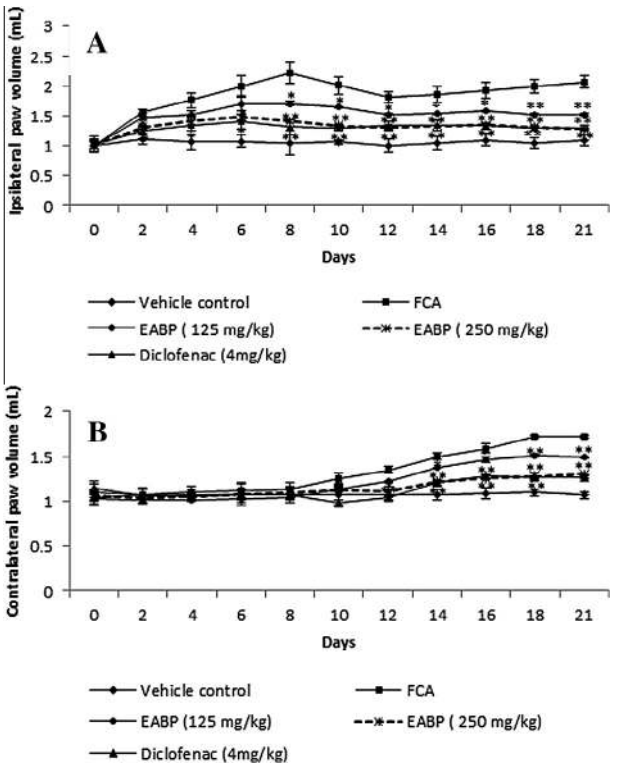

Figure 6 Effects of EABP (125 and 250 mg/kg) on (A) ipsilateral paw and (B) contralateral paw. Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction in paw volume was analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to FCA control.

formaldehyde injection into the rat paw.[44] In the present investigation, EABP inhibited the paw oedema, induced by formaldehyde injection. The effect may be due to certain changes in the inflammatory response which are comparable with diclofenac.

The anti-arthritic effect of EABP was further confirmed by Freund's Complete Adjuvant arthritis in rats. The FCA model is a well-established rat model to study the inflammation.[45] FCA consists of inactivated and dried mycobacterium, which effectively stimulates cell mediated immunity and ultimately leads the immunoglobulin production. The FCA induced arthritis shows pronounced swelling in the hind paw (Primary arthritis) which persists for a week and where generation of prostaglandin occurs. After few days, swelling in the contralateral as well as in front paw was seen (Secondary chronic arthritis) along with appearance of arthritic nodules in the ear and tail.[46,47] RA is an autoimmune disorder of unknown aetiology that is characterized by release of various inflammatory mediators. In recent investigations role of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interlukin-1b (1L-1b), 1L-6, 1L-8, MCSF, interferons and platelet derived growth factor (PDGF) has been revealed for the limb and joint swelling along with pain, joint destruction, deformity and disability.[48,49] The present study revealed that, paw volume was increased in adjuvant treated rats. EABP administration delayed the onset and attenuated the severity of disease as revealed by a decrease in volume of both paws via inhibition

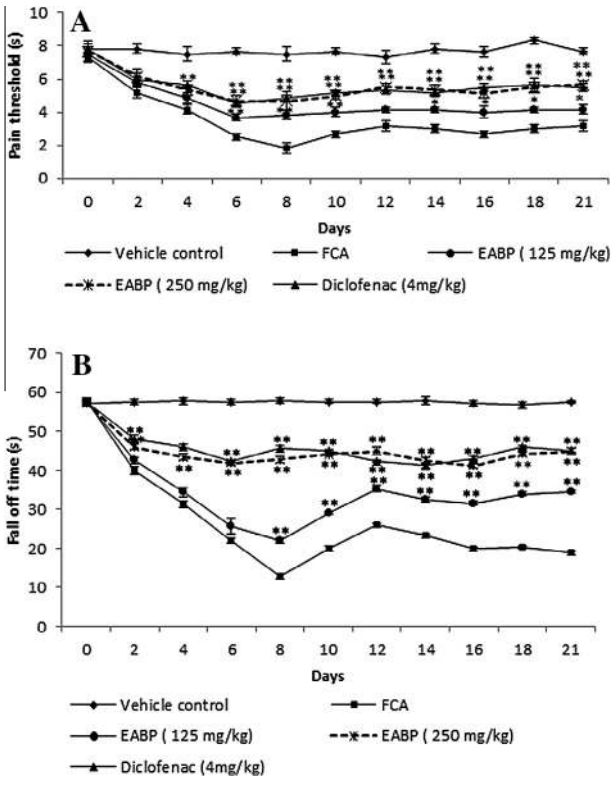

Figure 7 Effects of EABP (125 and 250 mg/kg) on (A) Pain threshold (s) and (B) Fall off time (s). Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction in fall off time and pain threshold were analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to FCA control.

of release of inflammatory mediators, thus indicating its anti-inflammatory potential. The effects shown by extract at dose 250 mg/kg were comparable to standard drug.

The diclofenac, a non-steroidal anti-inflammatory drug was used for comparison because it is commonly prescribed for the treatment of arthritis and its action is mainly through the inhibition of cyclooxygenase and prostaglandin production.[50,51] In the present study diclofenac sodium prevented the spread of adjuvant induced arthritis which is consistent with previous reports of various researchers.[52,53]

The severity of arthritis was expressed as arthritic score is the index of joint inflammation, being the sum of measures of four paws subtracted by measures before immunization.[54] Arthritic score of EABP treated groups was significantly lower than FCA induced rats, thus distinguish the immunosuppressive effects of fraction from its anti-inflammatory effect. Progression of disease status and response to anti-inflammatory therapy are indirectly linked with change in body weight.[55] RA is associated with weight loss and loss of lean body mass, known as rheumatoid cachexia which leads to the decreased physical activity, muscle strength and decreased daily performance.[56–58] Some researchers have reported the altered metabolic activities in the arthritic rats.[59] Due to inflammatory condition, intestinal absorption of ^{14}C-glucose and ^{14}C-leucine was reduced and treatment with anti-inflammatory drugs has improved the decreased absorption capacity.[60,61] In the present investigation, the FCA treated rats showed less body weight gain as compared with diclofenac and extract treated arthritic rats. Thus, body weight gain may be due to the restoration of the absorption capacity of the intestine that shows effective management of rheumatoid cachexia.

It has been evidenced by previous studies that Eddy's hot plate is a quantitative method for determination of hyperalgesia related to behaviours.[36,62] Inhibiting controls caused by long standing nociceptive input from the contra lateral

Table 1 Effect of EABP (125 and 250 mg/kg) treatments on thymus and spleen weight.

Groups	Dose (mg/kg)	Spleen weight (mg/100 g b.wt.)	Thymus weight (mg)
Vehicle control	–	185.5 ± 4.09	103.5 ± 2.01
FCA control	–	249.16 ± 2.71	81.00 ± 0.81
EABP	125	231.50 ± 1.56**	84.50 ± 0.76
EABP	250	212.00 ± 1.06**	89.50 ± 1.33**
Diclofenac	4	209.83 ± 4.20**	91.00 ± 0.96**

Values are plotted as the mean ± SEM, $n = 6$ in each group; significant change was analysed by one-way analysis of variance followed by Dunnett's multiple comparison test using Graph pad Instat Software; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to FCA control.

Table 2 Effect of EABP (125 and 250 mg/kg) on haematological profile.

Treatment	Dose (mg/kg)	RBC ($\times 10^6$/μL)	WBC ($\times 10^3$/μL)	Hb (g/dL)	ESR (mm)
Vehicle control	–	8.05 ± 0.12	11.48 ± 0.16	13.21 ± 0.10	3.05 ± 0.07
FCA control	–	7.58 ± 0.11	14.15 ± 0.18	11.56 ± 0.18	9.43 ± 0.12
EABP	125 mg/kg	8.00 ± 0.07	12.88 ± 0.61	12.40 ± 0.37	6.31 ± 0.19**
EABP	250 mg/kg	7.96 ± 0.08	12.85 ± 0.72	12.43 ± 0.33	4.08 ± 0.12**
Diclofenac	4 mg/kg	8.00 ± 0.12	12.21 ± 0.43	13.08 ± 0.09**	3.53 ± 0.10**

Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction was analysed by one -way analysis of variance followed by Dunnett's multiple comparisons test using Graph pad Instat Software; $^{**}P < 0.01$ compared to FCA control.

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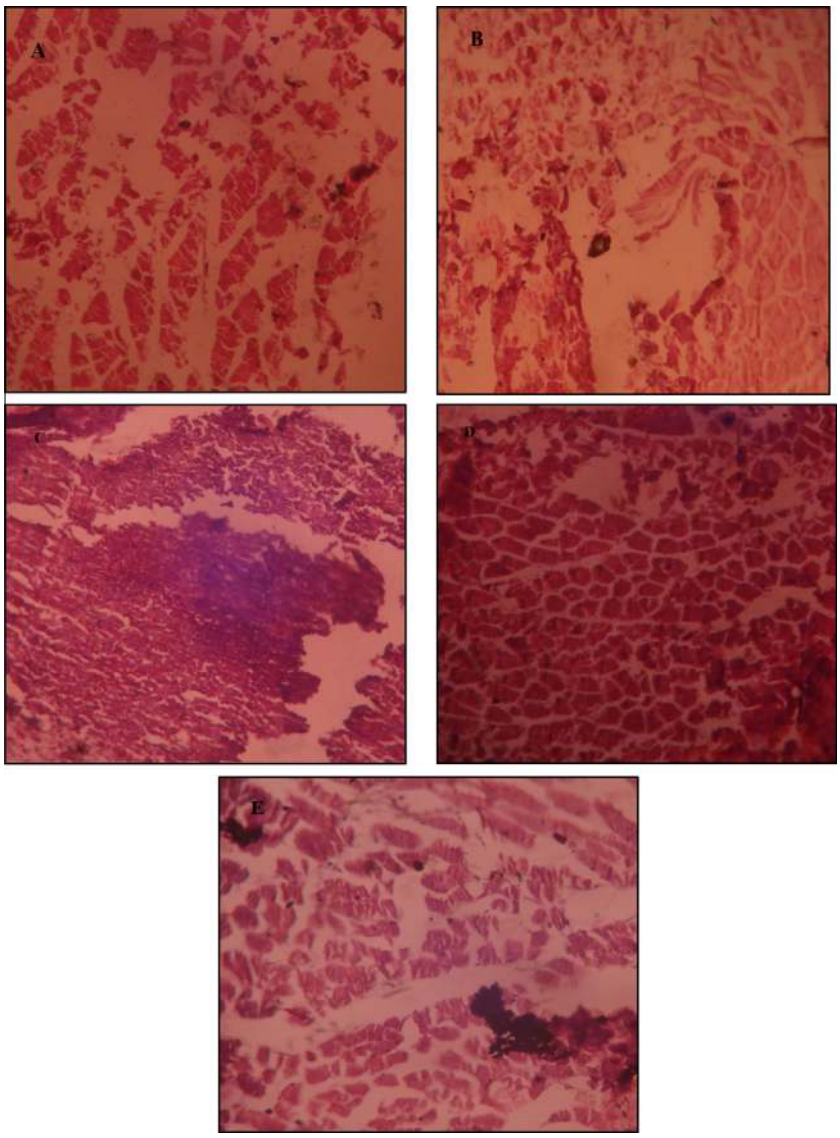

Figure 8 Histology of the arthritis developing 21 days after immunization with FCA compared with unimmunized Sprague Dawley rats. (A) Vehicle control; (B) FCA control; (C) EABP (125 mg/kg); (D) EABP (250 mg/kg); (E) Diclofenac (4 mg/kg). Magnification × 100; thickness: 5 μm.

arthritic limb is probably involved in the alterations occurred after an acute pain stimulus in the non-affected limb. Study of an earlier researcher shows that increased pain sensitivity is linked with decreased hyperalgesia latency .[36] In the present investigation we observed that EABP showed significant improvement in nociceptive threshold.

The spleen is a vital organ which serves as the available source of cells and antibody formation, known to be involved in immunological response in adjuvant arthritis.[63] In the spleen of arthritic rats, increased cellularity is observed. Due to splenomegaly, increase in the weight of spleen occurs.[64] EABP attenuated the increased weight of spleen and decreased weight of thymus may be due to the immune-stimulant effect.

The erythrocyte sedimentation rate is elevated in various stress conditions, cell necrosis and inflammation.[65] The ESR is an indirect method for the measurement of inflammation in the body. Due to protein production in inflammation, erythrocytes move closer, stack up in a group, become denser and settle faster. With the increase in erythrocyte settling rate, erythrocyte sedimentation rate also increases.[66] The significant low level of ESR in the EABP and diclofenac treated arthritis rats indicates their anti-inflammatory potential.

Histopathological studies of the paws contribute towards the treatment of arthritis with EABP despite evidence of pathology in the arthritic animals treated with the reference drug.

The anti-arthritic effect of fraction (EABP) of chloroform extract of *B. prionitis,* established in this study could be attributed to the presence of flavonoids, triterpenoid, saponins, tannins and steroids detected after phytochemical screening of the fractions. Triterpenoids are known to inhibit histamine release from mast cells and exert anti-inflammatory effects. Non-specific anti-arthritic activity may be due to the combined effect of the different phytoconstituents present.

6. Conclusions

On the basis of the results obtained in the present study we suggest that possibly, the anti-arthritic potential of EABP may be through protection of synovial membrane, vascular permeability, prevention of cartilage destruction, thereby improving the health status through haematinic properties. The effect may be due to the inhibition of phospholipase A_2 and prostaglandins due to similar effect of diclofenac. The results obtained in the present study demonstrate beneficial effects of plant during recovery from arthritis by including Hb, ESR and body weight along with clinical signs including paw oedema, thermal hyperalgesia and histopathological examination. The study established that the ethyl acetate fraction of *B. prionitis* possesses anti-arthritic activity in Sprague Dawley rats.

However, further studies are needed to identify and isolate the possible phytoconstituent(s) involved in the anti-arthritic activity, which would facilitate the use of *B. prionitis* in inflammatory disease.

Conflict of interest

None.

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