Original Article

Experimental evaluation of anti-inflammatory effect of topical application of entada phaseoloides seeds as paste and ointment

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Citation: Dawane JS, Pandit VA, Rajopadhye BD. Experimental evaluation of anti-inflammatory effect of topical application of entada phaseoloides seeds as paste and ointment. **North Am J Med Sci** 2011; 3: 513-517.
doi: 10.4297/najms.2011.3513.

Abstract

Aims: The study was to study the anti-inflammatory effect of topical application of different formulations of seed pulp of entada phaseoloides. **Method and Material**: After removing the shell, entada phaseoloides seeds were powdered. Paste was prepared with water and ointment with polyethylene glycol & Carbowax 3350. 32 Wister rats of either sex weighing 140-200 gram were divided into four groups, Group-I vehicle, Group-II entada phaseoloides paste, Group-III entada phaseoloides Ointment, Group-IV Diclofenac sodium Ointment. Arthritis was induced by injecting 0.1ml Complete Freund's adjuvant in sub plantar region of the left hind paw. Drug treatment was started on the same day and given for 12 days. Paw volume was measured with Plethysmometer on day 0, 1, 5, 12 and 21 for both the paws. Bodyweight and Gait was observed throughout the study. **Results:** Localized inflammatory reaction developed in all the rats in 24 hours. In control group, there was no resolution of swelling even in 21 days. Both EP formulations showed significant ($P < 0.001$) anti-inflammatory activity as compared to control. entada phaseoloides ointment was equi-effective to that of Diclofenac sodium on 12th day. entada phaseoloides paste was significantly ($P < 0.05$) more effective than Diclofenac sodium on 21st day. **Conclusion:** Both the formulations of entada phaseoloides have anti-inflammatory activity and entada phaseoloides paste is significantly more effective than diclofenac sodium.

Keywords: Complete Freund's adjuvant, plethysmometer, anti-inflammatory.

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Introduction

Arthritis is one of the inflammatory condition affecting both sexes and all ages involving damage to the joints. Inflammation is a protective and defence mechanism of the body. Its main purpose is to destroy the injurious agent and/or minimize its ill effect by limiting spread [1]. Inflammatory response occurs in two distinct phases. An acute phase characterized by local vasodilatation, increased capillary permeability and release of inflammatory mediators like histamine, serotonin, prostaglandins etc. Chronic phase is characterized by infiltration of leukocytes and phagocytic cells [2]. This results in tissue degeneration and fibrosis. So, inflammation involves two basic processes-inflammatory responses and healing [1].

There are many types of arthritis, of which rheumatoid and osteoarthritis are the most common forms. In all types of arthritic pain, inflammation and functional restriction are the presenting manifestations [3]. Naturally, symptomatic relief is the major aim of treatment. Presently many steroidal, non-steroidal and immunosuppressive drugs are used to control pain and inflammation [4]. They are associated with a variety of adverse effects. These drugs can cause iatrogenic diseases which can be as difficult to manage as the disease itself [5].

Entada phaseoloides (Mimosaceae) is a gigantic climber tree with twisted and angled stem. Seeds of this plant commonly called as Gilla (Sanskrit), Hathibij (Hindi), Garambi (Marathi) and Gogo are traditionally used

worldwide for medicinal purpose[6]. The seeds are hard, circular, chocolate brown with their sides flattened about 5 cm across. Paste of the seed pulp is used as an herbal medicine to reduce inflammation and pain of joints and lymph nodes. In addition seeds are reported to have emetic, anthelmintic and antimalignant activities [6, 7]. No reference is available where this preparation is tested for its topical anti-inflammatory activity using modern medicine parameters. Therefore, the purpose of present study was to find out the anti-inflammatory potential of entada phaseoloides seed pulp after topical application in animal models of arthritis.

Materials and Methods

The study was started after obtaining approval from Institutional Animal Ethics Committee, approval Letter No. IAEC/BVDUMC/01/2008-2009.

Collection and Authentication of Plant Material

Entada phaseoloides seeds were obtained from M/s Gopal Govind Lokhande, 764 Budhwar Peth, Near Phadke Houdh, Pune 411002 known vender of ayurvedic and unani medicine. Authenticated in Agharkar Research Institute, Pune-411004.

Drugs and Chemicals

Complete Freund's Adjuvant obtained from Sigma Chemical Co., USA.; Voveran Emulgel obtained from pharmacist of Novartis India Ltd-Mumbai, Polyethylene glycol 400 and 3350 Analytical grade (ANALAR) from local supplier Sharad chemical Agencies, Pune., Pentobarbitone Sodium from Loba Chemie Industries, Mumbai.

Formulations Used

(1) Paste of entada phaseoloides (EP) seeds powder [6, 7]
Seed pulp (EP) was powdered and sieved through mill (No. 80). Fresh paste was prepared by addition of water in the powder (5 gm/5 ml) and mixed to make uniform paste.

(2) Ointment of EP seeds powder [9, 10]
Polyethylene Glycol (PEG) Ointment base was prepared by mixing PEG 400 (60%) & 3350 (40%). 5gm PEG mixture was warmed to 65 ^{0}C, stirred while cooling until congealed. 5 gm EP powder was incorporated in 5 gm ointment base using tile and spatula. This preparation was divided into ten equal parts on the tile and one part is applied topically to each rat every day.

Standard Drug

Market preparation of Diclofenac ointment as Voveran Emulgel was used as positive control.One hundred gram of Voveran Emulgel contains 1.16 g of diclofenac diethyl ammonium (equivalent to 1 g DS), isopropyl alcohol, propylene glycol, perfume, Cream 45, and other additives.

Animals

Albino Wistar rats of either sex weighing 140-200 gm were used for the study. Animals were procured from Central Animal House recognized by CPCSEA (Regd. No. 258) of B.V.D.U. Medical College, Pune. Housing 12 hrs day and night cycle maintained. The rats were fed with commercial rat diet and Aquaguard water ad libitum. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethical Committee Guidelines.

Groups

Rats were randomly divided into four groups of 8 animals in each. Group-I Control treated with ointment base. Group-II EP paste, Group-III EP Ointment and Group-IV Diclofenac Sodium ointment All topical formulations were gently applied to the plantar surface of the left hind paw. Ointment base was applied to control rats.

Procedure

Evaluation of the anti-inflammatory activity was done by measurement of edema size resulting from Complete Freund's adjuvant injection in the left hind paw region of the body [11]. Normal paw volume and body weight was recorded on day 0. Induction of Inflammation was done with Complete Freund's adjuvant. After anesthesia with 6 mg/100 gm, Pentobarbitone i.p., 0.1 ml of Complete Freund's adjuvant i.e. complete fraction of Mycobacterium tuberculosis suspended in mineral oil was injected in the sub-plantar tissue of the left hind paw. Topical treatment was started, as per groups on the same day and continued up to day 12.

Parameters tested

Body weight was measured on day 0, 1, 5, 12 and 21. Gait of all the animals was observed and scored according to, 0-Three legged gait, 0.5-Marked limping & 1-Normal gait [11]. Paw volume was measured with Plethysmometer on day 0, 1, 5, 12 and 21[13]. Paw volume in test group was compared with control and standard.

Statistical Analysis

The statistical package, Graph Pad Prism 5 was used to analyze all results. Values are expressed as mean ± S.E.M.. One way ANOVA followed by post hoc analysis (Dunnett's test) was used for analysis of data and for comparisons between treated and control groups. $P < 0.05$ was considered significant.

Results

Localised inflammatory reaction due to Complete Freund's adjuvant (CFA), as evident by increase in paw volume, started in 24 hrs, peaked on day 5 and reduced by day 12. In control group, paw volume kept on increasing over the observation period of 21 days. In group II, III & IV increase in paw volume was significantly less ($P \leq .001$) till day 12 in comparison with control. On day 21, only group II showed significant ($P \leq .001$) reduction in paw volume (Table 1). In Acute Phase**,** as compared to control group, all drug treated groups were effective in reducing paw volume. However, in chronic phase**,** only EP paste showed significant effect.

Table1 Effect of topical application of entada phaseoloides on rat paw volume in adjuvant induced arthritis model

Paw volume left	Group I (Control)	Group II (EP Paste)	Group III (EP Ointment)	Group IV (Diclofenac Ointment)
Day 0	1.29±0.10	1.22±0.07	1.24 ±0.07	1.27±0.05
Day 1	1.55±0.08	1.31±0.13***	1.39±0.14**	1.38±0.05**
Day 5	2.22±0.23	1.85±0.15**	1.93 ± 0.11*	1.92±0.29*
Day12	2.11±0.22	1.31±0.11***	1.38±0.11***	1.29±0.12***
Day21	2.58±0.16	1.92±0.13***	2.36 ± 0.30	2.38 ± 0.32

*: $P \leq 0.05$,**: $P \leq 0.01$, ***: $P \leq 0.001$ in comparison with control. n=8. Values indicate mean ± SEM. Treatment was given from day 0-12.

Table 2 Effect of topical application of entada phaseoloides on body weight of rats in adjuvant induced arthritis model

Weight	Group I (Control)	Group II (EP Paste)	Group III (EP Ointment)	Group IV (Diclofenac Ointment)
Day 0	145.13±4.97	144.75±4.71	145.25±6.16	147.37±3.93
Day 1	145.13±4.97	144.75±4.71	145.25±6.16	147.37±3.93
Day 5	146.00±6.53	159.75±8.19***	147.25±3.01	148.37±5.26
Day 12	153.75±5.09	166.25±5.92***	155.5±5.95	157.00±4.75
Day 21	160.12±8.95	172.88±12.02*	163.37±6.65	164.87±5.87

*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$ in comparison with control. n= 8, Values indicate mean ± SEM. Treatment was given from day 0-12.

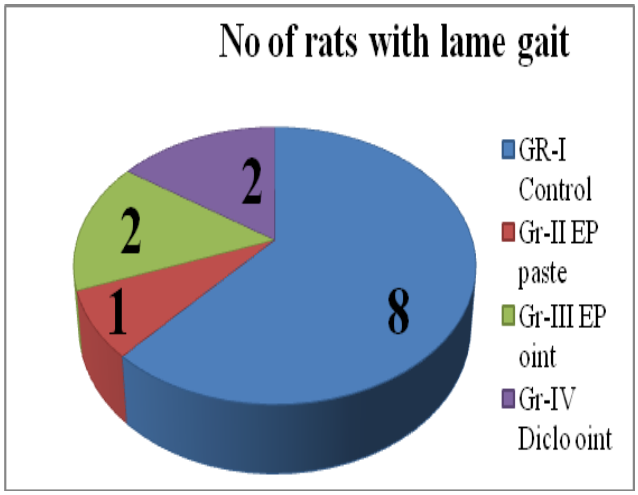

Fig. 1 Effect of topical application of entada phaseoloides on gait of rats in adjuvant induced arthritis model

Fig. 2 Three legged gait of control rat

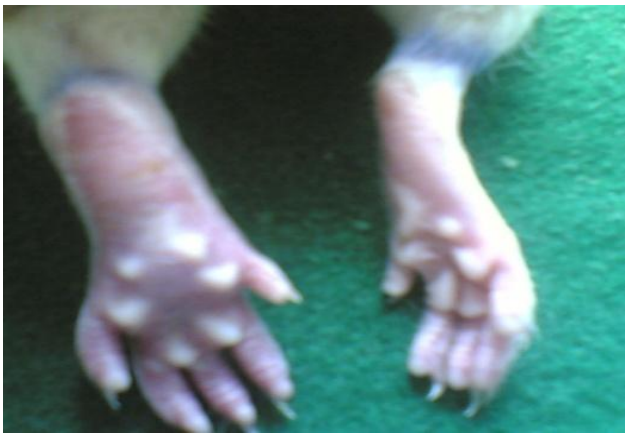

Fig. 3 Paw of control rat on day 5

Mean weight of all the groups is comparable at day 0. From day 5 onwards, mean animal weight in group II was significantly more when compared to that in other groups (Table 2). The difference was highly significant on day 5 and 12 ($P \leq .001$).

Lame gait was seen in all the control animals. Two animals from Group III (EP ointment), Group IV (Diclofenac ointment) and only one animal of Group II developed lame gait (Figures 1-3).

Discussion

Present study was planned to evaluate the anti-inflammatory effect of topical application of EP formulations in animal models of arthritis.

Oral methanolic extract of EP seeds was effective in sub-acute inflammation [8]. Oral administration of EP is difficult because of its emetic action. EP is used topically as freshly prepared paste before use by practitioners of traditional & ayurvedic medicine for rheumatoid arthritis and osteoarthritis [6, 7]. Topical anti-inflammatory agents used commonly are available in ointment forms i.e. ready to use formulations. The delivery of drug through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks, and non invasive nature of the treatment [25, 26]. Therefore, in the present study, paste and ointment formulations of EP were studied.

Adjuvant induced arthritis in rats, a chronic inflammatory disease characterized by infiltration of the synovial membrane and associated with destruction of the joints, resembles rheumatoid arthritis in humans in terms of immunological and biochemical features [12]. EP in the form of paste is reported to be useful in treating joint inflammation [6, 7]. It can be used in many types of arthritis but its main utility is in rheumatoid and osteoarthritis to reduce pain and inflammation. So, animal models selected was-Complete Freund's adjuvant induced arthritis.

After injection of Complete Freund's adjuvant, animals were observed for paw edema, gait & weight gain. Within

a day of injection of Complete freund's adjuvant, acute inflammatory response gets initiated and injected paw becomes red and edematous [14, 15]. This acute phase reaction continues involving synovium and intra-articular tissues till day 5. From day 5, inflammation spreads to para-articular tissues and also involves bone in subchondral region (pannus formation). Local joint inflammation continues if left untreated. After about 12 days, inflammation becomes chronic and extensively damages joint at the site of injection. Joints at non-injected sites also get affected [12, 23, 24]. This spreading chronic inflammation resembles rheumatoid arthritis **[**14**]**. Since the treatment was given as topical application, inflammatory changes in the joint at the injection site were studied in acute as well as chronic phase.

Comparing the results of paw oedema, EP paste was found to be highly significantly effective in reducing inflammation from day 1. EP ointment was equi-effective to that of diclofenac ointment when compared to control but was less effective than paste on day 1. On day 5 also, EP paste was more effective than ointment. On day 12, EP paste, EP ointment and Diclofenac ointment were equi-effective.

EP paste and ointment were effective in reducing paw oedema similar to Diclofenac sodium. This indicates the efficacy of EP formulations in acute inflammation. This observation is contrary to the lack of effect on acute inflammation reported after oral EP [8], and is consistent with report of efficacy of topical application of EP paste [6, 7]. Difference in the results after oral and topical application is probably because of less availability of EP after oral administration.

Topical application was stopped on day 12, but significant reduction in paw edema persisted with EP paste till day 21. This effect was not observed with EP and Diclofenac ointment. In Complete Freund's adjuvant induced arthritis both anti-inflammatory and immunosuppressant drugs are effective [3-5]. Anti-inflammatory drugs are effective in acute phase and immunosuppressant in chronic phase. Saponins shows wide-ranging cytostatic effects against cancer cells [20]. Saponins are important constituents of EP [16]. EP is reported to have anti-malignant action[7], and all antimalignant drugs produce immunosuppression [3, 4, 7]. Since EP was effective in both the phases it may be having both anti-inflammatory and immunosuppressant actions.

The membrane-permeabilising activity showed by a large number of Saponins, whether triterpenoid or steroid. Strong foaming of aqueous solutions is the characteristic of Saponins. Foaming occurred in paste but not in ointment. This could be the factor responsible for increasing availability of EP in paste formulation leading to better efficacy [21].

After injection of complete Freund's adjuvant in left hind paw, the gait of all animals was changed. All rats in control group could not ground the left hind paw and had three legged gait as observed on day 5 and 12. Lame gait was present in two animals each of EP and diclofenac ointment groups. Only one animal of EP paste treated group had lame gait. All the animals were limping, though they could touch the ground with the affected leg.

Limping is the effect of pain and tenderness in left hind paw. Lame gait in all control animals is well correlated with more inflammation is control group. Gait of EP paste treated animals was minimally affected in comparison with other groups. Near normal gait indicates less inflammation and less pain.

A change in body weight of rats was also measured as one of the parameter to assess the course of the disease and the response to therapy of anti-arthritic drugs [19]. Reduced weight gain or weight loss are the indirect parameters of assessment of severity of arthritis [11]. It is reported that anorexia due to fever and pain, gastrointestinal disturbances and disabilities interfering with self feeding are responsible for not gaining weight as in the normal animals [19].

On day 12, EP ointment and diclofenac ointment group showed marginal weight gain as compared to control. EP paste, on the other hand, showed highly significant weight gain on day 12 which gradually reduced till day 21. This weight gain though was not comparable to that in the normal animals i.e. 30 - 50 gram in 21 days.

Considering that anti-inflammatory action of all three formulations was comparable on day 12, the difference in weight gain with EP paste alone may be due to some additional action. Sapogenin which is present in EP could be a steroid [7]. Steroids are known to have mood elevating action (euphoria) [3]. This may give the feeling of general well being leading to increased food intake and weight gain. Saponins are reported to have beneficial effect on the efficiency of feed utilisation and growth in animals [20]. Increase in weight gain with EP paste may be due to increased availability of EP in this formulation.

Two sulphur containing amides present in EP 'Entadamide A' and 'Entadamide B' are shown to inhibit 5-lipooxygenase (5 LOX) enzyme, [17, 18]. High concentrations of Leukotrienes have been observed in the arthritic joints. Which may be a probable cause of symptomatic relief in arthritis; further studies are required to find out exact mechanism of action.

Conclusion

EP paste and ointment have potent anti-inflammatory actions and is comparable to that of diclofenac ointment. The present study revealed, both the formulations of EP are effective in acute inflammation Only EP paste is effective in chronic inflammation. EP paste is more effective than ointment formulation. Supportive parameters like Near normal gait indicates Less inflammation and Healthy weight gain signifies General

well being. This study supports the topical use of pulp of EP seeds in inflammatory conditions.

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