

Anti-inflammatory and analgesic activities of stem bark extracts of *Eugenia jambolana*

Sir,

The world is facing an explosive increase in the incidence of many systemic diseases and hence cost effective complementary therapies are needed.^[1] Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development. The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non narcotics (NSAIDS), and have known toxic and lethal effects.^[2] On the contrary, herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times.^[3] It is therefore, essential that efforts be made to introduce new medicinal plants, to develop cheaper and effective drugs.^[4] *Eugenia jambolana* Lam. (Myrtaceae) has been claimed to reduce different ailments in the folk medicine. We have investigated the anti-inflammatory and analgesic activities of petroleum ether, ethyl acetate, and methanol extracts of *E. jambolana* stem bark.

The barks of mature plants were collected from the Soraba region of Shimoga district, Karnataka, India, and the taxonomic identification of the plant was done by Dr. Y. L. Ramachandra, Department of Biotechnology, Kuvempu University, Shankaraghatta (Voucher specimen number E/002/2008). Freshly collected whole stem bark of *E. jambolana* was shade-dried, powdered, and extracted successively in petroleum ether, ethyl acetate, and methanol (LR grade, Merck, India) using the Soxhlet apparatus.

Healthy, inbred male Albino rats of Wistar strain (150 – 200 g) and male Swiss albino mice (25 – 30 g) were used for the

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study. The experiments were carried out taking appropriate measures to minimize pain or discomfort, in accordance with the guidelines laid down by CPCSEA (INDIA), with regard to the care and use of animals for experimental procedures, and with due clearance from the Institute Animal Ethics Committee. Based on the acute toxicity study,^[5] 500 mg/kg, 500 mg/kg, and 200 mg/kg body weight was taken as the therapeutic dose of petroleum ether (EJP), ethyl acetate (EJE), and methanol (EJM) extracts of stem bark of *E. jambolana* (EJ), respectively.

Acute inflammation was induced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats, 30 minutes after oral administration of the drugs. The degree of paw volume was measured initially and then at intervals of one, two, three, and four hours after the carrageenan injection, by using the plethysmometer. Diclofenac sodium 5 mg / kg was used as the standard drug. The anti-inflammatory effect of different test extracts was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = [(V_c - V_t)/V_c] \times 100$$

Where, V_c represents the inflammatory increase in paw volume in the control group, and V_t represents the increase in paw volume in drug-treated animals.

The extracts were tested for analgesic activity using two methods, namely, acetic acid-induced writhing and the tail flick method. The description of the methodology is as follows:

Mice were injected with 10 ml/kg of 3% aqueous acetic acid intraperitoneally (i.p) 30 min after treatment of *E. jambolana* petroleum ether (EJP), ethyl acetate (EJE), and methanol (EJM) extracts (500, 500, and 200 mg/kg, respectively) or aspirin 30 mg/kg orally, to all groups, as shown in Table 1. The number of writhing episodes of an individual mouse was recorded for 20 min after acetic acid treatment.

In the tail flick method, the mice were treated with different crude extracts orally or with pentazocine 25mg/kg i.p. After 30 min the animals were held firmly to immerse its tail in a water bath that was maintained at 54°C. The time required for the violent jerk of the tail, was recorded to assess the response to the noxious stimulus.

The results were expressed as mean $\pm$ SEM. The statistical significance was determined by using the Student's *t*-test and one-way Analysis of Variance (ANOVA), followed by Tukey's multiple pairwise comparison test. The level of significance was considered to be at $P < 0.05$.

The anti-inflammatory activity of the *E. jambolana* stem bark extracts were measured against acute paw edema induced by carrageenan and the results are shown in Figure 1. The results were comparable to those of diclofenac sodium as a standard anti-inflammatory drug. The extracts of *E. jambolana* stem bark exhibited a varying degree of activity. The methanol extract (EJM), however, showed a promising result at the dose of 200 mg/kg after four hours, where it caused 30.7% (0.52 ± 0.01) inhibition. Furthermore, the control group did not show any inhibition even after four hours. Carrageenan-induced inflammation is a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents. Edema formation in the rat paw is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins, whereas, the second phase is due to the release of prostaglandin and slow reacting substances.^[6] Similar observations were made in the present investigation where a sudden peak was noticed at three hours. Furthermore, EJM was more effective and comparable to the standard drug diclofenac sodium, which corresponded with the previous findings.^[7] The results of our study indicate that *E. jambolana* is effective against acute inflammatory disorders. The carrageenan-induced inflammation model is a predictive test for anti-inflammatory agents acting against mediators of acute inflammation.^[8]

Table 1: Analgesic activity of different extracts of *E. jambolana* stem bark on acetic acid-induced writhings in mice

Treatment	Dose mg / kg	No. of writhings	Writhing inhibition
Control	---	28.5 $\pm$ 1.06	
Aspirin	30	9.8 $\pm$ 0.48*	65.6%
EJP	500	16.8 $\pm$ 0.98*	40.3%
EJE	500	15.0 $\pm$ 0.58*	43.4%
EJM	200	12.5 $\pm$ 0.76*	56.2%

n = six per group. Values are mean $\pm$ SEM. * $P < 0.01$ compared to control (a) EJP – Petroleum ether extract; (b) EJE – Ethyl acetate extract; (c) EJM – Methanol extract

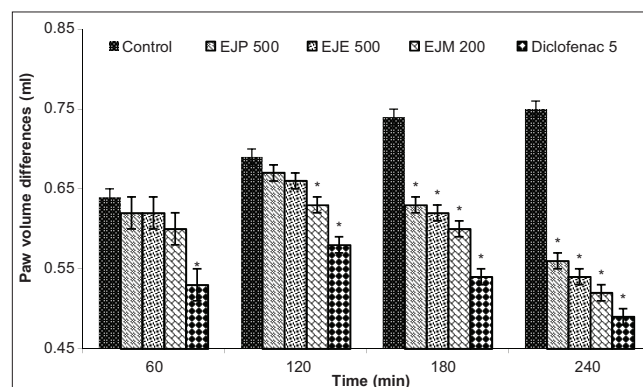


Figure 1: Effect of different extracts of *E. jambolana* on carrageenan-induced rat paw edema

Table 2: Analgesic activity of different extracts of *E. jambolana* stem bark on tail flick response in mice

Treatment	Dose mg/kg	Time required for flicking (s)
Control	-	3.0 ± 0.27
Pentazocine	25	10.2 ± 0.48*
EJP	500	4.3 ± 0.36**
EJE	500	5.8 ± 0.31**
EJM	200	7.7 ± 0.88**

n = six per group. Values are mean ± SEM. *P < 0.01, **P < 0.001 compared to control, (a) EJP – Petroleum ether extract; (b) EJE – Ethyl acetate extract; (c) EJm – Methanol extract

The results of different extracts on acetic acid-induced writhings indicate a significant reduction in writhing with all the test extracts [Table 1]. EJm showed an appreciable percentage of inhibition (56.2 %), which was comparable to the standard drug Aspirin (65.6 %). Moderate activity was observed with EJE (43.4 %) and EJP (40.3 %). The time required for the flicking tail response was found to be delayed in drug-treated animals as compared with the control [Table 2]. EJm (7.7 ± 0.88) was more active with an increase in time for flick response than the EJE (5.75 ± 0.31) and EJP (4.33 ± 0.36), in reducing the intensity of analgesia. The constriction response of the abdomen, induced by acetic acid, is a sensitive method for peripheral analgesic agents. This response was believed to be mediated by the prostaglandin pathways.^[9] The interference of the acetic acid induced writhing response mediated by extracts explored the potential antinociceptive action. However, the reduced number of writhings alone did not provide proof of whether the antinociceptive action was central or peripheral. To understand the mode of action, the extracts were examined using the tail-flick response, which was generally considered a central action. In the tail-flick model, the extracts increased the pain threshold significantly and this indicated the involvement of a higher center.^[10] It was through these mechanisms of action that the analgesic activity of *E. jambolana* bark could be explained.

The current study presents the analgesic and local anti-inflammatory effects of *E. jambolana* and justifies the use of this plant by traditional medical practitioners.

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