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Anti-inflammatory Activity of *Eugenia jambolana* and *Trigonella foenum graecum* in Experimental Animal Model

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Abstract

The present study was carried out to evaluate the anti-inflammatory effect of *Eugenia jambolana* and *Trigonella foenum graecum* in abino rats. Animals were classified into seven groups; T1 served as vehicle control, which received 0.1 ml gum acacia, while T2 received acetylsalicylic acid at 150 mg/kg orally. The ethanolic extract of *E. jambolana* in gum acacia was administered orally in groups T3 and T4 at 100 and 200 mg/kg orally respectively. Aqueous extract of *T. f. graecum* in gum acacia was administered orally at a dose rate of 100 mg/kg, 200 mg/kg and 400 mg/kg to T5, T6 and T7 respectively. Paw oedema was induced by injecting 0.1 ml of 1% (w/v) carrageenan sub-cutaneously (s/c). The percentage inhibition of oedema in T2, T3, T4, T5, T6 and T7 were observed to be 62, 43, 64, 57, 58 and 62 percent respectively. The results of the present study revealed that the treatment groups showed a significant reduction in paw volume in a dose dependent manner indicating their anti-inflammatory action, which had provided a proof for the scientific validation of their ethno pharmacological property.

Keywords: Anti-inflammatory; *Eugenia jambolana; Trigonella foenum graecum;* Paw

Introduction

Inflammation is the tissue response to an injury involving a localized increase in the number of leukocytes and a variety of complex mediator molecules. Biosynthesis of eicosanoids has been implicated in the pathophysiology of cardiovascular diseases, arthritic conditions, cancer, respiratory diseases etc. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with an alleged folkloric use as pain relievers and anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Ravi *et al*., 2009).

Eugenia jambolana (*E. jambolana*), belonging to the family Myrtaceae, has been reported to have hypoglycemic, neuropsycho-pharmacological, antibacterial, anti-HIV and anti-diarrheal effects (David *et al*., 2010). The bark is astringent and is used in sore throats, bronchitis, asthma, ulcers and dysentery. The seeds are used in the treatment of diabetes.

Trigonella foenum graecum (*T. f. graecum*), commonly known as Fenugreek, is an annual herb belonging to the family Leguminosea, widely grown in India, Egypt, and Middle Eastern countries. *Trigonella foenum graecum* is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicine. Fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Indian and Chinese medicine. Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, hypotensive and antioxidant activity (Hassan *et al*., 2006). The present study was undertaken to evaluate the anti-inflammatory effect of ethanolic extract of leaves of E. jambolana and aqueous extract of seeds of *T. f. graecum* in albino rats.

Materials and methods

Plant materials

The leaves of *E. jambolana* were collected from the college campus. The seeds of *T. f. graecum* were purchased from local market at Thrissur, Ker-

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Preparation of extracts

The leaves of *E. jambolana* were air-dried at room temperature and coarsely powdered using an electrical pulverizer. The powder obtained was extracted using a soxhlet apparatus using ethanol. The ethanolic extract was then concentrated in a rotary vacuum evaporator under reduced pressure and temperature. The aqueous extract of seeds of *T. f. graecum* was prepared by taking 100g of the coarse seed powder in one liter of water and subjecting to boiling for 30 minutes with constant stirring. The extract was filtered through a muslin cloth and then kept in boiling water bath for the complete evaporation of the water.

Animals

Albino rats of either sex used for the study were purchased from the Small Animal Breeding Station of the faculty. They were maintained as per the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals, Animal Welfare Division, Government of India.

Chemicals

Carrageenan and acetylsalicylic acid used in the study were purchased from Sigma-Aldrich, U.S.A.
Anti-inflammatory activity in Carrageenan-induced paw oedema model

The experimental design was approved by the Institutional Ethics Committee of the Faculty. The anti-inflammatory activity was assessed using carrageenan-induced paw oedema model in rats (Winter *et al*, 1962). Forty two healthy rats of either sex weighing about 150-200g were divided into 7 groups comprising six in each group. T1 served as vehicle control, which received 0.1 ml gum acacia, while T2 was treated with acetylsalicylic acid at 150 mg/kg orally. The ethanolic extract of *E. jambolana* in gum acacia was administered orally in groups T3 and T4 at 100 and 200 mg/kg orally respectively. Aqueous extract of *T. f. graecum* in gum acacia was administered orally at a dose rate of 100 mg/kg, 200 mg/kg and 400 mg/kg to T5, T6 and T7 respectively. An hour after vehicle/drug administration, paw oedema was induced by injecting 0.1 ml of 1% (w/v) carrageenan subcutaneously (s/c) into the sub-plantar region of the right hind paw of the rats in all the groups. The paw volume was recorded up to 3 hours at hourly interval. The percentage inhibition of oedema was observed.
% inhibition = Vc-Vtx100/Vc where Vc – % increase in paw volume of control at 3rd hour, Vt – % increase in paw volume of test at 3rd hour

Statistical analysis

The statistical analysis was performed using one way analysis of variance test (ANOVA) followed by paired‘t’ test.

Results

The results are presented in Table 1. The percentage inhibition of oedema in T2, T3, T4, T5, T6 and

Table 1. Anti-inflammatory activity of ethanolic extract of *E. jambolana* and aqueous extract of seeds of *T. f. graecum*

	Treatment	Increase in paw volume (ml) (N.=6)		% inhibition in paw volume
		0 (min.)	180 (min.)	
T1	Gum acacia	0.69 ± 0.04	1.85 ± 0.02	---
T2	Acetylsalicylic acid– 150 mg/kg	0.67 ± 0.03	0.92 ± 0.02	62
T3	*E. jambolana* – 100 mg/kg	0.66 ± 0.02	1.03 ± 0.01	43
T4	*E. jambolana* – 200 mg/kg	0.65 ± 0.04	0.88 ± 0.01	64
T5	*T. f. graecum* – 100 mg/kg	0.69 ± 0.01	0.98 ±0.03	57
T6	*T. f. graecum* – 200 mg/kg	0.65 ± 0.01	0.92 ± 0.03	58
T7	*T. f. graecum* – 400 mg/kg	0.66 ± 0.03	0.91 ± 0.04	62

Data are expressed as mean ± SE. $P<0.05$ significantly different from control

T7 were observed to be 62, 43, 64, 57, 58 and 62 percentages respectively. The present work revealed that ethanolic leaf extract of *E. jambolana* at doses 100 and 200 mg/kg showed significant reduction in the paw oedema in a dose dependent manner ($P<0.05$). Similarly, T. f. graecum at the doses of 100, 200 and 400mg/kg, significantly reduced the paw oedema throughout the entire period of observation in comparison to control ($P<0.05$). Even though both the extracts showed anti-inflammatory activity, the percentage inhibition shown by the 200 mg/kg treatment group of ethanolic leaf extract of *E. jambolana* (T4) was higher than the 400 mg/kg treatment group of aqueous extract of seeds of *T. f. graecum* (T7) and comparable with that of the acetylsalicylic acid (standard drug) treated group ($P>0.05$) suggesting higher anti-inflammatory activity for the ethanolic extract of leaves of *E. jambolana*.

Discussion

Carrageenan induced paw edema in rats has been accepted as a useful phlogistic tool for investigating anti-inflammatory agents. It is suggested that there are biphasic effects in carrageenan induced oedema. The early hyperemia results from the release of histamine and serotonin and the delayed phase of carrageenan induced edema results mainly from the potentiating effects of bradykinin on mediator release, and also from prostaglandins, which produce edema after the mobilization of leukocytes (Garcia-Pastor *et al*., 1999).

Presence of flavonoids in the leaves of *E. jambolana* has been reported (Timbola *et al*., 2002). Mahmoud *et al*. (2001) identified acylated flavonol glycosides along with polyphenols. Presence of saponins and flavonoids as the major compounds in T. f. graecum (Shang *et al*., 1998, Raju *et al*., 2004) can explain anti-inflammatory activity of the plant. The seeds of T. f. graecum contain flavonoids (Shang *et al*., 1998), alkaloids (Jain and Madhu, 1988) and salicylate (Swain *et al*, 1985). Flavonoids act as potential inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase as well as being antioxidants (Rao *et al*, 2005, Sharififar *et al*., 2009) . The possible mechanism of action by which the ethanolic extract of leaves of *E. jambolana* provide higher anti-inflammatory activity might be explained due to the presence of flavonoids inhibiting cyclooxygenase and lipoxygenase pathway thereby down regulating the biosynthesis of eicosanoids.

The results of the present study revealed the anti-inflammatory effect of ethanolic extract of leaves of *E. jambolana* and aqueous extracts of seeds of *T. f. graecum*. The results indicated that treatment with 200 mg/kg ethanolic extract of leaves of *E. jambolana* had higher anti-inflammatory activity than treatment with 400 mg/kg of aqueous extract of seeds of *T. f. graecum*. This study validates scientifically their ethno pharmacological property and may aid in the treatment strategies of many disease conditions in which there is involvement of eicosanoids.

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