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RESEARCH ARTICLE

Evaluation of anti-inflammatory activity of *Eugenia singampattiana* Bedd leaf

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Abstract

The anti-inflammatory effect of ethanol extract of *E. singampattiana* leaf administered orally at doses of 150 and 300 mg/kg, were evaluated *in vivo* using carrageenan induced paw edema to examine the acute effect of the plant extract. The ethanol extract of *E. singampattiana* showed significant reduction in the paw edema volume (59.5%) at a dose of 300 mg/kg after 3h carrageenan injection. The phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins, phenols, glycosides and xanthoprotein.

Introduction

Inflammation is a defensive reaction of the body against infections and injuries. Edema formation, leukocyte infiltration and granuloma formation represent typical feature of inflammation (Gorzalczany et al., 2011). Non – steroidal anti-inflammatory drugs (NSAIDS), steroidal drugs and immune-suppressant drugs which have been usually used in the relief of inflammatory diseases worldwide for a long time are often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcer (Valiollah et al., 2009). Therefore, now-a-days the development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary. For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases; but there is a lack of scientific evidence (Shukla et al., 2010).

E. singampattiana Bedd belong to the family Myrtaceae. It is commonly known as "Kattukorandi" by *Kanikkar* tribals of Agasthiarmalai, Biosphere Reserve, Western Ghats, Tamil Nadu, India. The paste prepared from the leaf of *E. singampattiana* is given to treat asthma and giddiness. Paste prepared from equal quantity of leaves and flowers is consumed by *Kanikkar* tribals to cure body pain and throat pain. Paste prepared from equal quantity of leaves, flowers and tender fruits are consumed by the *Kanikkars* to relief from leg sores and rheumatism. Paste prepared from equal quantity of stems, leaves and flowers is consumed with palm sugar to get relief from gastric complaints (Viswanathan et al., 2006). *E. singampattiana* leaf extracts were reported for the biological activities such as antitumor, antidiabetic, antihyperlipidaemic and *in vitro* antioxidant activity (Kala et al., 2011; Kala et al., 2012; Tresina et al., 2012).

However, perusal of literature survey reveals that, anti-inflammatory activity of *E. singampattiana* is totally lacking and hence, the present investigation was undertaken. The main objective of the present study is to evaluate the anti-inflammatory activity if *E. singampattiana* leaf.

Materials and Methods

Plant Material

The leaves of *Eugenia singampattiana* Bedd were freshly collected from well grown healthy plants inhabiting in the natural forests of Karaiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for phytochemical screening and anti-inflammatory studies

The *E. singampattiana* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *E. singampattiana* leaves was packed in a Soxhlet apparatus and extracted with ethanol The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures (Brindha et al., 1981; Anonymous, 1990; Lala, 1993).The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for anti-inflammatory studies.

Animals

Adult Wistar albino rats of either sex (150-200g) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2ºC) and light and dark (12:12h). Rats were feed standard pellet diet (Goldmohur brand, Ms Hindustan Lever Ltd., Mumbai, India) and water *ad libitum.*

Acute toxicity study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Carrageenan induced hind paw oedema

Albino rats of either sex weighing 150-200g were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows, Group I - Control (normal saline 0.5ml/kg), Group II – Leaf extract of *E. singampattiana* (150 mg/kg, p.o.), Group III – leaf extract of *E. singampattiana* (300mg/kg, p.o.) and Group IV-Indomethacin (10mg/kg). All the drugs were administered orally.

After one hour of the administration of the drugs, 0.1ml of 1% w/v carageenan solution in normal saline was injected into the subplantar tissue of the left hind paw and the right hind paw of the rat was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 60min., 120min., 180min. The percentage increase in paw oedema of the treated groups was compared with that of the control and the inhibitory effect of the drugs were studied. The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of the inflammation.

The percentage inhibition of the inflammation was calculated from the formula:

Percentage Inhibition = D_o-D_t / D_o ×100, where D_O was the average inflammation (hind paw oedema) of the control group of rats at a given time; and D_t was the average inflammation of the drug treated (i.e extracts or reference indomethacin) rats at the same time.

Results

The phytochemical screening of ethanol extract of *E. singampattiana* leaf revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed the nontoxic nature of the ethanol extract of *E. singampattiana*.

The inhibitory effect of the ethanol extract of *E. singampattiana* on carrageenan induced paw edema is shown in Table 1. For each of the two doses of extract tested (150 and 300 mg/kg) the ethanol extract exerted considerable inhibitory effect on paw increase 1 hour after carrageenan administration with about a 55% inhibition for the dose 300 mg/kg. The maximum inhibition 59.5% (p<0.01) elicited by the ethanol extract of *E. singampattiana* was recorded 3 hours after carrageenan injection. Indomethacin which is a reference drug showed a similar inhibitory effect 3 hours after carrageenan administration (60.1%).

Table 1 Anti inflammatory activity of ethanol extract of *Eugenia singampattiana* leaves

Group	Treatment (mg/kg)	Paw volume in ml± SEM and percentage of inhibition				
		0 min	30 min	60 min	120 min	180 min
Group I	0.5 ml saline	0.565±0.04	0.651±0.06	0.682±0.007	0.711±0.001	0.780±0.01
Group II	150	0.478±0.07	0.481±0.001 (26.1)	0.421±0.001 (38.2)*	0.386±0.001 (45.7)**	0.331±0.04 (57.6)**
Group III	300	0.526±0.06	0.524±0.05 (19.5)	0.519±0.04 (23.9)*	0.378±0.05 (44.0)**	0.316±0.05 (59.5)**
Group IV	10	0.499±0.01	0.520±0.02 (20.0)	0.412±0.07 (39.6)	0.353±0.03 (50.3)	0.311±0.02 (60.1)

*No. of animal / in each group 6 Data expressed in mean ± SEM * p < 0. 05 when compared to control. ** p < 0.01*

Discussion

Carrageenan induced edema is a suitable experimental animal model commonly used for the study of acute inflammation and is believed to be biphasic. In general, the first phase (1-2h) involves inflammation mediated by the release of serotonin and histamine and increased synthesis of prostaglandins in the surroundings of the damaged tissues. The second phase (3-5h) is the result of the release of kinins mainly prostaglandins (Crunlehorn and Meacock, 1971). In this study, the ethanol extract of *E. singampattiana* exerted considerable inhibitory effect on carrageenan induced paw edema in rats starting from the first hour after administration. This effect was dose-dependent and maximum inhibition induced by the extract was recorded after 3h with the highest doses (300 mg/kg) of the *E. singampattiana* leaf extract. Similar inhibitory effects were observed after carrageenan injection with indomethacin, a potent non-steroidal anti-inflammatory drug which acts by inhibiting cyclooxygenase. Therefore, the present results suggests that, the inhibitory effect of the ethanol extract of *E. singampattiana* on carrrageenan induced paw edema may be due to the suppression of the release of mediators including histamine, serotonin, bradykinin and prostaglandins responsible for the first and second phase of acute inflammation induced by carrageenan. There are also evidences that compounds inhibiting the carrageenan induced edema are effective in inhibiting the enzyme cyclooxygenases (Selvam and Jachak, 2004). Based on these reports, the inhibitory effect of *E. singampattiana* extract on carrageenan induced inflammation could be mediated via this mechanism.

Phytochemical analysis of *E. singampattiana* revealed the presence of flavonoids, saponins and phenols. Saponins and flavonoids have previously been reported to have anti-inflammatory activities (Mohammed *et al*., 2004; Aquila *et al*., 2009). Such compounds may be responsible in part for the described anti-inflammatory activity of *E. singampattiana* extract.

According to the present study, it can be concluded that, the ethanol extract of *E. singampattiana* leaf possesses anti-inflammatory effect. Further investigations are required to isolate and active principles present in the extract and to determine their extract mechanism of action.

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