

## Research Article



## Evaluation of Anti-Inflammatory Activity of *Eugenia floccosa* Bedd leaf

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### ABSTRACT

The anti-inflammatory effect of ethanol extract of *E. floccosa* leaf administered orally at doses of 150 and 300 mg/kg, were evaluated *in vivo* using carrageenan induced paw edema were used to evaluate the acute effect of the plant extract. The ethanol extract of *E. floccosa* showed significant reduction in the paw edema volume (60.9%) 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.

**Keywords:** *E. floccosa*, anti-inflammatory, carrageenan, saponin.

### INTRODUCTION

Inflammation is considered as a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses<sup>1</sup>. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases<sup>2</sup>. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

*Eugenia floccosa* Bedd is one of the medicinally important plants belongs to Myrtaceae family. The ethanol extract of *E. floccosa* has been reported for its anti-tumour activity, antidiabetic, antihyperlipidaemic and *in vitro* antioxidant activity<sup>3-6</sup>

The objective of this investigation was to ascertain the scientific basis of its use in treatment of inflammation on which there is no previous data available. Hence, in the present study effort has been made to establish the scientific validity to the anti-inflammatory property of *E. floccosa* leaf extract using carrageenan induced paw edema in experimental rats.

### MATERIALS AND METHODS

#### Plant Material

The leaves of *Eugenia floccosa* Bedd were freshly collected from the well grown healthy plants inhabiting the natural forests of Kothiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamilnadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamilnadu, India. A voucher specimen was deposited in Ethnopharmacology Unit,

Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamilnadu.

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

The *E. floccosa* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *E. floccosa* 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<sup>7-10</sup>. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antiinflammatory 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±2°C) 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<sup>11</sup>. 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. floccosa* (150 mg/kg, p.o.), Group III – leaf extract of *E. floccosa* (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 carrageenan 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 =  $D_0 - D_t / D_0 \times 100$ , where  $D_0$  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 AND DISCUSSION

The phytochemical screening of ethanol extract of *E. floccosa* 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. floccosa*.

The inhibitory effect of the ethanol extract of *E. floccosa* 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 50% inhibition for the dose 300 mg/kg. The maximum inhibition 60.9% ( $p < 0.01$ ) elicited by the ethanol extract of *E. floccosa* 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%).

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltrate by leucocytes and the third one by granuloma formation. In the present study, anti-inflammatory activity was determined by using inhibition of carrageenan induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins<sup>12-15</sup>. In the present study, the ethanol extract of *E. floccosa* leaf showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner. This response tendency of the extract in carrageenan induced paw edema revealed good peripheral antiinflammatory properties of the ethanol extract. This anti-inflammatory effect of ethanol extract of *E. floccosa* may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory activity<sup>16</sup>. The presence of flavonoid identified might be responsible for the antiinflammatory activity in ethanol extract. Tau-Muurolol,  $\alpha$ -Cadinol, phytol and oleic acid were reported in the ethanol extract of *E. floccosa* leaf by GC-MS analysis<sup>17</sup>. These compounds may have the role in anti-inflammatory effect. Further studies will be carried out to isolate and characterize other anti-inflammatory chemical constituents present in the ethanol extract of this plant.

**Table 1:** Antiinflammatory activity of ethanol extract of *Eugenia floccosa* leaves

| Group     | Treatment (mg/kg) | Paw volume in ml $\pm$ SEM and percentage of inhibition |                         |                          |                           |                           |
|-----------|-------------------|---|-------------------------|--------------------------|---------------------------|---------------------------|
|           |                   | 0 min   | 30 min                  | 60 min                   | 120 min                   | 180 min                   |
| Group I   | 0.5 ml saline     | 0.565 $\pm$ 0.04  | 0.651 $\pm$ 0.06        | 0.682 $\pm$ 0.007        | 0.711 $\pm$ 0.001         | 0.780 $\pm$ 0.01          |
| Group II  | 150               | 0.499 $\pm$ 0.01  | 0.520 $\pm$ 0.02(20.0)  | 0.412 $\pm$ 0.07(39.6)   | 0.353 $\pm$ 0.03(50.3)    | 0.311 $\pm$ 0.02 (60.1)   |
| Group III | 300               | 0.482 $\pm$ 0.03  | 0.491 $\pm$ 0.002(24.5) | 0.434 $\pm$ 0.07(36.3)*  | 0.381 $\pm$ 0.001(46.4)** | 0.334 $\pm$ 0.06 (57.2)** |
| Group IV  | 10                | 0.407 $\pm$ 0.08  | 0.531 $\pm$ 0.05(18.5)  | 0.479 $\pm$ 0.002(29.7)* | 0.374 $\pm$ 0.02(47.4)**  | 0.305 $\pm$ 0.02 (60.9)** |

No. of animal / in each group 6 Data expressed in mean  $\pm$  SEM \*  $p < 0.05$  when compared to control. \*\*  $p < 0.01$

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## REFERENCES

1. Kumar V, Abbas AK and Fausto N, Pathologic basics of disease. (eds) Robbins and Cotran. 7<sup>th</sup> edition. Elsevier Saunders. Philadelphia, Pennsylvania. 2004, pp. 47-86.
2. Sosa S, Balicet MJ, Arvigo R, Esposito RG, Pizza C and Altiner GA, Screening of the tropical anti-inflammatory activity of some central American Plants. J. Ethnopharmacol, 8, 2002, 211-215.
3. Kala SMJ, Tresina Soris P and Mohan VR, Antitumour activity of *Eugenia floccosa* Bedd and *Eugenia singampattiana* Bedd leaves against Dalton ascites lymphoma in swiss albino mice. Int. J. PharmTech Res, 3, 2011, 1796-1800.
4. Kala SMJ, Tresina Soris P and Mohan VR, Pharmacochemical characterization of *Eugenia floccosa* Bedd. J.Econ. Tax. Bot, 36, 2012a, 320-323.
5. Kala SMJ, Tresina Soris P and Mohan VR, Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia floccosa* Bedd leaves in alloxan induced diabetic rats. J. Basic Clinic. Pharm, 3, 2012b, 235-240.
6. Tresina PS, Kala SMJ, and Mohan VR, HPTLC finger print analysis of phytochemicals and *in vitro* antioxidant activity of *Eugenia floccosa* Bedd. Biosci. Discover, 3, 2012, 296-311.
7. Brinda P, Sasikala P and Purushothaman KK, Pharmacognostic studies on Merugan kizhangu. Bull. Med. Ethnobot. Res, 3, 1981, 84-96.
8. Kala MJS, Balasubramanian T, Mohan VR and Tresina PS, Pharma chemical characterization of *Eugenia singampattiana* Bedd. Advan. BioRes, 1, 2010, 105-108.
9. Vasantha K, Priyavardhini S, Tresina PS and Mohan V, Phytochemical analysis and antibacterial activity of *Kedrostis foetidissima* (Jacq.) Cogn. Biosci. Discover, 3, 2012, 06-16
10. Priyavardhini S, Vasantha K, Tresina PS and Mohan VR, Efficacy of phytochemical and antibacterial activity of *Corallocarpus epigaeus* Hook.f. Int. J. PharmTech Res, 4, 2012, 35-43.
11. OECD. Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris. 2002.
12. Vane J and Booting R, Inflammation and the mechanism of action of anti-inflammatory drugs. J. Fed. Amer. Soc. Exper. Bio, 1, 1987, 89-96.
13. Kavimani S, Mounissamy VM and Gunasegaran R, Analgesic and anti-inflammatory activities of Hispidulir isolated from *Helichrysum bracteatum*. Ind. Drugs, 37, 2000, 582.
14. Nivsarkar M, Mukherjee M, Patel M, Padh H and Bapu C, *Launaea nudicaulis* leaf juice exhibits anti-inflammatory action in acute and chronic inflammation models in rats. Ind. Drugs, 39, 2002, 290.
15. Patil VV and Patil VR, Evaluation of anti-inflammatory activity of *Ficus cania* Linn. leaves. Ind. J. Nat. Prod. Resour, 2, 2011, 151-155.
16. Mascolo N, Autore G, Capasso F, Menghini A and Fasulo MP, Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytother. Res, 1, 1987, 28-31.
17. Mary Jelastin Kala S, Tresina Soris P and Mohan VR, GC-MS determination of bioactive components of *Eugenia floccosa* Bedd. (Myrtaceae). Int. J. Pharma and Biosci, 3, 2011, 277-282.

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