



**ANTI-INFLAMMATORY ACTIVITY OF LEAF AND BARK OF *HUGONIA MYSTAX* L.
(LINACEAE)**

G. Rajeswari¹, M. Murugan² and V.R. Mohan^{2*}

¹ V.O.C. College of Education, Tuticorin, Tamil Nadu.

²Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

Abstract:

Objective: In the present study anti-inflammatory activity of ethanol extract of leaf and bark of *Hugonia mystax* were investigated. **Methods:** The anti-inflammatory activity of ethanol extracts leaf and bark of *H. mystax* were evaluated by carrageenan induced rat paw edema to determine its effect on chronic phase of inflammation models in rats. **Results:** Preliminary phytochemical analysis of ethanol extracts of leaf and bark showed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Maximum inhibition (83.91%) was obtained at the dose of 500 mg kg⁻¹ of *H. mystax* leaf after 3 hours of drug treatment in carrageenan induced paw edema, whereas, indomethocin produced 84.82% of inhibition. **Conclusion:** The present study suggests that *H. mystax* leaf and bark extrats possess strong anti-inflammatory property so it has immense scope as an effective source to develop drug for the treatment of inflammatory related diseases.

Key words: Anti-inflammatory, paw edema, *Hugonia mystax*.

Introduction:

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmic fluid and blood cells. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, many studies have been continuing an inflammatory

disease and the side effects of the currently available anti-inflammatory drugs are the major problem during their clinical uses. The most commonly used drug for management of inflammatory conditions are non steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers^{1,2}. Therefore, nowadays the development of newer and more substantial anti-inflammatory drugs with lesser side effect is necessary³. For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

For Correspondence:

vrnathan@gmail.com

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The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India^{4,5}. This plant *Hugonia mystax* is locally known as Modirakanni. Ethnobotanically, the fruits are used by the tribals of Kalakad Mundanthurai for the treatment of Rheumatism⁶. Roots were used as anthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were reported^{7,8,9,10}. Roots of *Hugonia mystax* were evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root extracts showed significant activity against various human pathogens¹¹.

Taking into consideration the medicinal value and utility, the present study has been initiated to evaluate the antiinflammatory studies on *Hugonia mystax* leaf and bark.

Materials and Methods

Plant Material: The leaf and bark of *H. mystax* L. were collected from Kodagiri, Nilagiri Biosphere Reserve, Western Ghats, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for anti-inflammatory activity: The dried leaf and bark of *H. mystax* were powdered in a Wiley mill. Hundred grams of plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for preliminary photochemical activity and anti-inflammatory activity.

Animals: Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature ($25\pm 2^{\circ}\text{C}$) and light and dark (12:12 h). Rats were fed with 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.

Anti-inflammatory activity

Carrageenan induced hind paw edema:

Albino rats of either sex weighing 150-200 grams 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 0.5 ml/kg), Group – II, III, IV and IV – *H. mystax* leaf and bark (250 and 500 mg/kg, p o. respectively), Group VI – Indomethacin (10 mg/kg, p.o.). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug.

After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw 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., 240min., 360min., and 480min. The percentage increase in paw edema of the treated groups

was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation.

$$\text{Percentage Inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where,

V_t = Percentage difference in increased paw volume after the administration of test drugs to the rats

V_c = Difference of increased volume in the control groups.

Statistical analysis: The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

Results

The photochemical screening of ethanol extracts of leaf and bark of *H. mystax* revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoids and xanthoprotein. Acute toxicity study revealed the non – toxic nature of the ethanol extracts of leaf and bark of *H. mystax*

In the present study, the anti-inflammatory activity of ethanol extract of leaf and bark of *H. mystax* were assayed in albino rats using carrageenan induced rat paw edema method. Table 1 shows the anti-inflammatory activity of ethanol extracts of leaf and bark of *H. mystax* significantly inhibited the rat paw edema at 3rd hour post carrageenan were 74.19 %, 83.91 % for 250 mg kg⁻¹ and 500 mg kg⁻¹ of leaf extract and 71.96%, 77.26% for 250 mg kg⁻¹ and 500 mg kg⁻¹ of bark extract respectively. The results were compared with indomethacin at 10 mg/Kg, which shows paw reduction of 84.82%.

Discussion

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of

prostaglandins in the damaged tissue surroundings. The late phase (3 h) is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages^{12,13}. Prostaglandin-E₂, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to redness and increased blood flow in areas of acute inflammation. The significant ($p < 0.001$) suppressive activity of the ethanol extracts leaf and of bark of *H. mystax* in late phase shows its potent anti-inflammatory effect. This result is quite similar to the one observed for indomethacin at 10 mg/Kg, which inhibited 84.82%. Therefore, it is suggested that the mechanism of action of the extract may be related to histamine and prostaglandin synthesis inhibition. Further studies will be carried out to isolate and characterize anti-inflammatory chemical constituents present in the methanol extracts of this plant.

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Table 1: Effect of leaf and bark of *H. mystax* extracts on the percentage inhibition of Carrageenan induced paw edema

Treatment Groups	Dose mg/kg	edema volume (ml)				% Inhibition after 180 min
		0 min	60 min	120 min	180 min	
CONTROL (Group-I)	Normal saline	33.89±1.94	81.632.53	113.842.68	132.632.93	-
Group-II	250 mg/kg	36.59±1.83	43.911.88**	48.162.04**	34.221.98**	74.19
Group-III	500 mg/kg	31.63±1.66	39.631.34**	26.841.05***	21.330.96***	83.91
Group-IV	250 mg/kg	34.91±1.84	48.641.13*	40.240.84*	37.182.16**	71.96
Group V	500 mg/kg	32.17±1.63	40.241.33**	37.280.76**	30.161.31***	77.26
Indomethacin (Group-VI)	10 mg/kg	29.84±0.84	31.521.08**	21.160.92**	20.130.86***	84.82

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P<0.01 *** P<0.001, Compared paw edema induced control vs drug treated rats