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Anti-Inflammatory activity of ethanol extract of *Hugonia mystax* Leaves

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Abstract

In the present study anti-inflammatory activity of ethanol extract of leaf of *Hugonia mystax* were investigated. Preliminary phyto chemical analysis of ethanol extract of *Hugonia mystax* showed the presence of carbohydrates, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins, amino acids. The anti-inflammatory activities of ethanol extract leaf of *Hugonia mystax* were evaluated by carrageenan induced paw edema to determine it's on chronic phase of inflammation model in rats. Maximum inhibition was obtained at the dose of 200mg/kg of *Hugonia mystax* leaf after 4 hours of drug treatment in carrageenan induced paw edema. Indomethacine used as a standard drug. The present study suggests that *Hugonia mystax* leaf possess significant anti-inflammatory activity.

Keywords: Anti-inflammatory, Paw edema, carrageenan, *Hugonia mystax*

1. Introduction

Hugonia mystax (family- Linaceae) is a woody evergreen species comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India [1, 2]. This plant *Hugonia mystax* is locally known as Modirakanni. The leaves of this species are used for skin disease and rheumatism. Roots were used as anthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation [3]. The preliminary phyto chemical screening showed the presence of carbohydrate, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins and amino acids.

2. Materials and Methods**2.1 Plant material**

Fresh leaves of *Hugonia mystax* were collected from Sirumalai hills, Dindigul district, Tamil Nadu. It was identified by a scientific officer and identification was confirmed by the botanical survey of India, Coimbatore.

**2.2 Preparation of plant extract**

Leaves of *Hugonia mystax* were dried in the shade for 2 weeks. Dried leaves were coarsely powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, ethanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator. The high yield was produced with the help of ethanol. The extract is preserved in a refrigerator at 4 °C. The ethanol extract of the leaves was selected for preliminary phyto chemical activity and anti-inflammatory activity.

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2.3 Phytochemical analysis

The phyto chemical analysis of ethanol extract of *Hugonia mystax*^[4] showed the presence of carbohydrate, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins and amino acids.

2.4 Animals

Adult wistar albino rats of either sex (150-200gm) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2 °C) and light and dark (12:12 h). Rats were fed with standard pellet diet and water *ad libitum*.

2.5 Acute toxicity study

Acute oral toxicity study was carried out as per stair case method^[5] (OECD 425 guidelines). In acute toxicity, no toxic symptoms were observed for the drug up to of 2000 mg/kg body weight. Albino rats of either sex 150-200 gm were used. The animals were fasted overnight prior to the acute experimental procedure. The animals were administered with aliquot doses of 100-250mg/kg extracts orally, suspended in Tween8 (1% w/v). The dose which caused no mortality and was tolerated was determined in a stepwise manner and the effective dose was found to be 100 mg/kg body weight. The dose of 100 mg/kg, 200 mg/kg was arbitrarily selected to study the anti inflammatory activity.

2.6 Anti inflammatory activity

2.6.1 Carrageenan induced hind paw edema^[6]

Albino rats of either sex weighing 150-200gms were divided into 4 groups of six animals each, the dosage of the drugs administered to the different groups was as follows.

Group I- control 1 ml/kg of 0.5% of CMC orally

Group II- Indomethacin 10mg/kg (0.5%w/v)

Group III & IV- *Hugonia mystax* leaf (100, 200mg/kg) p.o respectively.

All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the administration of the drugs, 0.1ml of 1%w/v carrageenan solution in CMC was injected into the subplantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw thickness was measured at 1 hour, 2 hour, 3, 4 hours after carrageenan injection by using vernier callipers.

The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drug was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition^[7] of the inflammation.

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.

2.7 Statistical analysis

The data were analysed using one way ANOVA followed by Dunnett's t-test. $P < 0.05$ was considered as statistically significant.

3. Results

The phyto chemical screening of ethanol extracts of leaf of *Hugonia mystax* revealed the presence of carbohydrate, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins, amino acids. Acute toxicity study revealed that non toxic nature of the ethanol extract of leaf of *Hugonia mystax*.

In the present study, the anti inflammatory activity of ethanol extract of leaf of *Hugonia mystax* were studied in albino rats using carrageenan induced rat paw edema method.

Table 1 shows the anti inflammatory activity of ethanol extracts of leaf of *Hugonia mystax* significantly inhibited the rat paw edema at 4th hour. The result was compared with indomethacin at 10 mg/kg, which shows paw reduction. The data represent the mean ± SEM (n=6) $P < 0.05$, $P < 0.01$, $P < 0.001$ compared with control.

4. 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 (1-2 hours) of the carrageenan model^[8, 9] is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3h) is sustained by prostaglandin release and mediated by bradykinin, leukotriens, polymorphonuclear cells and prostaglandins produced by tissue macrophages.

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.

5. Conclusion

The significant ($P < 0.01$) suppressive activity of ethanol extract leaf of *Hugonia mystax* shows it's potent anti inflammatory effects. Therefore, it is suggested that the mechanism of action of the extract may be related to histamine and prostaglandins^[10] synthesis inhibition. So it has immense scope as an effective source to develop drug for the treatment of inflammatory related diseases. Further studies will be carried out to isolate and characterize anti inflammatory chemical constituents present in the ethanol extract of this plant.

Table 1: Anti inflammatory activity of ethanol extract of leaf of *Hugonia mystax*

Treatment	Paw thickness (mm)			
	1 hour	2 hour	3 hour	4 hour
Control (cmc)	5.5 ± 0.004	5.9 ± 0.003	6.5 ± 0.003	7.4 ± 0.003
Indomethacin	5.0 ± 0.003 ^b (9.09%)	4.8 ± 0.005 ^b (18.64%)	4.3 ± 0.005 ^b (33.84%)	3.9 ± 0.003 ^b (47.29%)
EHM (100 mg/kg)	5.3 ± 0.003 ^b (3.63%)	5.6 ± 0.005 ^b (5.08%)	5.1 ± 0.007 ^b (21.53%)	4.6 ± 0.004 ^b (37.02%)
EHM (200 mg/kg)	5.1 ± 0.02 ^c (7.27%)	5.0 ± 0.03 ^b (15.25%)	4.9 ± 0.02 ^b (24.61%)	4.3 ± 0.04 ^c (41.89%)

EHM- Ethanol extract of *Hugonia mystax*

Each value is SEM 5 individual operations * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared paw edema induced control vs drug induced rats

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