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ANTINOCICEPTIVE AND ANTIINFLAMMATORY ACTIVITIES OF STEM BARK OF AN ENDANGERED MEDICINAL PLANT, *HILDEGARDIA POPULIFOLIA* (ROXB.) SCHOTT AND ENDL.

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ABSTRACT

This study examined the antinociceptive and antiinflammatory activities of the stem bark of the traditional medicinal plant, *Hildegardia poulifolia* (Sterculiaceae), an endangered species. Methanolic bark extract of this species was investigated for the antinociceptive effect by using acetic acid-induced writhing and hot plate methods in albino mice and antiinflammatory activity by carrageenan, formalin and histamine induced paw edema in rats. The dose level at 200mg/kg body weight of test extract of *H. populifolia* showed significantly better antinociceptive and antiinflammatory activities. The experimental data demonstrated that methanol extract of stem bark of *H. populifolia* possess remarkable antinociceptive and antiinflammatory activities. Hence, the bark of this species may be a potential bioresource for the manufacturing the drugs of antiinflammatory property.

KEYWORDS: **antinociceptive, antiinflammatory activity, *Hildegardia populifolia*.**

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INTRODUCTION

India is known as the "Emporium of medicinal plants" owing to high species richness which is mainly due to different bioclimatic zones. Plant derived natural products such as flavonoids, terpenes and alkaloids have received considerable attention in recent years due to their side effects free diverse pharmacological properties including antiinflammatory, antipyretic and analgesic properties[1,2]. Many plant derived drugs are being used against diseases in various systems of medicine such as ayurveda, unani and sidha. Despite this fact only little number of species have been scientifically explored and validated. *Hildegardia populifolia* (Sterculiaceae) is an endangered tree species distributed in certain tropical deciduous forests of Tamil Nadu and Andhra Pradesh. It is a semi-xerophytic tree, resistant to drought and grows on the steep rocky, dry and poor stony soils[3]. The stem bark is generally prescribed for dog bite and cure malaria in traditional medical practice at Krishnagiri and Dharmapuri districts of Tamil Nadu and Chithur district of Andhra Pradesh[4]. However, no scientific studies are made to confirm these properties. Hence, an attempt was made in the present study to know the antinociceptive and antiinflammatory properties of stem bark of *H. populifolia.*

MATERIALS AND METHODS

Plant materials

The stem bark of about ten years old *H. populifolia* tree species was collected from the campus of Forest Genetic Division, Bhavanisagar, Erode District, Tamil Nadu, India. The plant was identified and confirmed by a voucher specimen with authentic specimen (15211 (MH)) deposited in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore, India. The stem bark was air dried and powdered. Extraction was made by using 50g of powdered plant material in the methanol solvent in soxhlet apparatus for 8 to 10 hours. The residues were collected and dried at room temperature, 30°C after which yield was weighed and then performed to activity.

Experimental animals

Albino mice (Swiss strain) and Wistar rats weighing respectively 25-30gm and 150-250gm either sex were obtained from the Small Animal Breeding Station, Munnuthy, Trissur, Kerala, India. The animals were maintained at room temperature of 25±2˚C with the relative humidity of 75±5% under 12 h dark and 12 h light cycle. The animals were kept in groups of 6, in separate polyvinyl cages (BIK industries, India) having the dimensions of 408 × 280 × 150 mm. The animals were maintained under standard husbandry conditions and had free access to diet and water and they were allowed to acclimatize to the environment for 7 days prior to the experimental session. They were divided into different groups each consists of six animals which were fasted overnight prior to the experiments. The experimental protocol was approved by the institutional Ethics Committee of Nandha College of Pharmacy (Reg.No: 688/2C/CPCSCA), Erode, India.

Acute oral toxicity studies

Acute oral toxicity studies were performed according to Organization for Economic Co-operation and Department (OECD)[5]. Swiss albino male mice (n=6/each dose) were selected by random sampling technique. Animals were fasted for 12 h with free access to water only. Methanol extract of *H. populifolia* (dissolved in distilled water) were administered orally at a dose of 5 mg/kg and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality could be observed in only one mice out of six animals, then the higher doses at 50, 300, 500, 1000 and 2000 mg/kg might be administered. General behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis,

analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for the first one hour and after 24 h of drug administration.

ANTINOCICEPTIVE ACTIVITIES

Acetic acid-induced writhing test in mice

This test was followed by using the method of Koster, et al.[6]. Swiss albino mice, weighing 18-25g were randomly divided into four groups, six animals each (n=6). Control group (Group I) received 10mL/kg normal saline orally. The reference group (Group II) received aspirin (10mg/kg dissolved in distilled water, p.o.) and groups III and IV were orally pretreated respectively with 100 and 200mg/kg methanolic stem bark extract of *H. populifolia.* All drugs were administered orally 30 minutes prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1mL/10g). Thirty minutes later the animals were placed on an observation table and observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. A reduction in the number of writhes is an indication of analgesic property.

Hot plate method

The hot plate test was employed to measure central analgesic activity by the method of Eddy and Leimback[7] with minor modifications. In this experiment, the hot plate was maintained at 55±0.5˚C. All animals were selected 24hr prior to experimentation on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 2 and 15 seconds, respectively. In order to avoid damaging the paws of the animals, the time standing on the plate was limited to 25 seconds. All the rats divided into four groups each comprising six animals. Group I normal rat served as control. Pentazocine at the rate of 10mg/kg was administered intraperitoneally as a reference standard (Group II). Group III and IV received methanolic stem bark extract of *H. populifolia* at the concentrations of 100 and 200mg/kg body weight respectively. Thirty minutes after administration of standard drug and extract, the animals were placed individually onto the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recordered as the reaction time or latency of pain response.

ANTI-INFLAMMATORY ACTIVITIES

Carrageenan-induced paw edema

The anti-inflammatory activity was evaluated by carrageenan induced rat paw edema model[8]. Wistar rats of either sex were weighed (170-230gm) and normal paw volumes of all the rats was measured initially and then divided into four groups each comprising six animals (n=6). Inflammation was induced in all rats by single sub plantar injection of 0.1mL freshly prepared 1% carrageenan in normal saline. Group I treated with carrageenan alone served as negative control. The rats of Group II were treated with 10mg/kg indomethacin[9], Groups III and IV respectively received the methanolic stem bark extract at the concentration of 100 and 200 mg/kg body weight orally one hour before the carrageenan injection. The change in paw thickness (mm) was measured using digital calibrated vernier caliper (Model 2061, Mututoyo Digimatic Caliper, Japan) before carrageenan injection and at 0, 1, 2, 3 and 4 hour after carrageenan injection. Change in paw thickness was considered as a measure of inflammation and was calculated as per cent inflammation inhibition.

Inflammation Inhibition (%) = (Control group mean-test group mean) / (Control group mean) ×100

Formalin induced paw edema

Wistar albino rats of 180-200g weight were used for the study and they were divided into four groups each contains six rats. Rats which were given no treatment served as control (Group I), Group II treated with indomethacin (10mg/kg) served as positive control. The methanolic stem bark extract at 100 and 200 mg/kg were administered orally for group III and IV respectively through oral administration. Thirty minutes after treatment, inflammation was produced by sub planter injection of 0.1mL of

(1% w/v) freshly prepared formalin in the right hind paw of rats. Before formalin injection, the paw volume each rat was measured separately by means of digital calibrated vernier caliper. Edema caused by formalin was measured at 0, 1, 2, 3 and 4 hour. The increase in paw thickness and percentage inhibition were calculated like carrageenan induced inflammation and compared with control group[10].

Histamine induced paw edema

Using the method of Perianayagam *et al.*[11], the paw edema was produced by subplantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. Rats were divided into four groups of six rats each as used in Carrageenan test. The paw volume was recorded before and at 0, 1, 2, 3 and 4 hour after the histamine injection. Group I treated with histamine alone served as negative control and the remaining three groups of the rats were pretreated with 10mg/kg indomethacin reference drug (Group II), methanolic bark extracts at 100 and 200mg/kg (Groups III and IV respectively). They were administered orally 1 hour before eliciting paw edema. The antiinflammatory activity was calculated as described for carrageenan-induced edema.

Data analyses

Values were expressed as mean of triplicate analysis of the samples (n=3) standard deviation (SD). Analysis of variance and significant differences ($p>0.05$ and $p>0.01$) among means were tested by one-way ANOVA and Dunnett multiple range test.

RESULTS

ACUTE ORAL TOXICITY

The methanolic stem bark extract of *H. populifolia* did not produce any mortality even at the highest dose employed (200mg/kg). Hence, selected doses of test extract of this plant viz., 100 and 200mg/kg were found to be safe and were used for further pharmacological studies.

ANTINOCICEPTIVE ACTIVITIES

Acetic acid induced writhing in mice

The methanol extract of *H. populifolia* induced significant decrease in the number of writhes when compared to the control (Table 1). The extract at 200 mg/kg and the standard drug, aspirin 10mg/kg exhibited higher antinociceptive power13.46 and 15.16 respectively which indicates that the extract has effective antinociceptive property as exhibited by reference drug used in this study.

Hot plate test

The result of the hot plate test showed that oral administration of *H. populifolia* bark extract (100-200mg/kg) increased the reaction time significantly ($p< 0.05$) from 9.54to 11.26 after 30 min of its administration. There were also significant increase in reaction time at 60 and 90 min post extract administration (Table 2).

Antiinflammatory activities

Carrageenan induced paw edema

The results of the carrageenan test (Table 3) showed that paw edema was significantly ($p<0.05$) reduced 2 to 4 h after the administration of the extract (100 and 200mg/kg) and the standard, indomethacin (10 mg/kg).

Formalin induced paw edema

The result obtained from this model showed that paw sizes of the animals were significantly reduced from the 3rd hour of oral administration of extracts (both 100 and 200mg/kg) and the standard, indomethacin (Table 3).

Histamine induced paw edema

The effect of the extract (200mg/kg) and the reference drug on histamine induced paw edema was most pronounced 2h after histamine injection; the anti-histaminic activity of the extract decreased with the increase of the dose of the extract (Table 3).

Table 1

Effect of different doses of the methanolic bark extract of Hildegardia Populifolia on acetic acid induced mice writhing. (Mean±SD)

Sl. No.	Group	Treatment	Number of writhing per 15 min.	Inhibition (%)
1.	Group I	10 mL/kg normal saline	35.52±0.72	-
2.	Group II	10mg/kg aspirin	15.16±0.78*	57.32
3.	Group III	100mg/kg *H. populifolia* stem bark extract	21.71±0.87*	38.88
4.	Group IV	200mg/kg *H. populifolia* stem bark extract	13.46±1.02*	62.11

Degrees of freedom 2.15: *P<0.05 of ANOVA followed by Dunnett test compared with control

Table 2

Effect of methanolic bark extract of Hildegardia populifolia on the hot plate test in swiss albino mice. (Mean±SD)

Sl. No	Group	Dose (mg/kg)	Before drug	Reaction time in seconds				
				0 min	30 min	60 min	90 min	120 min
1.	Control	-	4.63± 0.63	4.95±0.36	4.03±0.42	3.82±0.62	3.07±0.46	2.48±0.25
2.	Pentazocine	10mg/kg	4.84±0.67	5.40±0.41	9.79±0.26*	10.64±0.56**	7.46±0.51	5.83±0.23
3.	Bark extract	100mg/kg	4.72±0.62	5.38±0.73	7.37±0.27	8.35±0.49*	6.43±0.47	4.68±0.42
4.	Bark extract	200mg/kg	4.96±0.59	6.07±0.45	9.54±0.24*	11.26±0.61**	8.52±0.54*	6.35±0.26

Degrees of freedom 2.15: *P<0.05, ** P<0.01 of ANOVA followed by Dunnett test compared with control

Table 3

Antiinflammatory activity of the methanolic bark extract of Hildegardia populifolia on carrageenan, formalin histamine-induced edema in the right hind paw of wistar rats. (Mean±SD)

Sl. No.	Tests	Group	Dose (mg/kg)	Rat paw edema volume at different time interval				
				0 h	1 h	2 h	3 h	4 h
1.	Carrageenan induced edema	Control	-	0.56±0.67	0.78±1.06	0.97±0.85	1.24±0.56	1.65±0.94
2.		Indomethacin	10mg/kg	0.49±0.84	0.60±0.92	0.44±0.83	0.26±0.72*	0.14±1.04**
3.		Bark extract	100mg/kg	0.52±1.07	0.78±0.79	0.42±0.72	0.36±0.25	0.30±0.43*
4.			200mg/kg	0.58±0.73	0.66±0.53	0.42±0.78	0.35±0.39	0.26±0.75**
5.	Formalin -induced edema	Control	-	0.21±1.03	0.49±0.17	1.21±0.29	1.28±0.1.08	1.37±0.72
6.		Indomethacin	10mg/kg	0.16±0.84	0.37±0.42	0.42±0.51*	0.35±0.82*	0.23±0.37**
7.		Bark extract	100mg/kg	0.15±0.63	0.56±0.51	0.60±0.32	0.53±1.13	0.47±0.81*
8.			200mg/kg	0.15±0.51	0.40±0.38	0.45±0.26*	0.37±0.49*	0.20±0.62**
9.	Histamine-induced edema	Control	-	1.19±0.64	1.41±0.85	1.58±0.30	1.80±0.53	1.99±0.64
10.		Indomethacin	10mg/kg	0.17±0.54	0.38±1.14	0.22±0.48*	0.19±0.46*	0.10±0.35**
11.		Bark extract	100mg/kg	0.34±1.07	0.58±0.88	0.50±0.50	0.41±0.78	0.33±1.13
12.			200mg/kg	0.14±0.44	0.31±0.94	0.23±0.21*	0.16±0.59*	0.12±0.36**

Degrees of freedom 2.15: *P<0.05, ** P<0.01 of ANOVA followed by Dunnett test compared with control

DISCUSSION

This is the first study in an endangered tree , *H. populifolia* to know the antinociceptive (acetic acid induced writhing model and hot plate test) and antiinflammatory activities in three inflammation models such as carrageenan induced hind paw edema, formalin induced hind paw edema and histamine induced hind paw edema. It is found that the methanolic bark

extract significantly inhibited the acetic acid induced writhing and enhanced the reaction time in a dose dependant manner. The bark extract of *H. populifolia* used in our study inhibited edema induced by carrageenan, formalin and histamine in dose-dependant manner, and its activity was comparable to the standard drug, indomethacin, a known cyclooxygenase inhibitor which indicates the existence of antiinflammatory properties in this plant. Many studies have demonstrated that antiinflammatory activity of several plant species has been attributed to their high sterol and triterpene[12] or flavonoids[13] and the antinociceptive property may be due to the different kinds of flavonoids[14]. As the study species *H. populifolia* is growing in dry habitats of stony substratum, it is expected to have more variety of above said secondary metabolites to withstand drought condition, the species might have both antinociceptive and antiinflammatory properties. However, further studies on secondary metabolites are needed to confirm this fact. Increased body temperature and pain are known as main symptoms of the body against an inflammatory stimulation. Hence, a drug possessing antiinflammatory activity may also exhibit antipyretic and analgesic properties[15]. The present study suggested that the methanol extracts of *H. populifolia,* may be used as a herbal remedy for the management of inflammation. However, the assessment of observed pharmacological effects with isolated individual chemical constituent merit further investigation for better understanding of the molecular mechanisms underlying antiinflammatory activity of the methanolic extract of the study species, *H. populifolia.*

CONCLUSION

The methanolic bark extract of *H. populifolia* possesses considerable level of antinociceptive and antiinflammatory activities. It has effectively reduced inflammation and relieved pain in rats. Therefore, this species may be treated as a potent source of drugs for the treatment of inflammatory disorders. Before going for commercialization by drug formulation, mass propagation technologies must be developed by stem cuttings or employing suitable tissue culture techniques both for sustainable conservation of the tree species and to meet the demand as well.

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