ORIGINAL ARTICLE

Evaluation of anti-inflammatory and analgesic activities of methanolic leaf extract of the endangered tree species, *Hildegardia populifolia* (Roxb.) Schott and Endl

Saradha Maran, Paulsamy Subramanium[1]
Department of Botany, Nirmala College for Women, [1]Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India

Objective: The present study was aimed at to evaluate the anti-inflammatory and analgesic activities of methanolic leaf extracts of the endangered medicinal tree species, *Hildegardia populifolia* on laboratory animal. **Methods:** The anti-inflammatory potential of the extract has been determined by using carrageenan, formalin and histamine induced paw edema assays in Wistar rats. Indomethacin was used as a reference drug. The analgesic activity was tested by using acetic acid induced writhing response and hot plate method in swiss albino mice. Aspirin and pentazocine were used as reference drugs respectively for these models. **Results:** The oral administration of leaf extract at doses, 100 and 200 mg/kg significantly ($P < 0.05–0.01$) inhibited the carrageenan, formalin and histamine induced inflammation. The acute treatment of the extract produced a significant ($P < 0.05–0.01$) antinociceptive effect in the animals of acetic acid induced pain and the animals tested in a hot plate method. Acute toxicity test showed that the plant may be safe for pharmacological uses. **Conclusions:** It is very clear that *H. populifolia* has both anti-inflammatory and analgesic activities and so may be used as pharmaceuticals.

Key words: Anti-inflammatory and analgesic properties, endangered species, *Hildegardia populifolia*

INTRODUCTION

Plants have been an important source of medicine for 1000's of years. Herbal medicine is still the mainstay of therapy for about 75–80% of the whole population in developing countries for primary health care.[1] This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries.[2] The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine.[3] World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases.[4] Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain.[5] However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. One such plant from Indian flora *Hildegardia populifolia* an indigenous medium sized tree belonging to the family, Sterculiaceae is found in few tropical deciduous forests of Tamil Nadu and Andhra Pradesh in India.[6] The plant possesses significant antimicrobial,[7-11] antioxidant[12,13] and antiinflammatory[14] activities. The fiber extracted from the bark is used for domestic purposes and the leaf extract is known to have healing properties and used for the treatment of dog bite and malaria in the traditional medical practice of Tamil Nadu and Andhra Pradesh.[15,16] The major phytoconstituents reported in this species are alkaloids, tannins, saponins, terpenoids, flavonoids and phenolics.[8] Since no scientific data are available to justify the traditional anti-inflammatory and analgesic potentials in leaf part of the plant, present study was planned to validate the therapeutic uses of this species in treatment of inflammatory and analgesic diseases.

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MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant material was collected from the campus of Forest Genetics Division, Tamil Nadu Forest

Address for correspondence: Dr. Paulsamy Subramanium, Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India. E-mail: paulsami@yahoo.com
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Department, Bhavanisagar, Erode District, Tamil Nadu, India. The species was confirmed in Botanical Survey of India, Coimbatore with the accession number 15211 (MH).

Preparation of Extracts

The leaves were washed in running tap water, and shade dried for 15 days. Then the dried leaves were pulverized into a fine powder using pestle and mortar. 50 g of fine powder was packed separately with No. 1 Whatman filter paper and placed in soxhlet apparatus along with solvent, methanol. 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 25–30 g and 150–250 g either sex were obtained from the Small Animal Breeding Station, Munnuthy, Trissur, Kerala. The animals were maintained at room temperature of 25 ± 2°C with 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 mm × 280 mm × 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 the organization for Economic Co-operation and Department.[17] 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) was administered orally at a dose of 5 mg/kg and mortality was observed 3 days. If the 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 color were observed for the first 1 h and after 24 h of drug administration.

Anti-inflammatory Activity

Carrageenan-induced Paw Edema

The anti-inflammatory activity was evaluated by carrageenan induced rat paw edema model.[18] Wistar rats of either sex were weighed (170–230 g) and normal paw volumes of all the rats were 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 0.1 mL freshly prepared 1% carrageenan in normal saline. Group I treated with carrageenan alone served as a negative control. The rats of group II were treated with 10 mg/kg indomethacin,[19] groups III and IV respectively received the methanolic leaf extract at the concentrations of 100 and 200 mg/kg body weight orally 1 h 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 h 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 180–200 g weight were used for the study, and they were divided into four groups each group contains six rats. Rats which were given no treatment served as control (group I), group II treated with Indomethacin (10 mg/kg) served as a positive control. The methanolic leaf extracts at 100 and 200 mg/kg were administered orally for group III and IV respectively through oral administration. Thirty minutes after the treatment, inflammation was produced by sub planter injection of 0.1 mL of (1% w/v) freshly prepared formalin in the right hind paw of rats. Before formalin injection, the paw volume for 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 h. The increase in paw thickness and percentage inhibition were calculated like carrageenan induced inflammation and compared with the control group.[20]

Histamine Induced Paw Edema

Using the method of Perianayagam *et al.*,[21] 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 per group, which were used as in Carrageenan test. The paw volume was recorded before and at 0, 1, 2, 3 and 4 h 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 10 mg/kg indomethacin reference drug (Group II), methanolic leaf extracts at 100 and 200 mg/kg (Groups III and IV respectively). They were administered orally 1 h before eliciting paw edema. The anti-inflammatory activity was calculated as described for carrageenan-induced edema.

Analgesic Activity

Hot Plate Method

The hot plate test was employed to measure central analgesic activity by the method of Eddy and Leimback[22] with minor modifications. In this experiment, the hot plate was maintained at 55 ± 0.5°C. All animals were selected 24 h prior to experimentation on the basis of their normal reaction time that is, pain response to the hot plate to the minimum and maximum of 2 and 15 s, respectively. In order to avoid damaging the paws of the animals, the time standing on the plate was limited to 25 s. All the rats divided into four groups each comprising six animals. Group I normal rat served as control. Pentazocine at the rate of 10 mg/kg was administered intraperitoneally (group II) as a reference standard. Groups III and IV received methanolic leaf extracts of *H. populifolia* at the concentrations of 100 and 200 mg/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 recorded as the reaction time or latency of pain response.

Acetic Acid-induced Writhing Test in Mice

This test was conducted using the method of Koster, *et al*.[23] Swiss albino mice, weighing 18–25 g were randomly divided into four groups, six animals each (n = 6). Control group (group I) received 10 mL/kg normal saline orally. The reference group (group II) received aspirin (10 mg/kg dissolved in distilled water, p.o.) and groups III and IV were orally pretreated with 100 and 200 mg/kg methanolic leaf extracts of *H. populifolia* extract, respectively. All drugs were administered orally 30 min prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1 mL/10 g). Thirty minutes later the animals were placed on an observation table and observed individually for counting the number of writhing they made in 15 min commencing just 5 min after the intraperitoneal administration of acetic acid solution. A reduction in the number of writhes is an indication of analgesic property.

Data Analyses

The percentage inhibitions were log-transformed before they were subjected to statistical analysis. Data are expressed as mean ± standard error. Statistical evaluation was carried out by One-way analysis of variance (ANOVA followed by Duncan's multiple range test). Statistical significance is expressed as *$P < 0.05$, **$P < 0.01$.

RESULTS

Acute Oral Toxicity

The methanolic leaf extract of *H. populifolia* did not produce any mortality at the highest dose employed. Selected doses of methanolic extract of this plant were found to be safe. Two doses of the leaf extract, of 100 and 200 mg/kg, p.o. were selected for further pharmacological studies.

Anti-inflammatory Activity

Carrageenan-induced Paw Edema

The anti-inflammatory activity of methanolic leaf extract of *H. populifolia* was measured at the dose of 100 and 200 mg/kg against acute paw edema induced by carrageenan [Table 1]. Carrageenan induced rat paw edema was markedly inhibited by the leaf extract of the study species (3.82 ± 0.71 at 200 mg/kg) and the standard drug indomethacin (10 mg/kg).

Formalin-induced Paw Edema

In the formalin induced paw edema method, the oral administration of leaf extract of *H. populifolia* in two different doses *viz*., 100 and 200 mg/kg significantly reduced the paw volume in a dose-dependent manner in comparison to control [Table 2]. The maximum effect was seen in the oral dose of 200 mg/kg of leaf extract that showed (4.37 ± 0.18) a significant ($P < 0.01$) reduction (46%) in paw volume than the control after 4 h. The anti-inflammatory activity performed by all doses of leaf extract at 200 mg/kg was comparable to the standard, indomethacin at 10 mg/kg, p.o. The maximum anti-inflammatory activity was observed after 3 hours from the administration of all the doses of the plant extract [Table 2].

Histamine-induced Paw Edema

The effect of the leaf extract of *H. populifolia* (200 mg/kg) and the reference drug on histamine-induced paw edema was most pronounced 2 h after histamine injection, while the 200 mg/kg dose of the leaf extract showed highest activity in 4 h duration after histamine administration. Further, it was observed that the anti-histaminic activity of the extract decreased with the increase of the dose of the extract administered [Table 3].

Table 1: Anti-inflammatory activity of the methanolic leaf extract of *H. populifolia* on carrageenan-induced edema in the right hind paw of Wistar rats

Group	Dose (mg/kg)	Before carrageenan	The rat paw oedema volume after injection of carrageenan				
			0 h	1 h	2 h	3 h	4 h
Control	-	3.96±0.67	4.52±1.06	4.74±0.85	4.93±0.56	5.20±0.94	5.61±0.78
Indomethacin	10	3.82±0.84	4.31±0.92	4.42±0.83	4.26±0.72	4.08±1.04*	3.96±0.43**
H. populifolia leaf extract	100	3.85±1.06	4.48±1.12	4.53±0.87	4.29±0.48	4.18±0.29*	4.00±0.39*
H. populifolia leaf extract	200	3.80±0.92	4.23±0.84	4.36±0.92	4.02±0.71*	3.94±0.84**	3.82±0.71**

*P<0.05, **P<0.01 versus control (values are mean±SE from 6 animals in each group). SE – Standard error; *H. populifolia – Hildegardia populifolia*

Analgesic Activity

Hot Plate Method

Results of the hot plate test are presented in Table 4 for the methanolic leaf extract of *H. populifolia*. The extract was found to exhibit a dose dependent increase in latency time when compared with control. The oral administration of leaf extract at 200 mg/kg significantly attenuated the hot plat thermal stimulation. The analgesic activity of the extracts was compared to the standard drug, pentazocine (10 mg/kg) which indicates that the methanolic leaf extract was found to be more effective.

Acetic Acid Induced Writhing in Mice

The methanolic extracts of *H. populifolia* leaves and the positive control, aspirin, induced significant decrease in the number of writhes when compared to the control [Table 5]. The extract administered at 200 mg/kg dose level showed a significant result than that of the standard drug. The results indicate that the leaf extract has slightly higher antinociceptive than the reference drug used in this study.

DISCUSSION

Carrageenan-induced edema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamines, bradykinins, leucotrienes and serotonins, all of which also cause pain and fever.[24] Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. Carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation.[25,26] The results of the present study indicate that the species, *H. populifolia* can be an effective medicinal source to cure in acute inflammatory disorders. The formalin-induced paw edema assay defines distinctive biphasic nociceptive response termed neurogenic and inflammatory phases.[27] The ability of methanolic leaf extract of *H. populifolia* on both phases shows that it contains most appropriate active anti-inflammatory principle compounds acting both centrally and peripherally. The extract also caused pronounced reduction in the edema produced by histamine. The result tends to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. The anti-histaminic effect of the extract decreased with the increase in the doses of the extract concomitantly. Histamine is an important inflammation mediator and potent vasodilator substance also increasing the vascular permeability.[28-30] As the extract effectively suppressed the edema produced by histamine, it is understood that the leaf extract of *H. populifolia* exhibited anti-inflammatory actions by inhibiting the synthesis and release or action

Table 2: Anti-inflammatory activity of the methanolic leaf extract of *H. populifolia* on formalin-induced edema in the right hind paw of Wistar rats

Group	Dose (mg/kg)	Before formalin	The rat paw oedema volume after injection of formalin				
			0 h	1 h	2 h	3 h	4 h
Control	-	4.46±0.75	4.67±1.03	4.95±0.17	5.67±0.29	5.74±0.1.08	5.83±0.72
Indomethacin	10	4.32±0.69	4.48±0.84	4.69±0.42	4.55±0.51*	4.49±0.82*	4.40±0.37**
H. populifolia leaf extract	100	4.35±0.62	4.46±0.80	4.81±0.70	4.72±0.35	4.63±0.23*	4.59±0.55*
H. populifolia leaf extract	200	4.31±0.76	4.40±0.42	4.66±0.25	4.51±0.67*	4.46±0.61**	4.37±0.18**

*P<0.05, **P<0.01 versus control (values are mean±SE from 6 animals in each group). SE – Standard error; *H. populifolia – Hildegardia populifolia*

Table 3: Anti-inflammatory activity of the methanolic leaf extract of *H. populifolia* on histamine-induced edema in the right hind paw of Wistar rats

Group	Dose (mg/kg)	Before histamine	The rat paw oedema volume after injection of histamine				
			0 h	1 h	2 h	3 h	4 h
Control	-	3.48±1.07	4.67±0.64	4.89±0.85	5.06±0.30	5.28±0.53	5.47±0.64
Indomethacin	10	3.40±0.85	3.57±0.54	3.78±1.14	3.62±0.48*	3.59±0.46*	3.50±0.35**
H. populifolia leaf extract	100	3.45±1.09	3.91±0.83	4.17±0.71	4.05±0.32	3.92±0.75	3.87±0.72
H. populifolia leaf extract	200	3.37±0.70	3.61±1.00	3.94±0.73	3.85±0.47	3.70±0.84	3.66±0.85*

*P<0.05, **P<0.01 versus control (values are mean±SE from 6 animals in each group). SE – Standard error; *H. populifolia – Hildegardia populifolia*

Table 4: Effect of methanolic leaf extract of *H. populifolia* on the hot plate test in Swiss albino mice

Group	Dose (mg/kg)	Before drug	Reaction time after administration of control/standard/extract in minutes				
			0 min	30 min	60 min	90 min	120 min
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
Pentazocine	10	4.84±0.67	5.40±0.41	9.79±0.26*	10.64±0.56**	7.46±0.51	5.83±0.23
H. populifolia leaf extract	100	4.41±0.52	5.62±0.33	7.00±0.35	8.13±0.24	6.35±0.32	4.08±0.36
H. populifolia leaf extract	200	5.01±0.41	5.71±0.39	8.93±0.18*	9.72±0.45*	6.47±0.18	4.92±0.34

*P<0.05, **P<0.01 versus control (values are mean±SE from 6 animals in each group). SE – Standard error; *H. populifolia – Hildegardia populifolia*

Table 5: Effect of different doses of the methanolic leaf extract of *H. populifolia* on acetic acid induced mice writhing test

Group	Treatment	Number of writhing per 15 min	Inhibition (%)
I	10 mL/kg normal saline	35.52±0.72	-
II	10 mg/kg aspirin	15.16±0.78*	57.32
III	100 mg/kg *H. populifolia* leaf extract	23.07±1.06*	35.05
IV	200 mg/kg *H. populifolia* leaf extract	17.24±1.05*	51.46

*P<0.05, **P<0.01 versus control (values are mean±SE from 6 animals in each group). SE – Standard error; *H. populifolia – Hildegardia populifolia*

of inflammatory mediators such as histamine, serotonin, and prostaglandins.

The extract increased reaction latency to thermal pain induced by the hot plate in mice, which is a specific central antinociceptive test.[31] It may be attributed to the inhibition of histamine or kinin pathway may reduce the pain.[32] The results of the present study also showed that the extract of *H. populifolia* exhibited a comparable magnitude of antinociceptive activity in both models of pain, which suggested that the phytochemical constituents are responsible for the analgesic effect. The analgesic activity of some flavonoids and terpenoids has already been reported, which suggests that these or similar constituents may be responsible for the analgesic effect of the extract.[33]

The extract significantly (P < 0.01) reduced the number of abdominal writhings induced by acetic acid in mice. Abdominal construction induced by acetic acid is used to screen the peripheral analgesic effect.[23] The acetic acid-induced writhing has been associated with an increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.[34] The results support the hypothesis of participation in the inhibition of prostaglandin synthesis since the nociceptive mechanism of abdominal writhing induced by acetic acid involves the process or release of arachidonic acid metabolites via cyclo-oxygenase (COX) and prostaglandin biosynthesis. The effect of the plant extract on acetic acid-induced abdominal writhing suggested that they might inhibit or modify responses to pain mediated by noiceptors peripherally.[35] In conclusion, the results of the present study indicate that the methanolic leaf extract of the study species, *H. populifolia* might contain constituents capable of relieving pain or modifying the responses to pain caused by either thermal or chemical stimulation of the noiceptors mediated by both central and peripheral mechanisms. In addition, the flavonoids are known to inhibit prostaglandin synthetase.[36] As the prostaglandins are involved in pain perception and are inhibited by flavonoids,[37] it could be suggested that reduced availability of prostaglandins by flavonoids present in *H. populifolia* might be responsible for its analgesic effect.

CONCLUSION

In conclusion, as the plant extract reduced significantly the formation of edema induced by carrageenan, formalin and histamine and reduced the number of writhes in acetic acid induced writhing models and increased reaction latency, the leaf of *H. populifolia* exhibited anti-inflammatory and analgesic activities in an appropriate manner. Further, no mortality was observed in the acute toxicity test, which showed that the plant is safe for use. The study has thus provided some justification for the folkloric use of this plant in several communities for conditions such as stomach ache, pain and inflammations.

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