Anti-inflammatory and Analgesic Effects of
Pongamia glabra Leaf Gall Extract

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Summary

The ethanol extract of Pongamia glabra Vent leaf gall (PG) was investigated for anti-inflammatory and analgesic activity at the doses (p.o.) of 100, 200, and 400 mg/kg body weight. For evaluation of inflammation carrageenan-, histamine- and serotonin-induced paw edema served as acute models and cotton pellet–induced granuloma served as a chronic model in rats. The acetic acid-induced writhing response and hot plate method using mice were used to assess analgesic activity. The higher doses of PG (200 and 400 mg/kg, p.o.) were inhibiting carrageenan, histamine and serotonin-induced paw edema as well as cotton pellet –induced granuloma successfully. In addition, PG (200 and 400 mg/kg, p.o.) significantly attenuated the writhing responses induced by an intraperitoneal injection of acetic acid. There were no significance changes in the reaction time by using hot plate method in mice. Furthermore, our phytochemical studies indicated that the ethanolic extract of leaf gall contains carbohydrates, protein, tannins, steroids, flavonoids, and mucilage. From acute oral toxicity studies (OECD-423 guidelines), no mortality was observed even at highest dose of PG (2000 mg/kg, p.o.).

Keywords: Pongamia glabra leaf gall, anti-inflammatory, analgesic, paw edema.

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Introduction

*Pongamia glabra.* Linn. (Papilionaceae) Leaf gall (Synonym, *Pongamia pinnata*) which may also be called as *Galdupa indica*, is a large tree found in tropical regions and costal forests of India, North Australia, Southeast Asia and Malaysia (1,2). In the Ayurvedic literature of India, different parts of the plant have been recommended as a remedy for various ailments, and have been used in traditional medicine for bronchitis, whooping cough and rheumatic joints (3). Aqueous extracts of the leaf have significant anti-ulcer activity (4), dried flowers of the plant was shown potent anti-diabetic activity in normal and non-insulin dependent diabetes mellitus patients (5). The seed and seed oil have been used for treating various inflammatory and infectious diseases such as lecderma, leprosy, lumbago, articular rheumatism, and muscular (6, 7). Extracts of roots, leaves and seeds of the *Pongamia glabra* have been reported to have anti-inflammatory and antidiarrhoeal activities (8-12). The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement (2, 13). In addition, the phytochemical examinations of this plant have indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, flavones, furanodiketones and flavonoid glucosides (14-21). In the light of above, the present study investigated the potential of ethanolic extract of *Pongamia glabra* leaf gall on as an anti-inflammatory and analgesic in rodents.

Material and Methods

Plant Material

The fresh *Pongamia glabra* leaf gall was collected during the month of June, 2004 from Erode situated in the state of Tamil Nadu (India). The plant material was taxonomically identified and authenticated by The Head, Department of Botany, Tamilnadu Agricultural University, Coimbatore. A voucher specimen (NCP/RHM/535/10) has been deposited at the herbarium of natural product research lab, Nandha College of Pharmacy, Tamilnadu, India for ready reference.

Preparation of the Extract

The freshly collected leaf galls were shade-dried, pulverized using a mechanical grinder and passed through 40 mach sieve. The powdered leaf galls (2.25 Kg) were extracted with 95% ethanol using a Soxhlet extractor, at room temperature. After exhaustive extraction, the ethanolic extract was filtered and concentrated by distillation process. A brownish-black colored residue was obtained (yield 17.8% w/w), which was kept in a desiccator. This ethanolic extract of *Pongamia glabra* leaf gall (PG) was used in further experiments.
Animals

All the experiments were carried out using male, Swiss Albino mice (25-30 g) and Wistar rats (150-200 g) were obtained from animal house, IRT Perundurai medical college, Erode, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Register Number: 688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Drugs and Chemicals

The drugs and fine chemicals were purchased from Sigma-Aldrich, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest pure and analytical grade.

Vehicle

Plant extract (PG) and indomethacin were suspended in 0.5% w/v carboxymethylcellulose sodium (CMC) and administered orally to animals. Carrageenan, histamine, serotonin and acetic acid diluted separately in normal saline and injected.

Preliminary Chemical tests

The extract was subjected to preliminary screening, for various active phytochemical constituents such as alkaloids, carbohydrates, steroids, protein, tannins, phenols, flavonoids, gum and mucilage, glycosides, saponins and terpins.

Acute Oral Toxicity Studies

Acute oral toxicity studies were performed (22) according to OECD-423 guidelines (acute toxic class method). Swiss mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The PG (suspended with 0.5% w/v CMC) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 300, and 2000 mg/kg.
Acute Anti-inflammatory Studies

Carrageenan, histamine and serotonin induced paw edema models were used for evaluating potential of PG on inflammation. For each model, rats were divided into five groups (n = 6). PG (100, 200, and 400 mg/kg) and indomethacin (10 mg/kg) were administered orally one hour before the subplantar injection of edematogenic agent. The control groups of animals were received vehicle (1 ml/kg) orally. A digital vernier caliper (Model 2061, Mututoyo Digimatic Caliper, Japan) used for measuring paw thickness (mm) of rats (23,24). Edema ($\Delta T$) was calculated as follows:

$$\Delta T = T_t - T_0$$

Where $T_t$ is the right hind paw thickness (mm) at time ‘t’, $T_0$ is hind paw thickness (mm) before subplantar injection.

Carrageenan Induced Edema in Rats

In this method, acute inflammation was produced by the subplantar administration of 0.1 ml of 1% w/v carrageenan in the right paw of the rat. The thickness (mm) of the paw was measured immediately and at 1, 2, 3, and 4 h intervals after the administration of the carrageenan (23-27).

Histamine and Serotonin Induced Edema in Rats

Edema in rats was induced by injecting 0.1 ml of 0.1% w/v histamine or 0.2% w/v serotonin in subplantar region of the right hind paw. The thickness (mm) of the paw was measured immediately and at 1, 2, 3, and 4 h intervals after the administration of the histamine or serotonin (28,29).

Cotton Pellet –induced Granuloma

The cotton pellets-induced granuloma in rats was studied according to the method D’Arcy et al(30). The animals were divided into five groups of six animals in each group. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group 1 served as control and received the vehicle (normal saline, 5 ml/kg). The PG extract at the concentration of 100, 250 and 500 mg/ kg was administered orally to groups 2, 3 and 4, respectively for seven consecutive days from the day of cotton pellet implantation. Group 5 received the standard drug indomethacin (10 mg/ kg) for the same period, on 8th day the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The antiproliferative effect of PG extract was compared with control.
Acetic acid-Induced Writhing Method

Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster et al. (31). Male Albino mice were divided into five groups of six animals each. The animals were pretreated with PG (100, 200 and 400 mg/kg, p.o.) or aspirin (200 mg/kg, p.o.) used as a standard drug, one hour prior to intraperitoneal injection of 1% v/v acetic acid (0.1 ml/10g). Five minutes after the intraperitoneal injection of acetic acid, the number of writhing during the following 10 minutes was counted. Control mice received drugless (0.5% w/v CMC; 10 ml/kg) vehicle (27,32).

Hot Plate Method

Five groups of six mice each were selected for the present study. Group 1 served as control and received the vehicle. The PG extract at the concentration of 100, 250 and 500 mg/kg was administered orally to groups 2, 3 and 4, respectively and group 5 received the standard drug pentazocine (30 mg/kg, i.p.). The mice were placed on Eddy’s hot plate kept at a temperature of 55 ± 0.5°C for a maximum time of 15 sec (33). Reaction time was recorded when the animals licked their fore-and hind paws and jumped; at before 0 and 15, 30, 45, and 60 min after administration of test drugs.

Statistical Analysis

All the results were expressed as Mean ± Standard Error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s t-test. P-values < 0.05 were considered as statistically significant.

Results

Preliminary Chemical Tests

Our phytochemical studies indicated that ethanolic extract of PG leaf gall contains carbohydrates, protein, tannins, sterols, flavonoids, and mucilage. Alkaloids, glycosides and proteins were showed negative.

Acute Oral Toxicity Test

*Pongamia glabra* leaf gall extract did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. All the doses (5, 50, and 300 mg/kg, p.o.) of PG were thus found to be non-toxic. Three doses (100, 200, and 400 mg/kg, p.o.) of PG were selected for further pharmacological studies.

Carrageenan Induced Edema in Rats

Subplantar injection of carrageenan in rats showed to a time-dependent increase in paw thickness (Fig.1); this increase was observed at 1 h and was maximal at 3 h after administration of carrageenan injection in the vehicle treated groups.
However, carrageenan-induced inflammation was significantly ($P < 0.001$) reduced in all phases of the experiment by treatment with PG 200 and 400 mg/kg as well as indomethacin. The lower dose of extract (100 mg/kg, p.o.) did not show any considerable change in paw edema as compared with vehicle treated group (Fig.1).

**Fig.1**

The effects of PG and indomethacin (INDO) on rat's hind paw edema induced by carrageenan (CARR).

Data represented as mean ± S.E.M. (n=6).

* $P< 0.001$ as compared with the CARR group.
Histamine and Serotonin Induced Edema in Rats

Fig. 2 and 3 show the effects of histamine and serotonin induced paw edema in rats. The maximal paw thickness was observed at 1h of after subplantar injection in vehicle treated groups of both the models. The animals treated with higher doses of PG (200 and 400 mg/kg, p.o.) as well as indomethacin (10 mg/kg, p.o.) produced statistically significant ($P < 0.01$) inhibition of the edema induced by histamine or serotonin in all the phases, when compared to the vehicle treated groups.

![Graph showing effects of histamine and serotonin induced paw edema in rats]

**Fig. 2**
The effects of PG and indomethacin (INDO) on rat’s hind paw edema induced by histamine (HIST).
Data represented as mean ± S.E.M. ($n=6$)
* $P<0.01$ as compared with the HIST group.
* $P<0.001$ as compared with the HIST group.
Cotton pellet – Induced Granuloma

The anti-inflammatory effect of PG was calculated depending on the wet and dry weight of cotton pellets. The continuous oral treatment (7 days) with plant extract (200 and 400 mg/kg, p.o.) and indomethacin (10 mg/kg i.p.) remarkably reduced ($P < 0.001$) the formation of granuloma by indicating the significant reduction in wet and dry weight of cotton pellets (Fig. 4).
Acetic acid-Induced Writhing Method

The ethanolic extract from leaf galls of *Pongamia glabra* strongly reduced the abdominal constrictions induced by the intraperitoneal administration of acetic acid solution. The treatment of animal with PG (200 and 400 mg/kg, p.o.) produced a significant (*P < 0.001*) and dose depend inhibition in abdominal writhes produced by acetic acid. The inhibition by PG (400 mg/kg, p.o.) was nearly similar to that produced by aspirin (200 mg/kg, p.o.), used as a standard drug (Fig.5).
Hot Plate Method

The extract at the all dose levels (100, 200 and 400 mg/kg, p.o.) did not show any significant changes in reaction time at throughout the observation period as compared with control animals. The mice treated with standard drug pentazocine (30 mg/kg, i.p.) showed significant ($P < 0.001$) increase in reaction time at 30, 45, and 60 min as compared with control (Fig.6).
Discussion

In living animal tissues, inflammatory processes involve the release of several mediators, including prostaglandins, histamines, thermo-attractants, cytokines, and proteinase and so on; as well as substances that regulate adhesion of molecules and the processes of cell migration, activation and degranulation (34).
Among several traditional claims, the usefulness of *Pongamia glabra* leaf gall in inflammation and pain has been emphasized more in literature. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to the above mentioned systemic pharmacological screening so far. In the present study, the anti-inflammatory activities of the ethanolic extract of *Pongamia glabra* leaf gall (PG) has been established in both acute and chronic inflammation models, which were employed.

Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products (35). Development of edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome (36, 37). Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclo-oxygenase. The result of the present study indicates that PG (200 and 400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation.

Histamine and serotonin are important inflammation mediators and they are potent vasodilator substances as well as increase the vascular permeability (38-40). In the present study, PG (200 and 400 mg/kg, p.o.) effectively suppressed the edema produced by the histamine and serotonin indicating that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators *viz.*, histamine, serotonin and prostaglandin might be involved in inflammation.

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation (41). Chronic inflammation occurs by means of the development of proliferative cells. These cells an either spread or granuloma form (42). Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (43). The ethanolic extract of PG showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory condition.

In order to distinguish between the central and peripheral analgesic action of PG, acetic acid induced writhing responses in mice were used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test the animals react with characteristic stretching behavior, which is called writhing. It was found that PG significantly inhibited the acetic acid induced writhing response. The abdominal constriction is related to the sensitzation of nociceptive receptors to prostaglandins. It is therefore possible that PG produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandins.
The hot plate method was originally described by Woolfe and Mac Donald (44). This test has been found to be suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance (45). The present findings of the study indicate that the PG did not show any changes in analgesic activity by using hot plate method.

In conclusion, the data obtained in this study demonstrated that *Pongamia glabra* extract might have analgesic and anti-inflammatory activities through peripherally. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

References


