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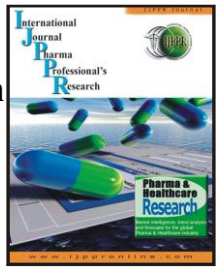

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF METHANOLIC EXTRACT OF *PONGAMIA PINNATA* STEM BARK

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Abstract

This study aims to evaluate the anti-inflammatory and analgesic potential of the methanolic extract of *pongamia pinnata* stem bark (PSBE) in different experimental animal models. PSBE (200, 500 and 1000 mg/kg) exhibited significant anti-inflammatory activity in acute (carrageenan induced hind paw edema) and chronic (cotton pellet granuloma) models of inflammation PSBE did not show any sign of toxicity and mortality up to a dose level of 10.125 g/kg, p.o. in mice. Both acute as well as chronic administration PSBE (200, 500 and 1000 mg/kg, p.o.) did not produce any gastric lesion in rats. The analgesic activity was tested by acetic acid-induced writhing response in albino mice and tail flick method in albino rats. Its methanolic extract shows the most effective anti-inflammatory activity at doses of 200, 500 and 1000 mg/kg significantly throughout the observation period. In the tail flick model, the PSBE in the above doses increased the pain threshold significantly after 30 min., 1, 2, and 4 hr. of administration. *pongamia pinnata* showed dose-dependent action in all experimental animal models.

Keywords: - *Pongamia pinnata*, Analgesic, Anti-inflammatory, acute toxicity

Introduction

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest Known diseases of mankind affecting the majority of population, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is the need of hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants [1]

Pongamia pinnata (Leguminosae), popularly known as 'Karanj' in Hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of South Eastern Asia and Australia[2]. The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations[3].A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scroflilous enlargement [2,4].While different extracts of roots and seeds (ethanol, petroleum ether, benzene extracts and others) of *Pongamia pinnata* have been reported to have anti-inflammatory activity[1,5,6], ethanolic extract of the leaves of *P. pinnata* has significant antibacterial activity against the tested bacteria *Vibrio* sp., *Pseudomonas* sp., and *Streptococcus* species [7].

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Materials and Method

Animals

Male Wistar rats (150–250 g) and either male or female Swiss albino mice (20–25 g) were used. These animals were obtained from colonies maintained at the Department of Pharmacy, GRD (PG) IMT, Dehradun, U.K. (India). The animals were housed in groups of 6–10 under environmentally controlled conditions with free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided *ad libitum*. The handling and use of animals were in accordance to the Guidelines of Institute Animal Ethics Committee, while using live animals. All the animals were acclimatized to the laboratory environment for 5 days before the experiment. Six animals (rats or mice) per group comprising of three males and three females, were used in each experiment, unless otherwise specified. The animals were fasted

overnight just prior to the experiment but allowed free access to drinking water.

Chemicals

Carrageenin, Sodium chloride was purchased from Sigma Chemicals Company, U.S.A, Pethidine was obtained from Bengal Immunity, Kolkata, and acetylsalicylic acid was purchased from Hi-Media Laboratories, Mumbai, India and all the other chemicals used were of the analytical and highest purity grade from standard companies. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

Plant Material

Fresh stem bark of *Pongamia pinnata* were collected from their natural habitats in and around Bareilly (Uttar Pradesh). The plant was authenticated by comparison with the herbarium and voucher specimen was lodged in the departmental herbarium of Botanical Research survey of India Dehradun. A voucher specimen has also been deposited in the herbarium of the institute for future references. Stem bark of *Pongamia pinnata* (500gm) were air dried at room temperature and powdered coarsely. Hundred gram of the pulverized plant was extracted with methanol using a soxlet apparatus. The extract was filtered, pooled and concentrated on rotavapour. The yield was 8.2% in powder extract. The extract of stem bark of *Pongamia pinnata* (PSBE) was administered as a suspension in 2% Gum acacia to the animals. Preliminary phytochemical screening method was carried out on the standard screening method[8].

Acute toxicity study (oral)

For acute toxicity, mice were divided into groups of eight animals each. One group served as a control and received (2 ml/kg, p.o.) alone. While the remaining groups were treated with increasing doses of the methanolic extract: of 3.0, 4.5, 6.75 and 10.125 g/kg respectively. All treated animals were closely observed for any abnormal or toxic manifestations and for mortality up to the end of 24 h in each group to calculate LD_{50} described by Weil (1952)[9]. Based on the results obtained from the preliminary toxicity study, the doses for further pharmacological studies were fixed to be 200, 500 and 1000 mg/kg, p.o.

Anti-Inflammatory Study

Carrageenin-induced hind Paw edema in rats

In present study anti- inflammatory activity was determined in albino rats of either sex according to the method[10].. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gam acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile) at '0' and '3' hours after the carrageenan injection(Table 1). Aspirin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug. The inhibitory activity was calculated according to the following formula:

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

Where C_t = paw circumference at time t, C_0 = paw circumference before carrageenin injection

Cotton pellet granuloma in rats

The effect of PSBE on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma rat model[11].Autoclaved cotton pellets weighing 35±1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with ether. The different groups of rats were administered the PSBE (200, 500 and 1000 mg/kg, p.o.) and ASA (300 mg/kg, p.o.) once daily for seven consecutive days from the day of cotton pellet insertion(Table 2). The control group received normal saline alone. On the eighth day, all the rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised and dried in hot air oven at 60 ℃ till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellet on 0 day (before start of experiment) from the weight of the cotton pellet on eighth day (at the end of experiment).

Acute ulcerogenic activity

The ulcerogenic potential of PSBE at three different doses (200, 500 and 1000mg/kg, p.o.) was tested in overnight fasted male rats. The control group was administered vehicle (1 ml/kg, p.o.), while the other group received standard drug, ASA (300 mg/kg, p.o.), respectively. All the animals were killed with anesthetic ether 5 h after the administration of test compounds. The stomachs were dissected out, incised along the greater curvature, and then put in diluted formaldehyde solution (2.5%). A few minutes later, mucosa of the stomach was observed for petechial hemorrhages and ulcers.

Chronic ulcerogenic activity

The experiment was carried out using male Wistar rats with free access to feed and drinking water throughout the period of experiment. The rats were administered vehicle (1 ml/kg, p.o.), PSBE (200, 500 and 1000 mg/kg, p.o.) and ASA (300 mg/kg. p.o.), once daily for 14 consecutive days. All the animals were sacrificed 24 h after the administration of the last dose of the drug and the stomachs were removed and examined as in the acute experiment[6,12].

Analgesic Activity

Acetic acid-induced writhing test

The prescreened animals were divided into groups as shown in (Table 3). Aspirin in doses suspended in 2% gam acacia was used as the standard drug. The drugs were autoclaved at $121^{O}C$ for 30 min and administered subcutaneously. Writhing was induced 30 min later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water[13] The number of writhes was counted for 30 min immediately after the acetic acid injection.

Tail flick method

The prescreened animals (reaction time: 3-4 sec) were divided into groups as shown in (Table 4). Pethidine 5 mg/kg acted as the standard drug. The drugs were administered intraperitoneally. The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage[14]. Acetylsalicylic acid is a well-known peripheral analgesic drug and was used as a positive control in the present investigation. The analgesic activity was calculated using the following formula:-

$$\% \text{ potential} = \frac{\text{Drug latancy (Test) - Base line lantency (Control)}}{\text{Base line latency (Control)}} \times 100$$

Statistical analysis

Results are expressed as mean ± S.E.M. statistical evaluations were made using ANOVA followed by t-test (Prism 3.0) and P values less than 0.05 were considered significant. Data are represented as mean ± S.E.M.

Result and Discussion

On all examination, all animals given PSBE at the doses of 3, 4.5, 6.75 and 10.125 g/kg, p.o. were devoid of toxic symptoms and mortality. The results of the present study suggest that the PSBE at doses of 200, 500 and 1000 mg/kg significantly suppressed carrageenan-induced paw edema in rats (Table 1). The results were found to be highly significant ($P<0.001$) in comparison to the control. The study of PSBE on proliferative phase of inflammation indicated that PSBE (200, 500 and 1000 mg/kg, p.o.) reduced the granuloma formation with percentage inhibition 8.48%, 16.47% and 21.23% as compared with ASA (300 mg/kg, p.o.), which showed inhibition on granuloma formation with the percent inhibition 43.95%. Significant analgesic activity in acetic acid-induced writhing (Table 3) and tail flick models (Table 4). The PSBE (200, 500 and 1000 mg/kg, s.c.) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner (Υ =0.99).

The anti-inflammatory effects of the extract on acute inflammatory process such as carrageenan-induced edema in rats paw was dose dependent[15]. At 500 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals was comparable to 1000 mg/kg of the extract. It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process[16]. Phytochemical screening of the methanolic extract shows the presence of flavonoids and saponins. Flavonoids act as an anti-inflammatory response in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins[17,18]. Further studies may reveal the mechanisms of action of *pongamia pinnata*.is responsible for its analgesic and anti-inflammatory activities.

Table 1: Anti-inflammatory effect of PSBE extracts on carrageenan-induced paw edema in rats

Groups	Dose orally (mg/kg, p.o.)	Change In Mean Paw Volume (ml)		Inhibition (%)	
		3h	4h	3h	4h
Control	---	0.88±0.20	0.91±0.24	----	-----
PSBE	200	0.54±0.03	0.42±0.02*	38.6%	53.8%
	500	0.42±0.12**	0.37±0.03**	52.3%	59.3%
	1000	0.36±0.02**	0.32±0.03**	60%	64.8%
ASA	300	0.21±0.01***	0.23±0.01**	76%	74.7%

n=6 in each group; Values are mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ significant from the control values.

Table 2: **Anti-inflammatory effect of PSBE on cotton pellet granuloma in rats**

Cotton pellet granuloma in rats			
Group	**Dose (mg/kg, s.c.)**	**Weight of cotton pellet granuloma(mg)**	**% of protection**
Control	----	109.67±2.21	–
PSBE	200	100.37±1.62	8.48%
	500	91.17±2.08*	16.87%
	1000	86.50±1.91**	21.23%
ASA	300	61.46±1.67***	43.95%

n=6 in each group; Values are mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ significant from the control values.

Table 3: Analgesic activity of PSBE on Acetic acid-induced writhing response in mice

Acetic acid-induced writhing response in mice			
Group	**Dose (mg/kg, s.c.)**	**No. of writhing movements**	**% of protection**
Control	----	56.8 ± 4.2	–
PSBE	200	34.5 ± 3.1**	33.39%
	500	32.5± 3.4**	37.25%
	1000	28.5 ± 2.8***	44.98%
ASA	100	20.2 ± 2.2***	61%

n=6 in each group; Values are mean ± SEM. **$p < 0.01$ significant from the control values, ***$p < 0.001$ significant from the control values.

Table 4: Analgesic activity of PSBE on tail flick response in rats

Group	**Drug dose mg/kg, p.o.**	**Dose Reaction time in seconds at time (h)**			
		30min	**1h**	**2h**	**3h**
Control	---	9.5±0.42	10.5±0.25	11.2±0.48	12.5±0.42
PSBE	200	11.6±0.49	10.9±0.38	11.4±0.61	12.7±0.66
	500	10.7±0.91	11.5±0.35	12.2±0.79	13.2±0.54
	1000	11.2±0.65	11.8±0.33	15.3±0.66**	18.8±0.54**
Pethidine	5	11.9±0.40*	12.2±0.35**	16.2±0.87**	20.2±0.70**

n= 6 in each group, each value is the mean ± S.E.M. *P< 0.05 compared to control, **P< 0.01 compared to control

Conclusion

The present study indicates the *pongamia pinnata* stem bark (PSBE) contained compounds, flavonoids and saponins. Its methanolic extract shows the most effective anti-inflammatory activity at doses of 100, 200 and 1000 mg/kg significantly throughout the observation period. The results shows in the present study provide evidence that the methanolic extract of *pongamia pinnata* stem bark possesses anti-inflammatory activity.

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