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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *PONGAMIA PINNATA* STEM BARK

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### Abstract

**Objective:** To evaluate analgesic and anti-inflammatory activities of aqueous extract of *Pongamia pinnata* stem bark (PSBA) in experimental animal models.

**Materials and Methods:** PSBA was evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema. The analgesic activity was tested by acetic acid-induced writhing response in albino mice and tail flick method in albino rats.

**Results:** The aqueous extract of *Pongamia pinnata* stem bark (PSBA) in doses of 300, 600 and 1000mg/kg showed 52.6, 54.3 and 56% inhibition of paw edema respectively at the end of three hours. The percentage of protection from writhing in acetic acid-induced writhing test was 47, 51 and 65.6 respectively. In the tail flick model, the aqueous extract of PSBA in the above doses increased the pain threshold significantly after 30 min, 1, 2 and 4h of administration. PSBA showed dose-dependent action of analgesic and anti-inflammatory activities in all the experimental animal models studied.

**Conclusion:** The present study indicates that PSBA has significant analgesic and anti-inflammatory properties.

**Key Words:** Carrageenan, writhing, tail flick, *Pongamia pinnata*.

### Introduction

Pain and inflammation are disabling accompaniments of many medical illnesses. It is the most common reason patients seek medical care. The control of both pain and inflammation assumes top priority for the physician.

Acute and Chronic inflammatory diseases are still one of the most important health problems in the World. Although inflammation is the unifying factor, it presents in various different forms, hence the treatment approach required for each of the inflammatory disease is unique.

Although several agents are known to treat painful inflammatory disorders, most common of them being NSAID's, their prolonged use often leads to gastrointestinal side effects ranging from dyspepsia to life threatening bleeding from ulceration. Upper gastrointestinal endoscopy studies have shown a 15-30% prevalence of ulcers in the stomach of patients taking NSAIDs regularly.<sup>1</sup> They are also one of the most common cause of Adverse Drug Reactions reported to drug regulatory agencies and are highlighted in many clinical and epidemiological studies.<sup>2</sup> Hence there is a continuous and ongoing research to identify more effective and safer agents in the therapy of pain and inflammation.

Therefore, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases. The added advantages of indigenous medicinal treatment would include its complementary nature to the conventional treatment making latter safer, well tolerated and economical remedy for inflammatory conditions.

Most people in rural areas of the World depend largely on herbs for the treatment of several ailments. This is because medicinal herbs constitute indispensable components of traditional medicine practice due to low cost, easy access and ancestral experience.

*Pongamia pinnata* (Family: Leguminosae) is a medium-sized, glabrous, semi-evergreen tree, growing up to 18 m or higher, with a short bole, spreading crown with grayish green or brown bark. Leaves are imparipinnate, alternate, and leaflets are 5-7 in number, ovate in shape and opposite in arrangement. This tree is popularly known as *Karanja* in Hindi, Indian Beech or *Derris indica* in English, and *Hongae* in Kannada. *P. pinnata* occurs all over India in the bank of rivers streams and planted as an avenue tree in gardens. The leaves of *P. pinnata* have been used in Ayurvedic medicine as digestive, laxative, anthelmintic, to cure piles, wound healing, relieving rheumatic pains, for cleaning ulcers in gonorrhoea and scrofulous enlargement. Previous studies have demonstrated that *P. pinnata* is rich in flavonoids and related compounds. Seeds and seed oil, flowers and stem bark yield karanjin, pongapin, pongaglabrone, kanugin, desmethoxykanugin and pinnatin.<sup>3</sup> Furanoflavonoid glucosides (pongamosides A-C) and flavonol glucoside (pongamoside D) have also been reported.<sup>4</sup> The crude extracts of this plant have shown various activities including antidiabetic<sup>5</sup>, antioxidant and anti-hyperammonemic<sup>6</sup>, antiulcer<sup>7</sup>, anti-diarrheal<sup>8</sup>, anti-plasmodial<sup>9</sup>, effects on various animal models. While different extracts of leaves, roots and seeds (petroleum ether, benzene, chloroform, acetone and ethanolic extracts) of *Pongamia pinnata* have been reported to have anti-inflammatory activity,<sup>10,11,12,13</sup> ethanolic extract of the leaves of *P. pinnata* has significant antibacterial activity against the tested bacteria *Vibrio* sp., *Pseudomonas* sp., and *Streptococcus* species<sup>14</sup>.

From the previous studies, Methanol extract of *Pongamia pinnata* stem bark showed significant anti-inflammatory and analgesic activity at the doses of 200, 500 and 1000 mg/kg, p.o.<sup>15</sup> However anti-inflammatory and analgesic studies on aqueous extract of the bark are sparse. Therefore the study was undertaken to evaluate the a) anti-inflammatory potential of aqueous extract of *Pongamia pinnata* stem bark (PSBA) on carrageenan- induced rat paw edema in albino rats b) analgesic activity using acetic acid-induced writhing test in albino mice and tail flick response in albino rats.

## Material and Methods:

**Preparation of extract:** The coarse powder of *Pongamia pinnata* stem bark was authenticated and procured from Mysore University. About 50g of the powder was subjected to Soxhlet extraction for 12 hrs using distilled water as solvent. The extract of PSBA was administered as a suspension in 2% gum acacia to the animals.

**Chemicals:** Pethidine, aspirin, acetic acid (Ranbaxy laboratories Ltd), Carrageenan (Sigma-Aldrich), and all other chemicals were of analytical grade.

**Acute toxicity study:** the animals were divided into different doses and treated with increasing doses of the aqueous extract: 3, 4, 5 g/kg. All the treated animals were observed for any abnormal or toxic manifestations and for mortality. Based on the preliminary toxicity study, the doses for our further study were taken as 300, 600 and 1000mg/kg.

**Animals:** Adult albino rats of either sex weighing between 150 to 250 grams will be randomly selected from Central animal facility, J S S Medical College, Mysore. Animals will be housed in 5 groups of 6 each, at an ambient temperature of 25±1°C with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee. The animals will be fasted overnight just prior to the experiment but allowed free access to drinking water.

## Anti-inflammatory model:

### Carrageenan induced rat paw edema Animal Model:<sup>16,17</sup>

The animals were divided into groups as shown in Table 1. The animals were pretreated with drugs orally 1 hr before the experiment. 0.05 ml of 1% carrageenan was injected aseptically into the subplantar surface of right hind paw of each rat. Paw edema was measured by Plethysmometrically (Ugo Basile, Italy) at '0' hour and at the end of '4' hours. The difference between the zero and 4 hours gives the actual edema. Percentage inhibition (protection) against edema formation was taken as an index of acute anti-inflammatory activity.

**Table 1: Effect of aqueous extract of *pongamia pinnata* stem bark (PSBA) on Carrageenan induced rat paw edema in Albino rats.**

<i>Group</i>	<i>Increase in paw volume (in ml)</i>	<i>Percent inhibition</i>
Control	0.66± 0.14	--
PSBA- 300mg/kg	0.326± 0.08*	51.5
PSBA- 600mg/kg	0.301± 0.05*	54.5
PSBA- 1000mg/kg	0.289± 0.03**	56.2
Aspirin- 100mg/kg	0.250± 0.04**	62

n=6 in each group, values expressed in mean ± SEM, \* $p < 0.05$ , \*\* $p < 0.01$  and compared to control.

It was calculated by: **The percent inhibition of edema = 100 x (1-  $V_t / V_c$ )**

Where,  $V_c$  = mean paw edema volume in the control group.

$V_t$  = mean paw edema volume in the drug treated group.

### **Analgesic models:**

#### **1) Tail flick method:**

The prescreened animals (reaction time: 3-4 sec) were divided into groups as shown in (Table 3). 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.<sup>18</sup>

The analgesic activity was calculated using the following formula:-

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

#### **2) Writhing Method:**

This is one of the most commonly used methods for measuring peripheral analgesic activity of a drug.

#### **Principle:**

In this method intraperitoneal (i.p) administration of noxious chemical substances to mice produce peritoneal irritation, this elicits writhing response. Each episode of writhing is characterized by internal rotation of feet, sucking

of belly, elongation of body, arching of the back, rolling on one side and remaining still of turning around and circling cage. Acetic acid most commonly used irritant.

### Acetic acid-induced writhing test: <sup>19</sup>

In this method, mice were divided in 3 groups of six each. The animals will be pretreated with drugs 30min prior to induction of writhing. Writhing is induced by administration of 0.1ml of a 0.6% acetic acid solution i.p into mice.

The mice are placed individually into glass beakers and five min are allowed to elapse. The mice are then observed for 20 min and the number of writhes is recorded per animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Percentage protection against abdominal constriction was taken as an index of analgesia.

It will be calculated as:

$$\frac{\text{Number of writhing in control group} - \text{Number of writhing in treated group}}{\text{Number of writhing in control group}} \times 100$$

### Statistical analysis:

The results will be analyzed by calculating the Mean values, Standard deviation and one way analysis of variance (ANOVA). Post-hoc comparisons were performed by applying Scheffe' test. The values will be compared at 0.05 level of significance to test the results of the study for the corresponding degrees of freedom.  $P < 0.05$  will be considered as significant.

### Results:

The results of the animal experiments are shown in tables-1, 2 & 3. In the acute inflammation model, the aqueous extract of *Pongamia pinnata* (PSBA) in the doses of 300, 600 and 1000mg/kg, p.o produced a dose-dependent inhibition of paw oedema. The test and the standard drugs produced significant inhibition of paw oedema as compared to the control. PSBA (300, 600 and 1000mg/kg, p.o) reduced the paw oedema with the percent inhibition of 52.6, 54.4, 56 % as compared with Aspirin which showed percent inhibition of 63% (Table-1).

The PSBA extract (300, 600 and 1000mg/kg, p.o) in a dose- dependent manner and the standard drug, Aspirin suppressed acetic acid-induced writhing significantly. The results were found to be highly significant ( $P < 0.01$ ) in comparison to the control.

**Table 2: Effect of aqueous extract of *pongamia pinnata* stem bark (PSBA) on Acetic acid- induced writhing response in Albino mice.**

Group	No. of writhing movements	Percent of protection
Control	57.17± 1.95	--
PSBA- 300mg/kg	33.180± 3.18*	36.4
PSBA- 600mg/kg	27.545± 6.23**	47.2
PSBA- 1000mg/kg	25.563±3.78**	51
Aspirin- 100mg/kg	17.946± 6.47***	65.6

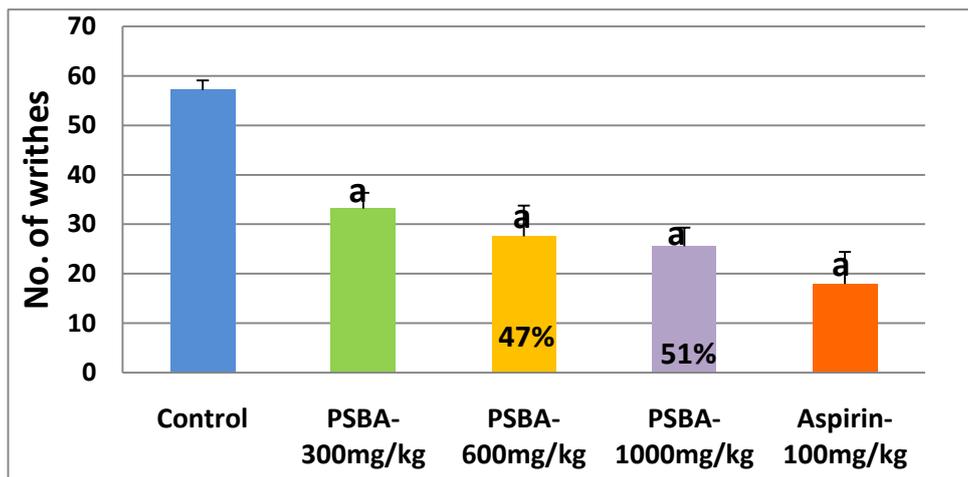
n=6 in each group, values expressed in mean ± SEM, \*P< 0.05, \*\*P< 0.01, \*\*\*P< 0.001 compared to control.

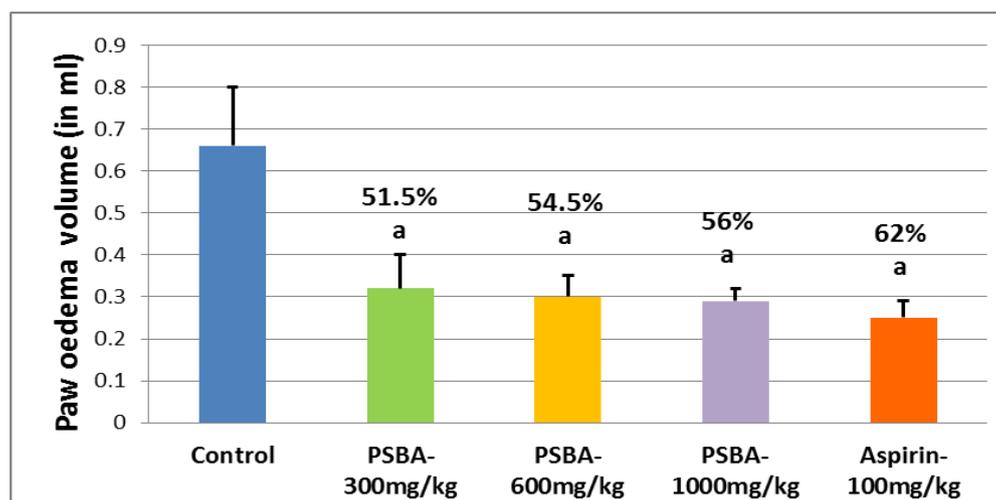
**Table-3: Effect of aqueous extract of *pongamia pinnata* stem bark (PSBA) on Tail flick response in Albino rats.**

Groups	Pre-drug reaction time (in secs)	Reaction time (in secs)			
		30 mins	1 hr	2hr	4hr
Control	3.2± 0.31	4.24± 0.6	4.22± 0.4	4.34± 0.5	4.22± 0.7
PSBA- 300mg/kg	3.66± 0.26	5.15± 0.2*	6.26± 0.9*	6.1± 0.7*	6.8± 0.8*
PSBA- 600mg/kg	3.72± 0.30	7.20± 0.3*	7.20± 0.7*	7.4± 0.8*	7.6± 0.8*
PSBA- 1000mg/kg	3.55 ± 0.22	7.04± 0.8*	8.12± 0.3*	8.2± 0.3*	8.05± 0.6*
Pethidine- 5mg/kg i.p	3.62± 0.19	8.11± 0.5*	8.37± 0.3*	8.32± 0.4*	8.1± 0.9*

n=6 in each group, values expressed in mean ± SEM, \*P< 0.05 compared to control.

In the tail flick model, there was no significant difference in the mean pre-drug reaction time between the different groups. Thirty min after drug administration, the reaction time increased significantly for the test and the standard groups when compared to the predrug reaction time. The test drug produced a dose-dependent increase in the reaction time at various intervals of observation. Preliminary phytochemical analysis of the PSBA extract revealed the presence of flavonoid compounds.

**Figure 1: Effect of PSBA (300, 600, 1000mg/kg) on acetic acid- induced abdominal constriction in mice.**

**Figure 2: Effect of PSBA (300, 600, 1000mg/kg) on Carrageenan- induced rat paw oedema.****Discussion:**

Carrageenan-induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experiment model exhibits a high degree of reproducibility.<sup>18</sup> Carrageenan induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas second phase is related to the release of prostaglandins and slow reacting substances which peak at 3 hr.<sup>21</sup> PSBA produced a dose-dependent inhibition of Carrageenan-induced paw oedema. The inhibition was however, less than that of the standard drug, aspirin. The anti-inflammatory activity in the present study is in concurrence with the previous study done with the methanolic extract of pongamia pinnata stem bark.<sup>22</sup>

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The pain response is generated indirectly via endogenous mediators like prostaglandins, which stimulates local peritoneal receptors. In the present study, PSBA significantly inhibited the acetic acid-induced pain response. The tail flick method has been found to be suitable for evaluation of centrally acting analgesics. In the tail flick model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of higher centers. However, the analgesic activity of PSBA was found to be more significant on acetic acid-induced model ( $P < 0.001$ ) than the tail flick model ( $P < 0.01$ ) and thus appears that the test drug inhibits predominantly the peripheral pain mechanism.

On preliminary phytochemical screening the aqueous extract of PSBA was found to contain flavonoid compounds. Flavonoids are known to target the enzymes in the synthesis of prostaglandins which are involved in late phase of

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acute inflammation and pain perception.<sup>23</sup> Hence, it may be concluded that PSBA possesses anti-inflammatory and analgesic activities that may be mediated by the presence of flavonoid compounds. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory and analgesic activities of PSBA.

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