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RESEARCH ARTICLE

ANTI -INFLAMMATORY AND ACUTE TOXICITY EFFECTS OF *PITTOSPORUM TETRASPERMUM* WIGHT AND ARN. ON RATS

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Abstract

The anti-inflammatory effect of *Pittosporum tetraspermum* was studied in carrageenan – induced oedema in albino rats. Among the plant parts tested the methanol extract of root bark and stem bark exhitbited significant (P<0.01) oedema suppressant activity. The potency of the extract of root bark was superior to the reference drug phenylbutazone. The result suggests that the bark of *P. tetraspermum* has potential anti-inflammatory activity that supports its use in folk-therapy for inflammatory disorders. The acute toxicity study proved that the crude extract of the bark was non-toxic up to 2g/kg per oral (p.o.).

Key words: Anti-inflammatory, Carrageenan, Acute-toxicity, Induced oedema
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Introduction

Pittosporum tetraspermum Wight and Arn. belongs to the family Pittosporaceae of the order Polygalineae. It is a small evergreen tree, growing up to 12 m height, found in the hills of South India. The bark is thin and light greenish grey coloured. Leaves are elliptic, subcoriaceous and glabrous. The leaf base is cuneate and the apex is acute. The petiole is 1.5 to 2 cm in length and the flowers are in corymbose. The capsule is ovoid, containing four globose, red seeds covered with a resinous viscid fluid. Bark is bitter and aromatic. When freshly cut, it emits a ginger like smell. It is an expectorant and possesses febrifuge and narcotic properties and is used to cure chronic bronchitis, leprosy and skin diseases. It is used as an antidote for snake poisoning. The paste of the root bark is applied to inflammatory dropsical and rheumatoid swelling.

Producing carrageenan – induced inflammation in the rat hind paw (acute inflammation) is a useful method for screening potential anti-inflammatory agents (Billingham and Davies, 1979; Flower *et al*., 1985). A wide variety of plants with potent anti-inflammatory activity are used in folklore medicines. The search for safe and effective anti-inflammatory drugs through the evaluation of medicinal plants known to be used in the treatment of inflammation disorder is continued even today. Hence an attempt is taken to evaluate the efficacy of *P. tetraspermum* as an anti-inflammatory agent in traditional healing system. In order to assess the tolerant capacity of the test organism an acute toxicity test was also conducted in the present investigation.

Methodology

P. tetraspermum was collected from Southern Western Ghats in Idukki District in Kerala, India, in 1997.The plant was identified using standard Flora and confirmed by the Botanical Survey of India, Coimbatore. The different parts of the plant (bark of stem and root, and leaves) were collected in sterile polyethylene bags and brought to the laboratory. The plant materials were washed with distilled water to remove the adhering dust particles. They were shade dried at room temperature (29 ± 2°C).

Extracts were prepared from shade dried and ground samples of the plant parts collected. About100 g powdered plant material from each sample was subjected to

successive extraction using n-hexane and methanol (3 times with 500 ml of each solvent). After extraction with nhexane, the powdered plant material was air dried overnight at room temperature for complete evaporation of extracting solvent before subsequent extraction with methanol. The individual extracts were collected, filtered and concentrated using rotary evaporator under reduced pressure at 40°C to obtain a crude extract which was collected in sterile container and stored at 4°C. Only methanol extract was used in the present study.

About 4-6 weeks old albino rats of either sex were procured from Tamil Nadu Veterinary University and Animal sciences, Madavaram milk colony, Chennai, India. Groups of six animals were kept in each cage measuring about 45 x 30 cm size, supplied by Tarson Private Limited, Mumbai, India. The cages were maintained in hygienic condition. Paddy husk was placed at the bottom of the cage to prevent infection and regularly replaced with fresh husk. The animals were fed with a standard Laboratory diet (Godrej Agro Food Industries, Bangalore, India) and tap water.

In the present investigation anti–inflammatory activity of *P. tetraspermum* was tested against carrageenan – induced paw oedema in rats (Winter *et al.* 1962). Wistar rats of either sex weighing between 100 – 150 g were used. The test groups were administered with methanol extracts of the leaf, stem bark and root bark of *P. tetraspermum* at the dose level of 100, 250 and 500 mg/kg body weight. Suspensions were made in 2% w/v gum acacia. All the test substances were administered orally by gavage. The administration of the plant extract test dose was started 5 days prior to the day of experimentation.The control group received 0.1 ml of normal saline and 1 ml of 2% gum acacia. The reference control group was given the reference drug phenylbutazone (80 mg/kg) (HiMedia, Mumbai, India). Oedema in the right hind paw of rat was induced by injecting 0.1ml of 1% (w/v) carrageenan (HiMedia, Mumbai, India) in saline (0.9%) into the footpad subcutanceously, after 1 hr of the administration of crude drug.

Dorso – ventral measurements of rat hind footpad (paw diameter) were measured using a sliding vernier caliper before and at 0.5, 3 and 5 hr after the carrageenan injection. The anti – inflammatory effect of the drug was evaluated as the degree of oedema inhibition. Inhibition of inflammation was calculated according to the formula :

$$\text{Percentage inhibition} = \left(1 - \frac{a-x}{b-y}\right) \times 100$$

where 'x' and 'a' are the mean paw diameter of the rats before and after the administration of carrageenan in the test or reference control group, whereas 'y' and 'b' are the mean paw diameter of rats before and after the administration of carrageenan in the control group (Al-Harbi *et al.,* 1994).
The results were expressed as the Mean ± Standard Deviation (SD) of six replicates (n = 6) and the data analyzed by student's t-test for significance of difference.
Acute toxicity and effects on gross behaviour (Singh *et al.,* 1984) were performed on groups of rats of either sex whose body weights ranged between 90 and 150g. The rats were maintained on a standard balanced diet and water *ad libitum*. Before the experiment they were made to fast overnight with water *ad libitum*. The rats were divided into two sets of control and test groups. The test groups on the first set received the different test suspensions of the root bark of *P. tetraspermum* in doses of 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, 7.5 and 10.0 g/kg body weight. Similarly the test groups in the second set, received the different test suspensions of methanol extract of the stem bark. Test substances were administered orally by gavage in 2% w/v gum acacia (2 ml/kg). An equivalent dose of the vehicle (gum acacia) was administered to the control groups. The animals were observed for apparent changes in general behaviour and for the assessment of mortality, for the next 24 hr, under normal environmental conditions, with free access to food and water.

Experiments on animals were performed in the Entomology Department, Loyola

College, Chennai in accordance with the guidelines and regulations set forth by Animal Welfare Board, Government of India and in accordance with the Internationally Accepted Principles for Laboratory Animal Use and Care.

Results and Discussion

The methanol extracts of plant parts (bark of stem and root and leaf) of *P. tetraspermum* showed anti-inflammatory activity on carrageenan-induced oedema in rats. Carrageenan increased the paw diameter in the control as well as in the treated animals. The rat's footpad became edematous soon after the injection of carrageenan. The pre-treatment with plant extracts resulted in a significant ($p<0.01$) dose dependent reduction of inflammation in carrageenan-induced paw oedema in rats. The inhibitory effect of *P. tetraspermum* plant parts and phenylbutazone at 5 hr after carrageenan-induced oedema is shown in (Fig. 1). Of the three plant parts tested the methanol extracts of root bark (100, 250 and 500 mg/kg) exhibited the highest oedema suppressant activity at all the concentrations tested (Table 1

Fig. 1. Inhibitory effect of *P. tetraspermum* plant parts and phenylbutazone, 5 hr after carrageenan-induced hind paw oedema in rats

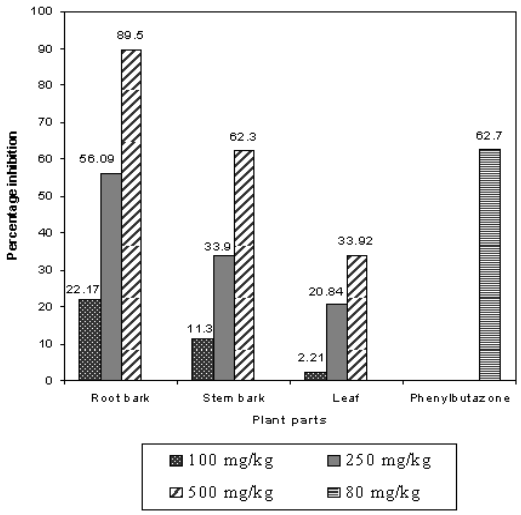
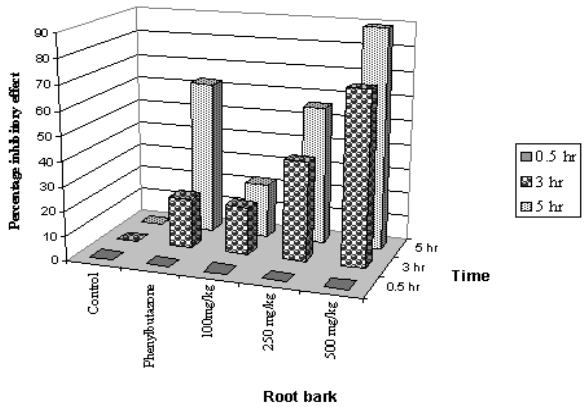


Table 1. Effect of *P. tetraspermum* root bark extract and phenylbutazone on carrageenan - induced rat hind paw oedema.

Treatment	Dose Mg/kg p.o	Paw diameter (mm) at different time intervals		
		0.5 hr	3 hr	5 hr
Control	Vehicle	7.05 ± 0.28	9.03 ± 0.28	8.54 ± 0.35
Phenylbutazone	80	7.07 ± 0.08	8.05 ± 0.06*	5.75 ± 0.04*
Root bark	100	7.05 ± 0.10	8.08 ± 0.12*	7.56 ± 0.14*
	250	8.05 ± 011	8.05 ± 0.11*	7.03 ± 0.09*
	500	7.55 ± 0.33	6.05 ± 0.27*	5.02 ± 0.24*

*significant at P<0.01

Fig. 2. Inhibitory effect of *P. tetraspermum* root bark and phenylbutazone against swelling induced by carrageenan



The percentage inhibition of inflammation after 3 hr and 5 hr of carrageenan injection was 70.2% and 89.5% respectively at 500 mg/kg dose level (Fig. 2& 3). At lower doses of 250 and 100 mg/kg, the percentage inhibition was 40% and 56.09% and 19.34% and 22.17% respectively after 3 hr and 5 hr of carrageenan injection. The methanol extract of root bark showed more potency as an anti-inflammatory agent than the reference drug phenylbutazone (80 mg/kg) that showed 62.7% inhibition after 5 hr of carrageenan injection.

The methanol extract of stem bark displayed dose dependent and significant ($p<0.01$) oedema suppressant activity (Table 2)

Fig. 3 The effect of *P. tetraspermum* root bark (500mg/kg p.o.) on carrageenan – induced rat hind paw oedema at 0.5 hr , 3 hr and 5 hr after carrageenan injection

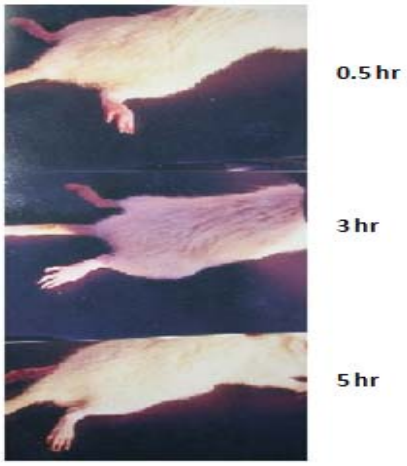
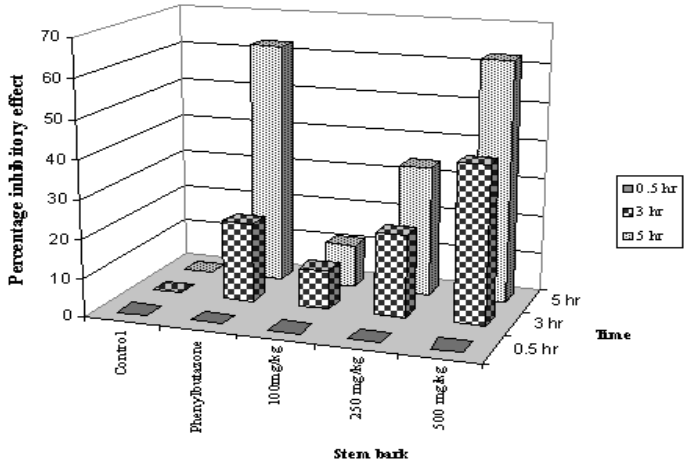


Table 2. Effect of *P. tetraspermum* stem bark extract and phenylbutazone on carrageenan - induced rat hind paw oedema.

Treatment	Dose mg/kg p.o.	Paw diameter (mm) at different time intervals		
		0.5 hr	3 hr	5 hr
Control	vehicle	7.05 ± 0.28	9.03 ± 0.28	8.54 ± 0.35
Phenylbutazone	80	7.07 ± 0.08	8.05 ± 0.06*	5.75 ± 0.04*
Stem bark	100	7.55 ± 0.33	9.05 ± 0.33*	8.55 ± 0.33*
	250	7.55 ± 0.37	7.52 ± 0.38*	6.53 ± 0.40*
	500	7.05 ± 0.16	7.02 ± 0.12*	5.75 ± 0.15*

*significant at P<0.01

Fig. 4. Inhibitory effect of *P. tetraspermum* stem bark and phenylbutazone against swelling induced by carrageenan



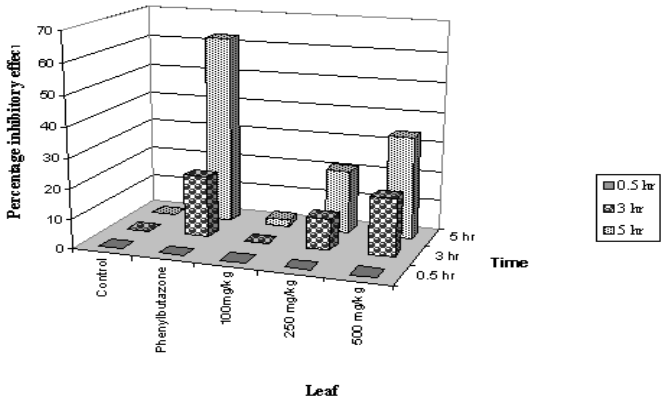
The leaf extract presented relatively low activity profile than the root bark and stem bark extracts (Table3). The methanol extract of leaf (100, 250 and 500 mg/kg) inhibited carrageenan – induced oedema by 2.21%, 20.84% and 33.92% respectively after 5 hr(Fig.5).

Table 3. Effect of *P. tetraspermum* leaf extract and phenylbutazone on carrageenan - induced rat hind paw oedema.

Treatment	Dose mg/kg p.o.	Paw diameter (mm) at different time intervals		
		0.5 hr	3 hr	5 hr
Control	vehicle	7.05 ± 0.28	9.03 ± 0.28	8.54 ± 0.35
Phenylbutazone	80	7.07 ± 0.08	8.05 ± 0.06**	5.75 ± 0.04**
Leaf	100	6.52 ± 0.37	9.55 ± 0.37	9.04 ± 0.38
	250	7.08 ± 0.17	8.55 ± 0.18*	7.65 ± 0.19**
	500	7.53 ± 0.04	8.54 ± 0.05*	7.51 ± 0.12**

* significant at P<0.05 and ** significant at P<0.01

Fig. 5. Inhibitory effect of *P. tetraspermum* leaf and phenylbutazone against swelling induced by carrageenan



From the results obtained it is evident that the methanol extracts of the root bark and stem bark showed significant anti-inflammatory activity (P≤ 0.01 and it is greater than or equal to that shown by the reference drug phenylbutazone. The above observation is in accordance with the anti - inflammatory activity of root bark of *Alstonia boonei* and *Rauvolfia vomitoria* on carrageenan-induced oedema where the extracts had no significant effect on the early phase of carrageenan-induced oedema but significantly reduced the late phase (Kweifio-Okai, 1991).

It was also observed that the percentage inhibition exhibited by different plant part extracts increased with increase in time interval and was found to be maximum at 5hr after carrageenan injection. This is in conformity with earlier reports (Kaith *et al*., 1996; Fernandez *et al*., 1997).

Acute toxicity and effect on gross behaviour

Methanol extracts of the root bark and stem bark of *P. tetraspermum* proved to be lethal at higher doses. Cent percent mortality was observed at the dose level of 5.0, 7.5, and 10 g/kg at the end of 24hr. Only 33.3% of the rats from the first set and 50% of the rats from the second set in the fifth test group (dose level of 3 g/kg) survived the observation period (24 hr) while the rest died. Oral administration of doses of root bark and stem bark upto 2.0 g/kg did not show any toxic symptoms in rats (Tables 4 and 5). In higher doses it was found to exert some depressant effect. It induced decrease in locomotor activity, consumption of food and water and passivity leading to death.

Table 4. Percentage mortality of rats after treatment with *P. tetraspermum* root bark

		n=6
Treatment	**% Mortality**	**% Survival**
Control	-	100
0.1g/kg	-	100
0.25 g/kg	-	100
0.5 g/kg	-	100
1.0 g/kg	-	100
2.0 g.kg	-	100
3.0 g/kg	66.6	33.3
5.0 g/kg	100	-
7.5 g/kg	100	-
10.0 g/kg	100	-

Table 5. Percentage mortality of rats after treatment with *P.tetraspermum* stem bark

		n=6
Treatment	**% Mortality**	**% Survival**
Control	-	100
0.1g/kg	-	100
0.25 g/kg	-	100
0.5 g/kg	-	100
1.0 g/kg	-	100
2.0 g.kg	-	100
3.0 g/kg	50	50
5.0 g/kg	100	-
7.5 g/kg	100	-
10.0 g/kg	100	-

The methanol extracts of root bark and stem bark of *P. tetraspermum* did not show any change in gross behaviour or toxic symptoms upto 2 g/kg p.o. The acute oral toxicity of the crude methanol extract of root and stem barks of *P. tetraspermum* on evaluation showed non–toxic effect upto 2 g/kg p.o. But in higher doses the extracts were found to exert depressant effect and in still higher doses they proved lethal. The result of the acute toxicity study showed that the methanol extracts of *P. tetraspermum* exhibited low toxicity and the oral administration of the extracts upto 2 g/kg p.o. concentration was devoid of toxic symptoms.

Conclusion

The anti-inflammatory effects of *P. tetraspermum* suggests that the use of this plant in folklore therapy may be justified. Among the plant parts tested the crude methanol extract of root bark and stem bark exhibited significant odema suppressant activity. The effect of root bark was superior to the reference drug phenylbutazone. The inhibitory activity shown by the herbal drug is an added information which may be of use in the established systems of traditional medicines in primary health care. The crude extract of the bark is found to be non-toxic upto 2g/kg p.o. The isolation and identification of the bioactive compound is in progress.

Acknowledgement

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