ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY OF THE FLOWERS OF *Pterospermum acerifolium*

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Summary

A methanol extract of the shade dried flowers of *Pterospermum acerifolium* (MEPA) was investigated for anti-inflammatory and antinociceptive activities at the doses (p.o.) of 200 and 400 mg/kg body weight. In acute oral toxicity studies (OECD-425 guidelines), no mortality was observed up to the highest dose of MEPA (2000 mg/kg, p.o). The extract produced a dose dependent inhibition of carrageenan – induced paw oedema and cotton pellet – induced granuloma in rats. At the same doses, antinociceptive potential was also observed with formalin - induced paw licking, acetic acid induced writhing and tail immersion models in mice. Further more, the phytochemical studies indicated that MEPA contains alkaloids, saponins, tannins, glycosides, phenols, flavonoids and oils. The results of the present study confirm the use of *Pterospermum acerifolium* traditionally as a remedy for pain and inflammation.

Keywords: *Pterospermum acerifolium,* anti-inflammatory, antinociceptive.

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Introduction

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) commonly known as "Dinner plate tree" (English) and "Muchukunda" (Hindi), is a large deciduous tree of about 24 m height and 2.5m girth. Flowers are large 12-15 cm in diameter, axcillary, solitary or in pairs. It is widely distributed in North Canada and in many parts India i.e. river banks of sub-Himalayan tracts, Dehradun, West Bengal, Assam and Manipur (1-2). In traditional system of medicine, the flowers are used as a general tonic, anti tumor agent, analgesic and for the treatment of diabetes, gastrointestinal disorders, leprosy, blood troubles, bronchitis, cough, cephalic pain, migraine and inflammation. The leaves are used as haemostatic and antimicrobial agent (3-6). In the light of the above findings, the present study was investigated to know the potential of methanol extract of *Pterospermum acerifolium* flowers (MEPA) as an anti-inflammatory and antinociceptive agent in experimental animal models.

Materials and Methods

Plant Material

The flowers of *Pterospermum acerifolium* (L) Willd were collected from the campus of Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India. The plant was identified and authenticated by, National Herbarium*, Botanical Survey of India*, Shibpur, Howrah, West Bengal, India. The voucher specimen was submitted in the Department of Pharmaceutical Sciences, B.I.T., Mesra, Ranchi under the voucher specimen no. CNH/I-I(36)/2006/Tech.II/638. The flowers were washed thoroughly with tap water and air dried in shade at room temperature.

Preparation of the Extract

The flowers were dried under shade, made into coarse powder and passed through 40 mesh sieve. The powdered flowers (1.5 Kg) were extracted with methanol using a Soxhlet extractor. After exhaustive extraction, the methanol was filtered and concentrated by distillation process. A brownish-black colored residue was obtained (yield 12.4% w/w), which was kept in a vacuum desiccator. The suspension of the extract was prepared in 2% v/v Tween 80 and was used for the entire experimental studies.

Animals

All the experiments were carried out using Swiss albino mice (25-30 g) and Wistar albino rats (150-200 g) which were obtained from the animal house of Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India. They were kept in polypropylene cages with paddy husk as bedding. The animals were housed at a temperature of $24^0C \pm 2^0C$ and relative humidity of 60 %– 70 %. A 10:14 light: day cycle was followed. All the animals were allowed free access to water and fed with standard commercial rat chow pellets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Reg. No: 621/02/ac/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 chemicals used were Carrageenan (SD Fine Chemicals Limited, Mumbai), Formaldehyde solution (37 - 41% w/v) (Universal Lab. Ltd., Mumbai), Methanol (BDH, Mumbai), Pentazocine (Pure Pharma Ltd., Mumbai), Indomethacin (M/s Jagsonpaul Pharmaceuticals Ltd., Faridabad).

Preliminary Chemical Tests

Phytochemical properties of the extract were tested using the following chemicals and reagents (7). Alkaloids with Mayer's and Dragendorff's reagents, saponins (frothing test), tannins ($FeCl_3$), glycosides (NaCl and Fehling's solution A and B), cardiac glycosides (Salkowski test), flavonoids (NaCl and HCl), anthraquinones (Borntrager's reaction) phenols ($FeCl_3$ and $K_3Fe(CN)_6$), and lipids (filter paper).

Acute Toxicity Study

Acute oral toxicity was performed in mice by following Organization for Economic Cooperation and Development (OECD) guidelines AOT No 425 [8].

Anti-inflammatory Activity

Carrageenan-induced paw oedema test

Four groups of six animals per group were used for the study. Group 1 served as control and received distilled water and Group 2 received the standard drug indomethacin orally at a dose of 10 mg/kg body weight. The plant extract was administered orally at doses 200 and 400 mg/kg to groups 3 and 4 respectively. The administration of extract and drugs was 30 min prior to injection of 0.05 ml, 1% carrageenan (9) in the right hindpaw subplantar of each rat. The paw volume was measured by vernier calliper. Prior to injection of carrageenan, the average volume ($V0$) of the right hindpaw of each rat was calculated from 3 readings which did not deviate more than 4%. At 0, 1, 2, 3, 4 and 5 h after injection of the phlogistic agent, only one reading was obtained for each rat (Vt). The percentage inhibition for each rat and each group was obtained as follows:

$$\text{Percentage inhibition} = \frac{(V_t - V_0)\text{Control} - (V_t - V_0)\text{treated}}{(V_t - V_0)\text{control}} \times 100$$

Cotton Pellet – induced Granuloma

The cotton pellet - induced granuloma in rats was studied according to the method of D' Arcy et al (10). The animals were divided into four groups with 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, 10 ml/kg). Group 2 received the standard drug indomethacin (10 mg/ kg) orally for the same period. The MEPA extract at the concentration of 200 and 400 mg/ kg was administered orally to groups 3 and 4, respectively for seven consecutive days from the day of cotton pellet implantation. On the 8^{th} 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 60^0C 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 MEPA extract was compared with control.

Anti-nociceptive Activity

Acetic acid induced writhing reflex

This study was performed according to the method of Gaertner et al. (11). Mice (four groups with six per group) were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. Thirty minutes prior to treatment with acetic acid, the control group received distilled water (p.o.), standard group received the standard drug pentazocine (10mg/kg) and the two test groups received the extract MEPA at 200 and 400 mg/kg, p.o. respectively. The writhing induced by the acid, consisting of abdominal constrictions and hind limbs stretching, were counted for 30 min after a latency period of 5 min. The percentage analgesic activity was calculated as follows:

Percentage analgesic activity =

$$\frac{N - N_I}{N} \times 100$$

Where N is the average number of stretching of control per group. N_I is the average number of stretching of test per group.

Formalin-induced pain

The procedure described by Santos et al. (12) was used but with slight modifications. Pain was induced by injecting 0.05 ml of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar region of the right hind paw. Rats were divided into four groups with six per group. Thirty minutes prior to injecting formalin, Group 1 received distilled water (p.o.) and served as control group. The standard drug pentazocine 10mg/kg was administered to Group 2. Groups 3 and 4 were given MEPA extract 200 and 400 mg/kg, p.o. respectively. These rats were individually placed in a transparent glass cage (25 cm × 15 cm × 15 cm) observation chamber. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15–30 min (second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively (13).

Tail immersion test

Tail immersion study was conducted as described by Aydin et al. (14). Rats (six per group) were used. This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55 ± 0.5^0C. Within a few minutes, the animal reacts by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0- and 10-min interval. The average of the two values was the initial reaction time (T_b). The control Group 1 received distilled water (p.o.), group 2 served as the standard group and received the drug Pentazocine (10mg/kg) p.o., the test groups 3 and 4 were given MEPA extract at doses of 200 and 400 mg/kg, p.o. The reaction time (T_a) for the test groups was taken at intervals 0.5, 1, 2, 4 and 6 h after a latency period of 30 min following the administration of the extract and standard (15). The cut-off time, i.e. time of no response was put at 120 s. The following calculation was:

Percentage analgesic activity =

$$\frac{T_a - T_b}{T_b} \times 100$$

Results

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the methanol extract of the flowers of *Pterospermum acerifolium* revealed the presence of alkaloids, saponins, tannins, glycosides, phenols flavonoids and oils whereas cardiac glycosides, and anthraquinones were absent.

Acute Oral Toxicity Study

In the acute toxicity study, methanol extract of the flowers of *Pterospermum acerifolium* did not produce any mortality even at the highest tested dose 2000 mg/kg, p.o. during the 24 hour period employed and also the animals showed no stereotypical symptoms associated with toxicity. Thus the two doses (200 and 400 mg/kg, p.o.) of MEPA were selected for further pharmacological studies.

Carrageenan-induced paw oedema

The average right back paw volumes and percentages of oedema are presented in Table 1. The percentages of inhibition are reported in Table 2. For the control group, the injection of the phlogistic agent caused localised oedema, 30 min later. The swelling increased progressively after 4 h to a maximum volume of 0.727±0.03 (73.09%). Rats pretreated with MEPA significantly decreased the carrageenan-induced oedema 30 min post-dosing beginning with 200 mg/kg and in a dose related manner. At 200 mg/kg, the extract showed significant inhibition of oedema formation after 3 h (35.86%). At 400 mg/kg, the extract achieved its maximal inhibitory effect (61.50%).

Cotton pellet –Induced Granuloma

The anti-inflammatory effect of MEPA 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.) significantly reduced ($P < 0.01$) the formation of granuloma by indicating the significant reduction in wet and dry weight of cotton pellets. The results are shown in Table 3.

Acetic acid induced writhing reflex

Pterospermum acerifolium significantly reduced writhings and stretchings induced by 0.6%w/v acetic acid at a dose of 10 ml/kg as shown in Table 4. The significant protective effect was dose dependent with 19.89% reduction ($P < 0.01$) observed for 200 mg/kg and 33.51% reduction ($P < 0.01$) seen for 400 mg/kg dose of MEPA.

Formalin-induced pain

The extract had analgesic effects on both first (0–5 min) and second phases (15–30 min) of formalin test as tabulated in Table 5. These phases corresponded to

neurogenic and inflammatory pains, respectively. Its neurogenic - induced pain blockade, started at 200 mg/kg (2.82%) but its effect is significant at 400mg/kg (9.78%). The extract was found to inhibit the inflammatory pain ($P < 0.01$) better than the neurogenic pain. Indomethacin was more significantly active (34.09%) on the second phase.

Tail immersion test

After a latency period of 2h following oral administration of the extract at a dose of 200 mg/kg (37.69%, $P < 0.01$), there was a significant reduction of painful sensation due to tail immersion in warm water and it was dose dependent, as shown in Table 6. The inhibitory effects of the extract became pronounced between 1 and 4h post-dosing and reached a maximum of 84.75% ($P < 0.01$) with the dose of 400 mg/kg. The antinociceptive property of the extract at 400 mg/kg was not as effective as that of pentazocine.

Table. 1. Anti-inflammatory effect of methanolic extract of *Pterospermum acerifolium* flowers on Carrageenan-induced paw oedema

S.No.	Treatment	Drug (mg/kg)	Paw oedema volume					
			0h	1h	2h	3h	4h	5h
1.	Control (distilled water)	10ml/kg	0.42±0.003	0.527±0.008 (25.48)	0.62±0.006 (47.62)	0.71±0.006 (69.05)	0.727±0.03 (73.09)	0.67±0.005 (59.52)
2.	Indomethacin	10mg/kg	0.383±0.003	0.413±0.003 (7.83)**	0.433±0.007 (13.05)**	0.457±0.006 (19.32)**	0.453±0.003 (18.28)**	0.48±.006 (25.32)**
3.	MEPA	200 mg/kg	0.397±0.009	0.493±0.007 (24.18)**	0.543±0.006 (36.77)**	0.583±0.007 (46.85)*	0.630±0.005 (58.69)*	0.580±0.005 (46.09)*
4.	MEPA	400mg/kg	0.38±0.005	0.427±0.012 (12.36)**	0.457±0.009 (20.26)**	0.5±0.010 (31.58)**	0.523±0.008 (37.63)**	0.52±0.010 (36.84)**

MEPA – Methanol Extract of *Pterospermum acerifolium* flowers; Control values at time zero was an average of three values; Percentages of oedema are in parentheses; Values are expressed as mean ±S.E.M; n=6; significance at $P < 0.05$* and 0.01** as compared to control.

Table. 2. Percentage of inhibition of inflammation of the methanolic extract of *Pterospermum acerifolium* flowers on carrageenan-induced paw oedema.

Treatment	Dose (mg/kg)	Interval (h)				
		1h	2h	3h	4h	5h
Indomethacin	10mg/kg	71.96	75.0	74.48	77.19	61.2
MEPA	200mg/kg	10.28	27.0	35.86	24.10	26.8
MEPA	400mg/kg	56.07	61.50	58.62	53.42	44.0

MEPA – Methanol Extract of flowers of *Pterospermum acerifolium*;
Values are expressed in percentage.

Table. 3. Anti-inflammatory effect of the methanolic extract of *Pterospermum acerifolium* flowers on cotton pellet-induced granuloma in rats.

Treatment	Dose	Granuloma Weight (mg)	
Control (normal saline)	10ml/kg	Wet Dry	177.0±1.528 60.333±1.856
Indomethacin	10mg/kg	Wet Dry	81.667±2.028** 25.0±1.155§
MEPA	200mg/kg	Wet Dry	125.33±2.603** 34.667±1.453§
MEPA	400mg/kg	Wet Dry	100.667±1.453** 23.33±1.202§

MEPA – Methanol Extract of flowers of *Pterospermum acerifolium*; Values are expressed as mean ±S.E.M; n=6; significance at P< 0.01** when wet weight of test compared with the wet weight of control and § $p < 0.01$ when dry weight of test compared with dry weight of control.

Table. 4. Antinocieptive effect of the methanolic extract of *Pterospermum acerifolium* flowers on acetic acid induced writhing response.

Treatment	Dose	Number of writhing within 30min	Percentage of inhibition
Control (distilled water)	10ml/kg	63.67±0.882	0
Pentazocine	10mg/kg	31.66±1.202**	50.27
MEPA	200mg/kg	51.0±1.155**	19.89
MEPA	400mg/kg	42.33±0.89**	33.51

MEPA – Methanol Extract of flowers of *Pterospermum acerifolium*;
Values are expressed as mean ±S.E.M; n=6; significance at P< 0.01** as compared to control.

Table. 5. Effect of methanolic extract of *Pterospermum acerifolium* flowers on formalin-induced pain in rats.

Treatment	Dose	Number of licks			
		0 – 5 min		15 – 30 min	
		Score of pain	Percentage inhibition	Score of pain	Percentage inhibition
Control (distilled water)	10ml/kg	61.33±0.8	0	131.0±1.155	0
Indomethacin	10mg/kg	48.6±0.89**	20.75	86.33±1.33**	34.09
MEPA	200mg/kg	54.6±0.33ns	10.97	119.67±0.8**	08.64
MEPA	200mg/kg	51.33±0.82**	17.93	98.33±1.764**	24.93

MEPA – Methanol Extract of flowers of *Pterospermum acerifolium*; Values are expressed as mean ±S.E.M; n=6; significance at P< 0.01** ; ns – statistically non significant as compared to the control.

Table. 6. Effect of the methanolic extract of *Pterospermum acerifolium* flowers on pain using tail immersion test.

Treatment	Dose	Latency period (hours)					
		0 h	1h	2h	3h	4h	5h
Control (distilled water)	10ml/kg	2.47±0.009	2.54±0.015 (2.83)	2.617±0.012 (5.95)	2.45±0.009(-0.809)	2.46±0.038 (-0.405)	2.54±0.046 (2.83)
Pentazocine	10mg/kg	3.16±0.035	6.213±0.024 (96.61)**	6.223±0.05 (96.93)**	5.74±0.04(81.64)**	4.623±0.12 (46.29)**	4.21±0.05 (33.22)**
MEPA	200mg/kg	2.3±0.153	2.543±0.02 (10.56)ns	3.167±0.12 (37.69)**	3.4±0.012(47.82)**	3.04±0.023 (32.17)**	2.383±0.03 (3.61)*
MEPA	400mg/kg	2.23±0.008	2.683±0.033 (20.31)**	3.85±0.026 (72.64)**	4.12±0.173 (84.75)**	3.97±0.024 (78.02)**	3.27±0.087 (46.64)**

MEPA – Methanol Extract of flowers of *Pterospermum acerifolium* ; Values are expressed as mean ±S.E.M and are in seconds; n=6; significance at P< 0.05* and 0.01** ; ns – statistically non significant as compared to the control; Percentages of latency of pain are in parentheses; Negative values indicate that the tails were withdrawn faster than the controls.

Discussion

The study indicated that *Pterospermum acerifolium* extract has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical (acetic acid and formalin, inflammatory phase) induced nociceptive stimuli. The centrally acting protective effects of the extract were corroborated by the first phase of formalin-induced pain and immersion tests results. The tail immersion test indicated that the pharmacological actions were mediated by mu opioid receptors rather than kappa and delta receptors (16). The fact that the neurogenic (0–5 min) algesia was blocked by the extract meant that it also acted through opioid receptors which were more centrally located than peripheral. Due to their central location, a higher therapeutic concentration (400 mg/kg) of the extract was therefore required for the analgesia as revealed by the first phase of formalin-induced pain test. The antiinflammatory effect of the extract on acute inflammatory process such as carrageenan-induced oedema in rat paw was dose dependent. At present, no literature has been found describing the side effects such as gastric ulcer, of this plant. These data validated the traditional uses of this plant to assuage pain as well as inflammatory diseases.

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