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Research Article

Anti-inflammatory, Analgesic and Anti-pyretic Activity of the Leaves of *Pterospermum acerifolium*

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Abstract
A methanol extract of the shade dried leaves of *Pterospermum acerifolium* (MEPA) was investigated for anti-inflammatory and analgesic activities at the doses (s.c, i.p) of 300 and 400 mg/kg body weight. In acute toxicity studies, no mortality was observed up to the highest dose of MEPA (2200 mg/kg, i.p). The extract produced a dose dependent inhibition of carrageenan-induced paw oedema in rats. At the same doses, analgesic activity was also observed with tail flick, tail immersion and acetic acid induced writhing models in mice. Furthermore, the phytochemical studies indicated that MEPA contains flavonoids and alkaloids. The results of the present study confirmed the scientific validation of the ethno pharmacological claim about anti-inflammatory, analgesic and anti-pyretic activity of the leaf extract.
Keywords: *Pterospermum acerifolium*, anti-inflammatory, analgesic, antipyretic

Introduction

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) commonly known as "Dinner plate tree" (English) and "Muchkunda" (Hindi), is a large deciduous tree of about 24 m height and 2.5 m 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 of 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* leaves (MEPA) as an anti-inflammatory, analgesic and antipyretic agent in experimental animal models.

Materials and Methods

Plant material

The leaves of *Pterospermum acerifolium (L)* Willd were collected from Asansol, West Bengal, 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 Pharmacology, Gupta College of Technological Sciences, Asansol under the voucher specimen no. CNH/ I-I/(289)/2008/Tech.II/331. The leaves were then air dried in shade at room temperature.

Preparation of the extract

The leaves were dried under shade, made into coarse powder and passed through 40 mesh sieve. The powdered leaves (1.5Kg) were extracted with methanol using a Soxhlet extractor. After exhaustive extraction, the methanol was filtered and concentrated by distillation process. A greenish colored residue was obtained, which was kept in a vacuum desiccator. The suspension of the extract was prepared in 5% 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 Gupta College of Technological Sciences, Asansol, West Bengal, India. They were kept in polyacrylic cages with paddy husk as bedding. The animals were housed under standard housing condition (room temperature 24-27ºc and humidity 60-65% with 12:12 light: dark cycles) and fed with standard diet (Tetragon Chemie Private Limited, Bangalore, India). The animals were fasted overnight before the experiment and given water ad libitum. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee

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(Reg. No: 955/A/06/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 (HiMedia Lab. Pvt. Ltd., Mumbai), Acetic Acid (Fisher Scientific, Mumbai), Methanol (BDH, Mumbai), Pentazocine (Pure Pharma Ltd., Indore), Paracetamol (Glaxo Smithkline, Mumbai) and Diclofenac (Voveran, Novartis, Mumbai).

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 and Flavonoids (NaCl and HCl).

Acute toxicity study

Acute toxicity study was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No 4258.

Anti-inflammatory activity

Carrageenan-induced paw oedema

Five groups of six animals per group were used for the study. Group 1 served as control and received 5% v/v Tween 80 at the dose of 10ml/kg. The plant extract was administered intra-peritoneally at doses 300 and 400 mg/kg to Group 2 and Group 3 respectively. Whereas Group 4 and Group 5 received the standard drug Diclofenac Sodium intra-peritoneally at doses 5 and 10 mg/kg respectively. The administration of extract and drugs was 30 min prior to injection of 0.05 ml, 1% carrageenan in the left hind paw subplantar of each rat. The paw volume was measured by Plethysmometer. Left paw volumes were measured after 1h, 2h 3h, 4h and 5h after Carrageenan injection. Percent inhibition of oedema by the extract and diclofenac sodium was calculated in each time interval by comparing with the control.[9]

Analgesic activity

Acetic acid induced writhing reflex

Five groups of six animals per group were used for the study. Group 1 served as control and received 5% v/v Tween 80 at the dose of 10ml/kg. The plant extract was administered intra-peritoneally at doses 300 and 400 mg/kg to Group 2 and Group 3, respectively. While Group 2 and Group 3 received the standard drug Diclofenac Sodium intra-peritoneally at doses 5 and 10 mg/kg, respectively. After 1h of administration of extract and drugs, intraperitoneal administration of 1%v/v acetic acid at a dose of 10ml/kg was done in each mouse. After 1h, writhing induced by the acid, consisting of abdominal constrictions and hind limbs stretching were counted for 10 min in each group of mice.[10]

Tail flick method

Five groups of six animals per group were used for the study. Group 1 served as control and received 5% v/v Tween 80 at the dose of 10ml/kg. The plant extract was administered intra-peritoneally at doses 300 and 400 mg/kg to Group 2 and Group 3 respectively. Whereas Group 4 and Group 5 received the standard drug Pentazocine subcutaneously at doses 5 and 10 mg/kg respectively. The basal reaction time was taken to radiant heat by placing the tip (last 1-2cm) of tail of each mouse on radiant heat source of Analgesiometer. The strength of current passing through the naked wire was kept constant at 6 ampere. The reaction time i.e. Flicking Response was recorded with a stopwatch. The reaction time was noted at 0, 5, 15, 30, 60, 90 and 120 min interval after Vehicle, Standard and Test Drug administration. The cut-off time, i.e. time of no response was put at 10sec.[11]

Tail immersion method

Five groups of six animals per group were used for the study. Group 1 served as control and received 5% v/v Tween 80 at the dose of 10ml/kg. The plant extract was administered intra-peritoneally at doses 300 and 400 mg/kg to Group 2 and Group 3 respectively. Whereas Group 4 and Group 5 received the standard drug Pentazocine subcutaneously at doses 5 and 10 mg/kg respectively. This involves immersing 3 cm of mice's tail in a water bath containing water at a temperature of 55 ± 0.5^0C. The time to flick the tail from water (reaction time) was recorded. The reaction time i.e. Flicking Response was noted at 0,5,15,30,60,90 and 120 min interval after Vehicle, Standard and Test Drug administration. The cut- off time, i.e. time of no response was fixed at 15 seconds to prevent thermal injury to the tail of the mice.[12]

Antipyretic Activity

Yeast induced pyrexia method

Five groups of six animals per group were used for the study. Group 1 served as control and received 5% v/v Tween 80 at the dose of 10ml/kg. The plant extract was administered intra-peritoneally at doses 300 and 400 mg/kg to Group 2 and Group 3 respectively. Whereas Group 4 and Group 5 received the standard drug Paracetamol intra-peritoneally at doses 100 and 150 mg/kg respectively. Initially a digital thermo- meter was inserted 3-4cm deep into the rectum, after fastened the tail to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10ml/kg of 15%w/v Brewer's

animals were returned to their housing cages. 19h after Yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately 5%v/v Tween 80, methanolic extract of *Pterospermum acerifolium* (W) Linn at the dose of 300 mg/kg body weight and 400 mg/kg body weight, Paracetamol at the dose of 100mg/kg body weight and 150mg/kg body weight was administered intraperitoneally into Group I, II, III, IV and V respectively. Rectal temperature of all the rats was recorded at 19h, immediately before extract or vehicle or paracetamol administration and again at 1h interval up to 23h, after Yeast administration.[13]

Carrageenan-induced paw oedema

The average left 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 Carrageenan caused localised oedema, 30 min later. The swelling increased progressively after 5h to a maximum volume of 0.89± 0.014(58.93%). Rats pretreated with MEPA significantly decreased the carrageenan-induced oedema 30 min post-dosing beginning with 300 mg/kg and in a dose related manner. At 300 mg/kg, the extract showed significant inhibition of oedema formation after 5h (75.75%). At 400 mg/kg, the extract achieved its maximal inhibitory effect (84.85%).

Acetic acid induced writhing reflex

Pterospermum acerifolium significantly reduced writhings and stretchings induced by the administration of 1%v/v acetic acid at a dose of 10ml/kg as shown in Table 3. The significant protective effect was dose dependent with 17.54% reduction ($P < 0.01$) observed for 300 mg/kg and 30.82% reduction ($P < 0.001$) in case of 400 mg/kg dose of MEPA.

Tail flick method

Pterospermum acerifolium significantly reduced painful sensation due to radiant heat from Analgesiometer and it was dose dependent as shown in Table 4. The time for peak analgesic activity was found to be 90min. The percentage increase in analgesic activity of the plant extract for 300 mg/kg at 90 min is 34.8 ($P < 0.001$); while the percentage increase in analgesic activity of the plant extract for 400 mg/kg at 90min is 50.5 ($P < 0.001$).

Tail immersion method

Pterospermum acerifolium significantly reduced painful sensation due to radiant heat from Analgesiometer and it was dose dependent as shown in Table 4. The time for peak analgesic activity was found to be 90 min. The percentage increase in analgesic activity of the plant extract for 300 mg/kg at 90 min is 33.4 ($P < 0.001$); while the percentage increase in analgesic activity of the plant extract for 400 mg/kg at 90 min is

Table 1: Anti-inflammatory effect of methanolic extract of *Pterospermum acerifolium* leaves on carrageenan-induced paw oedema

Treatment	Dose (mg/kg)	Paw Odema Volume (ml)					
		0 hr	1st hr	2nd hr	3rd hr	4th hr	5th hr
Control (5% v/v Tween 80	10 ml	0.56 ± 0.01	0.71 ± 0.02 (26.78)	0.8 ± 0.02 (42.86)	0.86 ± 0.02 (53.57)	0.88 ± 0.02 (57.14)	0.89 ± 0.01 (58.93)
MEPA	300	0.59 ± 0.01	0.69 ± 0.01 (16.95)	0.745 ± 0.01 *(26.27)	0.74 ± 0.01 ***(25.42)	0.72 ± 0.01 ***(22.03)	0.67 ± 0.01 ***(13.56)
MEPA	400	0.54 ± 0.01	0.63 ± 0.01 (16.67)	0.66 ± 0.01 **(22.22)	0.64 ± 0.01 ***(18.52)	0.62 ± 0.01 ***(14.81)	0.59 ± 0.01 ***(9.26)
Diclofenac	5	0.55 ± 0.01	0.64 ± 0.01** (16.36)	0.68 ± 0.01** (23.64)	0.66 ± 0.01 ***(20.00)	0.62 ± 0.02 ***(12.73)	0.59 ± 0.02 ***(7.27)
Diclofenac	10	0.51 ± 0.01	0.60 ± 0.01** *(17.64)	0.65 ± 0.01 ***(27.45)	0.61 ± 0.01 ***(19.61)	0.57 ± 0.01 ***(11.761)	0.54 ± 0.01 ***(5.88)

MEPA- Methanol extract of Pterospermum acerifolium leaves; Percentages of oedema are in parentheses; Values are expressed as Mean± SEM; n=6; significance at P<0.1, P<0.01**, P<0.001*** as compared to control.*

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

Treatment	Dose (mg/ kg)	Paw Odema Volume (ml)				
		1st hr	2nd hr	3rd hr	4th hr	5th hr
Control (5% v/v Tween 80	10 ml	-	-	-	-	-
MEPA	300	33.33	35.42	50	59.37	75.75
MEPA	400	40	50	66.67	75	84.85
Diclofenac	5	40	45.83	63.33	78.12	87.88
Diclofenac	10	40	41.67	66.67	81.25	90.91

33.4 ($P < 0.001$); while the percentage increase in analgesic activity of the plant extract for 400 mg/kg at 90 min is 49.4 ($P < 0.001$).

Yeast induced pyrexia method

The intra-peritoneal administration of methanolic extract of leaves of *Pterospermum acerifolium (W)* Linn at a dose of 300 mg/kg and 400 mg/kg showed significant antipyretic activity at 23h after Yeast administration as compared with Paracetamol at a dose of 100mg/kg and 150 mg/kg with probability factor $P < 0.001$; $n = 6$ as shown in Table 6. The initial and final rectal temperatures in the groups treated with MEPA at 300 mg/kg and MEPA 400mg/kg at 23h after Yeast administration were 38.33 ± 0.255 and 37.18 ± 0.252, 38.43 ± 0.156, and 37.08 ± 0.201, respectively.

Results

The results of the pharmacological experiments including anti-inflammatory, analgesic, and antipyretic activity have been cited in the following tables subsequently after necessary statistical treatment.

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 acetic acid induced writhing response method. The centrally acting analgesic effect of the extract was determined from tail flick and tail immersion methods. The tail flick and tail immersion methods indicated that the pharmacological actions were mediated by mu opioid receptors rather than kappa and delta receptors.[14] The anti-inflammatory effect of the extract on acute inflammatory process such as carrageenan-induced oedema in rat paw was dose dependent. It has also been found that anti-pyretic effect of the extract on yeast induced pyrexia in rats 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 in the treatment of pain, inflammation and fever.

Table 3: Analgesic effect of the methanolic extract of *Pterospermum acerifolium* leaves on acetic acid induced writhing response

Treatment	Dose (mg/ kg)	Number of writhing within 10 min	Percentage of inhibition
Control (5% v/v Tween 80	10 ml	35.17 ± 1.01	-
MEPA	300	29.00 ± 1.41**	17.54
MEPA	400	24.33 ± 1.36**	30.82
Diclofenac	5	11.50 ± 0.76**	67.30
Diclofenac	10	7.67 ± 0.75**	78.19

MEPA- Methanol extract of Pterospermum acerifolium leaves; Values are expressed as Mean± SEM; n=6; significance at P<0.01, P<0.001** as compared to control*

Table 4: Effect of methanolic extract of *Pterospermum acerifolium* leaves on pain using tail flick method

Treatment	Flicking response of the tail						
	0 hr	5 min	15 min	30 min	60 min	90 min	120min
Control (5% v/v Tween 80	0.48 ± 0.08	0.53 ± 0.07 (0.5)	0.56 ± 0.08 (0.8)	0.58 ± 0.08 (1.0)	0.62 ± 0.07 (1.4)	0.63 ± 0.06 (1.5)	0.65 ± 0.07 (1.7)
MEPA (300 mg)	1.2 ± 0.08*	4.2 ± 0.05* (30)	4.39 ± 0.07 *(31.9)	4.51 ± 0.07* (33.1)	4.64 ± 0.06* (34.4)	4.68 ± 0.05* (34.8)	4.65 ± 0.07* (34.5)
MEPA (400 mg)	1.34 ± 0.09*	5.51 ± 0.06* (41.7)	5.68 ± 0.06* (43.4)	5.91 ± 0.08* (45.7)	6.20 ± 0.01* (48.6)	6.39 ± 0.09* (50.5)	6.32 ± 0.09* (49.7)
Pentozocine (5 mg/kg)	1.32 ± 0.06*	8.13 ± 0.17* (68.0)	8.38 ± 0.15* (70.5)	8.78 ± 0.16* (74.5)	9.13 ± 0.17* (78.0)	9.31 ± 0.13* (79.8)	9.18 ± 0.12* (78.4)
Pentozocine (10 mg/kg)	1.38 ± 0.06*	8.27 ± 0.07* (68.9)	8.51 ± 0.14* (71.3)	8.88 ± 0.15* (75.0)	9.25 ± 0.15* (78.7)	9.44 ± 0.11* (80.6)	9.28 ± 0.10* (79.0)

MEPA- Methanol extract of Pterospermum acerifolium leaves; Percentage increase in analgesic activity are in parentheses; Values are expressed as Mean± SEM; n=6; significance at P<0.001 as compared to control*

Table 5: Effect of methanolic extract of *Pterospermum acerifolium* leaves on pain using tail immersion method

Treatment	Flicking response of the tail						
	0 hr	5 min	15 min	30 min	60 min	90 min	120min
Control (5% v/v Tween 80	0.49 ± 0.067	0.52 ± 0.069 (0.3)	0.55 ± 0.070 (0.6)	0.57 ± 0.065 (0.8)	0.60 ± 0.065 (1.1)	0.62 ± 0.072 (1.3)	0.59 ± 0.063 (1.0)
MEPA (300 mg)	1.24 ± 0.058*	4.23 ± 0.05* (29.9)	4.4 ± 0.067 *(31.6)	4.45 ± 0.069* (32.1)	4.52 ± 0.058* (32.8)	4.58 ± 0.064* (33.4)	4.56 ± 0.063* (33.2)
MEPA (400 mg)	1.38 ± 0.075*	5.55 ± 0.062* (41.7)	5.64 ± 0.065* (42.6)	5.79 ± 0.057* (44.1)	6.03 ± 0.076* (46.5)	6.32 ± 0.059* (49.4)	6.25 ± 0.058* (48.7)
Pentozocine (5 mg/kg)	1.44 ± 0.06*	8.07 ± 0.17* (68.0)	8.29 ± 0.107* (68.5)	8.47 ± 0.084* (70.3)	8.76 ± 0.079* (73.2)	9.19 ± 0.073* (77.5)	9.11 ± 0.069* (76.7)
Pentozocine (10 mg/kg)	1.51 ± 0.074[a]	8.17 ± 123[a] (66.3)	8.45 ± 0.107[a] (69.4)	8.58 ± 0.084[a] (70.7)	8.88 ± 0.097[a] (73.7)	9.31 ± 0.063[a] (78.0)	9.14 ± 0.085[*] (76.3)

MEPA- Methanol extract of Pterospermum acerifolium leaves; Percentage increase in analgesic activity are in parentheses; Values are expressed as Mean± SEM; n=6; significance at P<0.001[*] as compared to control

Table 6: Effect of methanolic extract of *Pterospermum acerifolium* leaves on fever using yeast induced pyretic rats method

Treatment	Rectal Temperature					
	0 hr	19 hr	20 hr	21 hr	22 hr	23 hr
Control	36.17 ± 0.17	38.25 ± 0.22	38.76 ± 0.11	38.58 ± 0.08	38.58 ± 0.08	38.65 ± 0.11
MEPA (300 mg)	36.07 ± 0.19	38.33 ± 0.26	38.1 ± 0.23	37.78 ± 0.22 *	37.78 ± 0.22 *	37.18 ± 0.25 **
MEPA (400 mg)	36.27 ± 0.218	38.43 ± 0.16	38.18 ± 0.15	37.75 ± 0.11 **	37.75 ± 0.11 **	37.08 ± 0.20 *
Paracetamol (100 mg/kg)	36.40 ± 0.24	38.6 ± 0.24	38.10 ± 0.23	37.67 ± 0.21 *	37.67 ± 0.21 **	36.88 ± 0.201 **
Paracetamol (150 mg/kg)	36.23 ± 0.21	38.58 ± 0.16	38.00 ± 0.23	37.58 ± 0.28 *	37.58 ± 0.28 **	36.67 ± 0.25 **

MEPA- Methanol extract of Pterospermum acerifolium leaves; Percentage increase in analgesic activity are in parentheses; Values are expressed as Mean± SEM; n=6; significance at P<0.01, P<0.001** as compared to control*

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