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Analgesic, Anti-inflammatory and Diuretic Activities of *Pisonia grandis*

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Abstract – In the present study, *Pisonia grandis* leaves were extracted with chloroform and methanol. The extracts were vacuum dried to yield the respective chloroform (CE) and methanol extract (ME). CE and ME were evaluated for analgesic, anti-inflammatory (acute and chronic) and diuretic activity at 2 dose levels (250 and 500 mg/kg). Significant analgesic and anti-inflammatory activities were associated with CE and ME. CE at the dose level of 500 mg/kg was found to exhibit equivalent chronic anti-inflammatory activity as diclofenac at 50 mg/kg dose level. Significant diuretic activity was exhibited by ME. Graded dose response for all the activities were observed for the extracts.

Key words: analgesic, anti-inflammatory, diuretic, *Pisonia grandis*

Introduction

In the alternative system of medicine, *Pisonia grandis* R. Br. leaves are used as analgesic, antiinflammatory and diuretic agent (Kirithikar and Basu, 1990; Nadkarni, 1976). The roots are used as purgative (Chopra *et al.*, 1956). There is no scientific documentation about the pharmacological properties of *Pisonia grandis* which promoted us to pursue a systematic pharmacological evaluation of *Pisonia grandis* leaves to verify their medicinal properties. In the present study, vacuum dried chloroform and methanol extracts of *Pisonia grandis* leaf were evaluated for their analgesic activity (Acetic acid induced writhing reflex method and Tail flick method), acute antiinflammatory activity by formalin induced pedal paw edema method, chronic antiinflammatory activity by freund's complete adjuvant induced inflammatory model and diuretic activity.

Materials and Methods

Plant material – *Pisonia grandis* leaves were collected from Chennai during September 2000. The plant was identified by Dr. P. Brinda, Botanist, Captain Srinivasamurthi Drug Research Institute for Ayurveda, Chennai where voucher specimen were deposited in the herbarium (P-144).

Extraction – Air-dried powdered leaves were extracted by maceration successively with chloroform and methanol for 72 hours. Then, the extract was vacuum dried using rotary vacuum flash evaporator to yield to solid residue of the respective extracts (i.e.) chloroform (CE) and methanol extract (ME).

Animals – Wistar albino mice (25-30 g) and wistar albino rats (150-200g) were obtained from the animal house department of the institution. The animals were maintained in colony cages at 25±2°C, relative humidity of 45-55%, maintained under 12 hr light and dark cycle (0600 to 1800 h-light; 1800 to 0600 h-dark). The animals were fed with standard animals feed (Hindustan Lever Ltd.) and water *ad libitum*. All the animals was acclimatized for a week before use. CE and ME were suspended in 1% carboxy methyl cellulose and administered to the animals.

Analgesic activity by writhing reflex method – The analgesic activity was determined by acetic acid induced writhing method (Ghosh, 1984) using wistar albino mice of either sex selected by random sampling technique. Paracetamol at a dose level of 100 mg/kg was administered as a standard drug for comparison. The extracts at 2 dose levels (250 and 500 mg/kg) were administered orally by gavage 15 min prior to administration of the writhing agent (0.6% v/v aqueous acetic acid-1 ml/100 g). The

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Table 1. Analgesic activity (writhing reflex method) of *Pisonia grandis* leaf extracts

Treatment	Dose (mg/kg)	Writhings	% Protection
CE	250	35.4 ± 0.72	16.31*
	500	29.5 ± 0.76	30.26*
ME	250	34.7 ± 0.46	17.97*
	500	24.6 ± 0.49	41.84*
Paracetamol	100	11.6 ± 0.88	72.57*
Control	–	42.3 ± 0.99	–

Significance level: *p<0.001 compared to control.

writhings produced in the animal were observed for 30 minutes and percentage protection was calculated for analgesic activity. The analgesic activity data (of writhing reflex method) are presented in Table 1.

Analgesic activity by tail flick method – The analgesic activity was also determined by tail flick method (Kulkarni, 1999) using wistar albino mice of either sex selected by random sampling technique. The basal reaction time to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. A cut-off period of 15 seconds was observed to prevent damage to the tail. Morphine at a dose level of 5 mg/kg (sc) was administered as standard drug for comparison. The extracts at 2 dose levels (250 and 500 mg/kg) were administered i.p. The reaction time was recorded at 15, 30, 60 and 120 min after the treatment. The analgesic activity data (of tail flick method) are presented in Table 2.

Acute Anti-inflammatory activity – The acute anti-inflammatory activity was determined by formalin induced pedal paw edema method (Laurence and Bacharach, 1964) in wistar albino rat of either sex by using Plethysmograph. Diclofenac sodium (50 mg/kg) was administered as a standard drug. The extracts were administered at 2 dose levels (250 and 500 mg/kg) orally by gavage 30 minutes prior to administration of formalin (0.1 ml of 1% w/v) in the plantar region of the paw. The paw volumes was measured at 15, 30, 60, 120, 180 and 240 min after formalin administration. The anti-inflammatory activity data are presented in Table 3.

Chronic anti-inflammatory activity – Chronic arthritis was produced by Freund's (complete) adjuvant induced chronic inflammatory model (Goel *et al.*, 1990) in wistar albino rats (n=6) of either sex selected by random sampling technique. Freund's complete adjuvant (1 ml, sc) was administered in the right hind footpad. Paw volume after 18th hr of injection was taken as a measure of acute inflammation and edema on the 14th day was taken as an index of chronic inflammation. The extracts were administered orally (250 and 500 mg/kg) by gavage one hour before adjuvant administration and then daily thereafter. The chronic anti-inflammatory data are presented in Table 4.

Diuretic activity – Male rats (n=12) were placed in four metabolic cages (Turner and Hebborn, 1971) in three replications. Extreme care was taken to avoid contamination of urine with fecal matters and food particles. All the animals received a priming dose of normal saline (25 ml/kg) orally one hour prior to compound administration. The extracts were administered at the dose of 250 and 500 mg/kg orally by gavage. Frusemide at the dose of 10mg/kg, i.p. served as standard. Urine was collected upto 24 hr after administration of the extracts. At the end of 24 hr, urine volume was measured and electrolyte (Na^+ and K^+)

Table 2. Analgesic activity (tail flick method) of *Pisonia grandis* leaf extracts

Treatment	Dose (mg/kg)	Basal reaction time (sec)	Reaction time (sec)			
			15 min	30 min	60 min	120 min
CE	250	2.67 ± 0.304	3.33 ± 0.31	5.5 ± 0.514**	7.17 ± 0.281***	7.67 ± 0.192***
	500	2.17 ± 0.366	3 ± 0.236*	4.83 ± 0.495**	7.33 ± 0.304***	8.83 ± 0.281***
ME	250	3 ± 0.236	2.83 ± 0.281	5.17 ± 0.495**	8.5 ± 0.391***	12.83 ± 0.281***
	500	2.83 ± 0.281	3.83 ± 0.281*	5.17 ± 0.366**	7.83 ± 0.281***	13 ± 0.577***
Morphine	5	2.67 ± 0.304	3.33 ± 0.192*	6.17 ± 0.152***	9 ± 0.333***	14.33 ± 0.304***

Significance level: *p<0.01, **p<0.005, ***p<0.001 compared to basal reaction time.

Table 3. Acute anti-inflammatory activity of *Pisonia grandis* leaf extracts

Treatment	Dose (mg/kg)	% Reduction of edema						
		15 min	30 min	45 min	60 min	120 min	180 min	240 min
CE	250	1.02	3.48	11.41*	20.18**	29.42**	39.24***	44.88***
	500	6.11	7.09	19.09**	29.09**	55.18***	71.71***	70.25***
ME	250	2.41	6.22	6.47	9.42	27.46**	29.42***	30.44***
	500	6.12	8.91	11.45*	22.48**	47.52***	60.98***	61.09***
Diclofenac	50	25.71**	33.42***	75.41***	79.63***	79.42***	80.76***	80.44***

Significance level: *p<0.1, **p<0.01, ***p<0.001 compared to control.

Table 4. Chronic anti-inflammatory activity of *Pisonia grandis* leaf extracts

Treatment	Dose (mg/kg)	Edema (% inhibition of edema)	
		18^{th} Hr	14^{th} Day
CE	250	1.226 ± 0.074 (15.33)*	0.941 ± 0.042 (28.82)***
	500	1.022 ± 0.041 (29.41)***	0.466 ± 0.038 (64.75)***
ME	250	1.241 ± 0.076 (14.30)*	1.004 ± 0.037 (24.05)***
	500	1.132 ± 0.069 (21.82)**	0.577 ± 0.047 (56.35)***
Diclofenac	5	0.966 ± 0.041 (33.28)***	0.455 ± 0.047 (65.58)***
Control	–	1.448 ± 0.084	1.322 ± 0.063

Significance level: *p<0.025, **p<0.05, ***p<0.001 compared to control

Table 5. Diuretic activity of *Pisonia grandis* leaf extracts

Treatment	Dose (mg/kg)	Urine volume in 24 hr (ml)	Urine electrolyte MEq/l (24 Hr)	
			Na^+	K^+
CE	250	8.01 ± 0.241	117 ± 6.14	76 ± 6.41
	500	7.43 ± 0.757	113 ± 7.13	78 ± 5.68
ME	250	10.21 ± 0.671*	175 ± 3.45**	145 ± 6.91**
	500	12.04 ± 0.482**	237 ± 7.05**	186 ± 7.29**
Frusemide	10	12.28 ± 0.478**	201 ± 1.85**	180 ± 5.78**
Control	–	8.25 ± 0.635	123 ± 7.15	74 ± 7.03

Significance level: *p<0.025 and **p<0.001 compared to control..

estimation was done on a flame photometer. The diuretic data are presented in Table 5.

Statistical analysis – All data was expressed as mean ± S.E. except acute anti-inflammatory activity (Table 3) and unpaired student-t-test (Spiegel and Meddis, 1980) was used for the statistical analysis.

Results and Discussion

It was observed that CE and ME exhibited significant analgesic activity (acetic acid induced writhing reflex method and tail flick method). The extracts exhibited graded dose response.

In acute anti-inflammatory model, significant activity of the extracts was only manifested after 45 min of formalin administration. CE (70.25% reduction of edema) was found to be more active than ME (61.09%). In chronic anti-inflammatory model, highly significant anti-inflammatory activity (p<0.001) was observed with CE and ME at 500 mg/kg dose level. CE at the does level of 500 mg/kg was found to exhibit equivalent chronic anti-inflammatory activity as Diclofenac at 50 mg/kg dose level. In both acute and chromic inflammatory models, CE and ME exhibited graded dose response. Significant diuretic activity and graded dose response was exhibited by ME. CE was completely devoid of diuretic activity at the experimental doses..

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