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ANTI-INFLAMMATORY, ANALGESIC AND DIURETIC ACTIVITY OF *POLYGONUM LANATUM* ROXB.

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The hexane (PLH), ethyl acetate (PLE) and methanol (PLM) extracts of dried whole plant parts of *Polygonum lanatum* Roxb. (Family, Polygonaceae) obtained by successive cold extraction, were subjected to evaluate anti-inflammatory, analgesic and diuretic activity in experimental animals. Oral administration of either PLH and PLM at a dose of 300 mg/kg body weight showed statistically significant ($p < 0.001$) inhibition of rat paw edema by 41.09% and 30.15%, respectively, which was comparable to that of standard drug phenylbutazone (42.15%). Compared to the inhibition of acetic acid-induced writhing by aminopyrine (69.94%, $p < 0.001$), treatment with either PLH, PLE or PLM elicited significant inhibition of acetic acid-induced writhing reflex by 44.80% ($p < 0.001$), 33.87% ($p < 0.01$) and 62.29% ($p < 0.001$), respectively. In addition, mild to potent diuretic activity was observed after oral administration of these extracts in *Swiss albino* mice.

Keywords: *Polygonum lanatum,* polygonaceae, analgesic activity, anti-inflammatory activity, diuretic activity.

INTRODUCTION

There is growing evidence suggesting the therapeutic potential of plant products. Herbal medicines are gradually becoming more popular through out the world (Wohlmuth, 2002). Herbs contain numerous secondary metabolites including terpenoids and flavonoids that are effective in the treatment and/or prevention of various chronic diseases such as heart disease, cancer, diabetes, and hypertension as well as other pathologic conditions. While inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, an uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Although a handful of synthetic anti-inflammatory drugs are readily available these days, the search for non-toxic anti-inflammatory substances of natural origin, especially from medicinal plants and dietary sources, has long been attempted.

Herbs belonging to the genus *Polygonum* L. (Family – Polygonaceae) has been used in Indian folk medicine as demulcent, pectoral, astringent, emetic, purgative, diuretic, insecticide, anthelmintic, analgesic and anti-inflammatory drugs (Kirtikar and Basu, 1980). In our previous studies, we have reported the analgesic and anti-inflammatory activity of extractives from different *Polygonum* species as well as that of terpenoids and flavonoids isolated from *P. viscosum* (Datta *et al.*, 2003; Datta *et al.*, 2004). The present study is aimed at investigating the biological activities of extracts of different polarities prepared from an indigenous medicinal plant *Polygonum lanatum* Roxb., which is widely distributed in Bangladesh and various parts of Eastern India, Nepal, Burma, Indonesia and the Philippines (Hasan, 1989). Our study revealed that the hexane (PLH) and methanol (PLM) extracts, but not the ethylacetate extract (PLE), of *P. lanatum* showed significant anti-inflammatory activity. Significant inhibition of acetic acid induced writhing reflex by PLH, PLE or PLM suggests that these extracts possess analgesic activity. Moreover, all extracts showed mild to potent diuretic activity.

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MATERIALS AND METHODS

Plant materials

Polygonum lanatum Roxb. was collected at flowering stage from Madanpur, Narayangonj, Bangladesh during April-May, 2001. A voucher specimen (accession number: DUH-3400) was kept in Dhaka University Herbarium, Dhaka, Bangladesh after identification of the plant. Collected plants, after cutting into small pieces, were sun-dried and pulverized into a coarse powder, and stored into an air-tight container.

Extraction

Pulverized coarse powder of dried whole plant *P. lanatum* Roxb. (800 gm) was extracted with *n*-hexane, ethyl acetate and methanol by successive cold extraction. Extracts were filtered off and evaporated to dryness *in vaccu* at low temperature and reduced pressure by rotary evaporator.

Sample preparation and animal treatment

Swiss albino mice (20-25 g) and *Long Evans* rats (140-160 g) of either sex were obtained from International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were given standard feed developed by ICDDR,B and water *ad libitum* and kept in the laboratory environment (12 h dark/12 h light cycle) for seven days for acclimatization. Animals were kept under fasting for overnight and weighed before the experiment. Extracts (PLH, PLE and PLM) were dissolved in normal saline by using 0.1% tween-80. Animals were randomly divided into eight groups, each consisting of six animals, of which six groups were given the test material PLH, PLE and PLM at doses of 150 and 300 mg/kg body weight by gavage, respectively. While one group of animals was treated with standard drugs [phenylbutazone (100 mg/kg body weight) for screening of anti-inflammatory activity, aminopyrine (50 mg/kg body weight) for analgesic activity study, and furosemide (5 mg/kg body weight) for assessing diuretic activity], another group of animals served as control receiving saline containing 0.1% tween-80.

Screening of anti-inflammatory activity

The effect of PLH, PLE and PLM on carrageenan (1%) induced inflammation in rat paw was investigated by following the method of Winter et al. (1962) with minor modifications. Half an hour after the oral administration of test materials, standard drug or saline to respective treatment groups, 1% carrageenan solution was injected to the sub-planter region of right hind paw of each rat. The volume of paw edema was measured at different time intervals for a period of 24 h by mercury displacement method. Traveling microscope (ELFO Scientific Apparatus, India) was used to record the volume of mercury displaced after immersion of the inflamed paw. Prior to immersion into mercury, the inflamed right hind paw was labeled with permanent ink to confirm uniform immersion during each episode of measuring paw volume. The average percent increase in paw volume was calculated and compared against the control group. Percent inhibition was calculated using the following formula.

$$\% \text{ inhibition of paw edema} = (1-V_t/V_c) \times 100$$

V_c and V_t represent average paw volume of control and treated animals, respectively.

Study of analgesic activity

Swiss albino mice (6-8 weeks) weighing between 20 to 25 g were used to study the analgesic activity by recording acetic acid-induced writhing reflex as described by Vogel and Vogel (1997). Animals of various groups were treated with either test extracts or standard drug 40 min prior to the intraperitoneal (i.p) administration of acetic acid solution (0.7%, 0.1 ml/10 gm body weight). After an interval of 10 min, numbers of writhing were counted for 10 min. The percent inhibition of writhing was measured using the formula,

$$\text{Percent inhibition of writhing} = (1-W_t/W_c) \times 100$$

where, W_C and W_t represent the average number of writhing produced by the control and test group, respectively.

Screening of diuretic activity

The diuretic activity of PLH, PLE and PLM was studied in *Swiss albino* mice following the method of Gujral *et al.* (1955), with modification of experimental animals from rats to mice, by comparing the urinary output in mice treated with different extracts of *P. lanatum* and standard diuretic drug furosemide. Animals were placed into metabolic cages according to treatment groups and allowed an adjustment period of 1 h. Treatment of animals was performed as described above. The urine volume for each group was measured at 1 h interval for total period of 4 h and the cumulative amount of urine volume was used to analyze diuretic activity by using the following formulae.

The volume of the urine excreted by each group was expressed as percent of the liquid fluid intake giving rise to a measure of urinary excretion (U.E.) as calculated by following formula.

Table 1
Effects of PLH, PLE and PLM on carrageenan-induced rat paw edema[¶]

Treatment	Dose (mg/kg)	*Increase in paw volume (Mean ± S.E.M.), n=6 (Percent inhibition of paw edema)				
		1 h	2 h	3 h	4 h	24 h
PLH	300	48.6 ± 2.441[a] (33.24)	60.6 ± 2.561[a] (36.34)	66.8 ± 2.518[a] (41.09)	74.2 ± 2.818[a] (38.87)	61.4 ± 3.026[NS] (9.97)
PLE	300	64.6 ± 2.874[c] (11.26)	80.0 ± 3.449[b] (15.96)	92.8 ± 2.557[a] (18.16)	100.6 ± 3.076[a] (17.13)	64.4 ± 2.959[NS] (5.57)
PLM	300	53.6 ± 2.891[a] (26.37)	67.8 ± 2.596[a] (28.78)	79.2 ± 3.292[a] (30.15)	86.4 ± 2.461[a] (28.83)	62.8 ± 4.054[NS] (7.917)
Standard (PBZ)	100	46.6 ± 1.568[a] (35.98)	54.2 ± 1.881[a] (43.06)	65.6 ± 2.135[a] (42.15)	78.8 ± 1.827[a] (35.09)	55.8 ± 2.154[b] (18.18)
Control	----	72.8 ± 1.462	95.2 ± 2.374	113.4 ± 2.158	121.4 ± 2.713	68.2 ± 2.083

[¶] Six animals per group. PLH, PLE and PLM indicate hexane, ethylacetate and methanol extract of *P. lanatum*, respectively.
[*] Time after carrageenan administration; paw volume is expressed in change of height (in mm) of Hg bath.
[a-c] Level of significance (calculated as compared to control using student's t-test)
a <0.001, b <0.01, c <0.05, NS = not significant

U.E. = (Total urinary out put/ Total liquid administered) x 100

The ratio of urinary excretion (U.E.) in test group and control group was denoted as diuretic action, which was used as the measure of degree of diuresis.

Diuretic action = (U.E. in test group / U.E. in control group)

Diuretic activity = (Diuretic action of drug / Diuretic action of urea)

RESULTS AND DISCUSSION

Effects of various extracts of P. lanatum on carrageenan-induced rat paw inflammation

The effect of PLH, PLE and PLM on carrageenan-induced rat paw edema was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The study revealed that after 3 h of carrageenan administration, PLH and PLM exhibited statistically significant (p <0.001) inhibition of paw volume by 41.09% and 30.15%, respectively, at a dose of 300 mg/kg body weight, which was comparable to that of standard drug phenylbutazone (42.15% inhibition, p <0.001) given *p.o.* at a dose of 100 mg/kg body weight. The PLH and PLM fractions exhibited 36.34% and 28.78% inhibition of paw volume at 2 h, respectively (Table-1). The PLE showed 18.16% inhibition of the rat paw volume at 3 h, which was not comparable to that of PLH, PLM or phenylbutazone. There was no significant inhibition of paw edema by the extracts at 150 mg/kg body weight (data not shown). Among the test materials PLH was found to be more active than PLM and PLE. Therefore, the order of anti-inflammatory response elicited by the *P. lanatum* extracts was PLH >PLM>PLE**.**

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Appleton *et al.*, 1995; Seibert *et al.*, 1994). The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Winter *et al.* 1962). The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin component (Crunkhorn and Meacock, 1971). Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at 3 h (Di Rosa and Willoughby, 1971). The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at 3 h and period thereafter (Vinegar *et al.*, 1969). Therefore, it can be inferred that the inhibitory effect of PLH and PLM on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by PLH could be attributed to the inhibition of histamine (Hirasawa *et al.*, 1991) and/or serotonin.

Table 2
Inhibition of acetic acid-inducing writhing reflex by PLH, PLE and PLM in *Swiss albino* mice¶

Treatment	Dose (mg/kg)	* Number of writhing (Mean ± S.E.M.) n =6	% Inhibition of writhing reflex	t-value
PLH	150	28 ± 1.483^{b}	23.49	3.238
	300	20.2 ± 1.463^{d}	44.80	6.200
PLE	150	32.2 ± 2.690^{NS}	12.02	1.265
	300	24.2 ± 2.222^{c}	33.87	3.962
PLM	150	29.2 ± 2.083^{a}	20.21	2.44
	300	13.8 ± 1.881^{d}	62.29	7.867
Aminopyrine	50	11.0 ± 0.894^{d}	69.94	10.765
Control	----	36.6 ± 2.204	------	-------

¶ Six animals per group. PLH, PLE and PLM indicate hexane, ethylacetate and methanol extract of *P. lanatum*, respectively; 0.7% (v/v) acetic acid (0.1 ml /10 g) was given i. p.; the number of writhings induced by acetic acid was counted for 10 min.
* Values are mean ± SEM; ^{d} $p<0.001$, ^{c} $p<0.01$, ^{b} $p<0.02$, ^{a} $p<0.05$, NS: Not Significant, when compared to control.

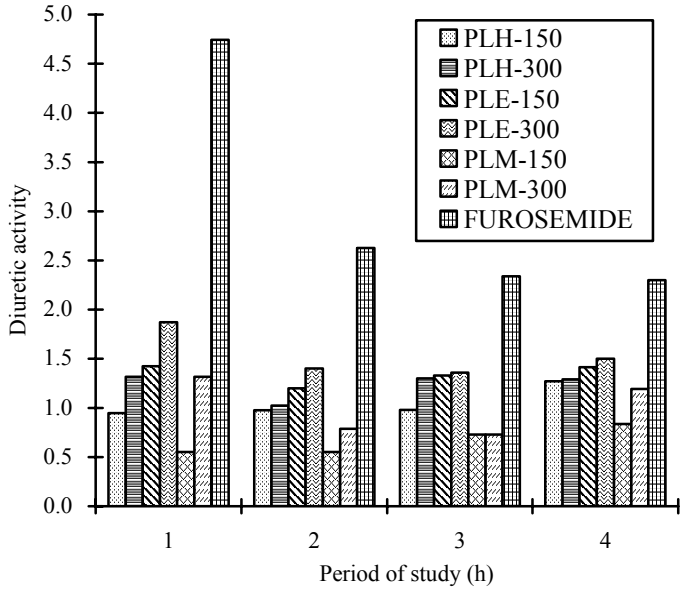

Fig. 1: Diuretic activity of various extracts of *P. lanatum*. Mice were given hexane, ethylacetate and methanol extract, prepared as described under Materials and Methods. Total urinary output was recorded and data were analyzed for determining diuretic activity according to the formulae described under Materials and Methods. PLH, PLE and PLM indicate hexane, ethylacetate and methanol extract of *P. lanatum*, respectively.

Suppression of acetic acid-induced writhing reflex by PLH, PLE and PLM

The effect of various extracts of *P. lanatum* on acetic acid induced writhing at doses of 150 and 300 mg/kg body weight was compared to that of aminopyrine at a dose of 50 mg/kg body weight. Significant analgesic effect of all extracts tested was observed at a dose of 300 mg/kg body weight (Table 2) only. While PLH, PLE and PLM exhibited inhibition of writhing reflex by 44.80% (p <0.001), 33.87% (p <0.01) and 62.29% (p <0.001) respectively, the inhibition of writhing by standard drug aminopyrine was 69.94% (p <0.001).

Table 3
Urinary output at different time intervals after oral administration of various extracts of *P. lanatum**

Treatment	Dose (mg/kg)	Urine volume (ml)			
		1 h	2 h	3 h	4 h
PLH	150	0.900	1.950	2.450	3.300
	300	1.250	2.050	3.250	3.350
PLE	150	1.350	2.400	3.3250	3.675
	300	1.775	2.800	3.400	3.90
PLM	150	0.525	1.100	1.825	2.170
	300	1.250	1.575	1.950	3.100
Urea	750	0.950	2.000	2.500	2.600
Furosemide	5	4.500	5.250	5.850	5.975
Control	---	2.200	2.775	2.875	4.400

*Six animals per group. PLH, PLE and PLM indicate hexane, ethylacetate and methanol extract of *P. lanatum*, respectively.

Diuretic activity of P. lanatum extractives

In order to determine the diuretic activity of the plant *P. lanatum*, effects of PLH, PLE and PLM on urination was investigated in *Swiss albino* mice. The urinary output at different hours of study has been presented in Table-3. Results of the experiment revealed that the extracts exhibited a dose dependent diuretic activity (Fig. 1). Among the three extracts, PLE elicited potent diuretic activity of 1.422 and 1.87 at the doses of 150 and 300 mg/kg body weight, respectively, at the first hour of study. The hexane and methanol extract of the plant showed a diuretic activity of 1.317and 1.317 at a dose of 300 mg/kg body weight at 1 h of study. According to Gujral *et al.*, (1955), the diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00 ~1.50, and little if it is between 0.72~1.00. In this respect, the ethyl acetate extract of *P. lanatum* showed moderate to good diuretic activity at doses of 150 mg/kg and 300 mg/kg body weight, whereas the hexane and methanol extract of the plant showed moderate diuretic activity at a dose of 300 mg/kg body weight.

Although preliminary biological study of different extractives of *P. lanatum* exhibited significant analgesic, anti-inflammatory and diuretic activity, the exact mechanisms underlying the observed pharmacological effects can be elucidated after isolation of active constituents using a wide range of experimental models. However, the present study suggests that the crude extracts of *P. lanatum*, may be used as herbal remedies to cure inflammatory disorders. The elucidation of mode of diuretic action, which may be the effect of test extracts either on loop permeability or reduction of antidiuretic hormone secretion or inhibition of carbonic anhydrase enzyme (Goodman and Gillman, 1975), needs further study. Our study suggests that the diuretic activity of PLE may be implicated for the treatment of certain cardiovascular disorders. Although no sign of toxicity was observed after 24 h of administration of PLH, PLE or PLM at a dose of 300 mg/kg body weight, detailed toxicological study should be performed before exploiting the extractives of *P. lanatum* for therapeutic purpose.

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