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Studies on diuretic and laxative activity of bark extracts of *Spondias pinnata* (Linn. f) Kurz

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ABSTRACT

The diuretic and laxative activity of different extracts of the barks of *Spondias pinnata* (Linn. f) Kurz (Family: Rubiaceae) were studied in Wistar albino rats. Furosemide (10 mg/kg, p.o.) and agar-agar (300 mg/kg, p.o.) were used as reference standards respectively for activity comparison. The chloroform and methanol extracts produced significant diuretic and laxative activity. On the other hand, the petroleum ether extract did not reveal significant activity. Urinary levels of sodium, potassium (by flame photometry) and chloride (by titrimetry) were estimated.

KEYWORDS: acute toxicity study, agar-agar, diuretic activity, furosemide, laxative activity, *Spondias pinnata*.

INTRODUCTION

Spondias pinnata (Linn. f) Kurz (Family: Rubiaceae) is a glabrous tree upto 10.5 m high with straight trunk and smooth ash coloured bark having pleasant aromatic smell found in the plain of Orissa, West Bengal and Bihar (1). In Ayurveda, the unripe fruits is believed to destroy "vata", enriches the blood and cures rheumatism. The leaves and bark are aromatic, astringent and useful in preventing vomiting, dysentery and diarrhoea (2, 3). The plant is reported to have anti-tubercular properties (4). The tribes of Orissa use the paste of the bark orally for treating diarrhoea in children. The paste is also used in adults for promoting diuresis in the adults. Reports on the biological activities on the bark is scarce. The present study was under taken to report the diuretic and laxative activities of the bark of *S. pinnata* and justify its use in the folklore remedies.

MATERIALS AND METHODS

Plant Material

The plant material (barks) was collected from the forests of Phulbani district of Orissa during June 2007 and identified by the taxonomists of the Botanical.

Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/I-I/(255)/2008Tech.II] has been kept in our research laboratory for further reference. After authentication, fresh barks were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extract

The powdered bark (500 g) was extracted successively with 2 lit each of petroleum ether (40–60° C), chloroform and methanol for 48 h in a soxhlet extractor. Following

extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods (5–6) were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Animals

Swiss albino mice (20–25 g) of either sex were used for acute toxicity study and adult Wistar albino rats (150–200 g) of either sex were used for evaluation of pharmacological studies. The animals were kept in standard polypropylene cages at room temperature of 34 ± 2 °C and at 60–65 % relative humidity during the experimental work. The institutional Animal ethics committee approved all the experimental protocols (Regd. No. 1212/ac/08/CPCSEA).

Acute toxicity study

The test was carried out as suggested by Ganapaty et al., 2002 (7). Selected animals were divided into different groups of six in each. The control group received normal saline (2 ml/kg, p.o.). The other groups separately received 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg/kg of the test extracts respectively in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Diuretic activity

The method of Lipschitz et al., 1943 (8, 9) was employed for the assessment of diuretic activity. In this method, male albino rats weighing between 150–200g deprived of food and water for 18 hours prior to the experiment, were divided into five groups of six rats in each. The animal groups were administered orally either with vehicle (1% Tween-80 in normal saline, 25 ml/kg) The first group of animals serving as control, received normal saline (25 ml/kg, p.o), the second group received furosemide (10 mg/kg, p.o) in saline (10); Group-III, IV and V received different extracts separately at doses of 300 mg/kg in a similar manner. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at $20^{\circ} \pm 0.5$ °C. The volume of urine collected was measured at the end of 5 h. During this period, no food and water was made available to animals. The parameters taken were the body weight before and after test period, total urine volume, concentration of Na^+ , K^+ and Cl^- in the urine. Na^+ and K^+ concentrations were determined by flame photometer and Cl^- concentration was estimated by

titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator (11–13). The results are depicted in Table I.

Laxative activity

The test was performed according to method of Bose et al., 2006 (14) on rats of either sex, fasted for 12 h before the experiment, but with water provided *ad libitum*. The animals were divided into five groups of six in each. The animal groups were administered orally with vehicle (1% Tween-80 in normal saline, 25 ml/kg), reference standard agar-agar (300 mg/kg, p.o.) in saline (7) and doses of extracts (300 mg/kg) in a similar manner. Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces. After 8 h drug administration, the faeces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 h (Table II).

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnett's t- test. A p-value less than <0.05 were considered to be significant. All the values were expressed as Mean \pm SEM.

RESULTS

Preliminary phytochemical screening

The results of the preliminary phytochemical screening of different extracts revealed presence of steroids, terpenoids, flavonoids, tannins, saponins and sugars in the test extracts.

Toxicological study

In acute toxicity study, it was found that the chloroform and methanol extracts induced sedation, diuresis, purgation, and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation.

Diuretic activity

Data represented in Table-I reveals that the chloroform and methanol extracts were produced significant increase in excretion of sodium, potassium and chloride ions at the tested dose level (300 mg/kg, p.o.). The order of activity of increase of urinary output was chloroform extract > methanol extract > petroleum ether extract. The order of activity of increase of urinary electrolytes

Table I: Diuretic Activity of Different Extract of Spondias Pinnata Bark.

Treatment	Dose	Urine Volume (ml)	Concentration of ions (mEq / l)			Na ⁺ / K ⁺ ratio
			Na ⁺	K ⁺	Cl ⁻	
Control	25 ml/kg	2.14 ± 0.15	52.33 ± 2.15	139.5±0.42	106.22±3.46	0.37
Furosemide	10mg/kg	7.16 ± 0.37**	96.33± 3.71**	163.67 ± 8.84**	136.96± 4.29**	0.58
Pet-Ether extract	300 mg/kg	2.72±0.22	58.5±2.09	135.16±0.98	110.67±4.25	0.43
Chloroform extract	300 mg/kg	4.7±0.29**	84.16±6.22**	159.67±1.78*	125.77±0.80**	0.52
Methanol extract	300 mg/kg	4.58±0.30**	74.83±1.24**	146.83±4.42	128.54±0.51**	0.50

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA;

*P<0.05

**P<0.01 when compared to control; Dunnet's

Table II: Laxative activity of different Extract of S.pinnata Bark.

Treatment	Dose	Faecal Output (g)	
		8h	8–16h
Control	—	8h	8–16h
Agar-agar	300mg/kg	0.848 ± 0.047	0.385 ± 0.055
Pet-Ether extract	300 mg/kg	1.053±0.046**	0.360 ± 0.041
Chloroform extract	300 mg/kg	0.735±0.045	0.321±0.028
Methanol extract	300 mg/kg	1.067±0.018**	0.336±0.034
		1.135±0.043**	0.232±0.031*

Values are expressed as mean ± S.E.M (n = 6). All columns are significant using ANOVA;

*P<0.05

**P<0.01 when compared to control; Dunnet's t-test.

excretion was found to be chloroform extract> methanol extract> petroleum ether extract.

Laxative activity

Results of the evaluation of laxative activity (Table-II) revealed that the chloroform and methanol extracts produced significant activity at the tested dose level (300 mg/kg, p.o.). The order of activity for various extracts was methanol extract>chloroform extract> petroleum ether extract.

DISCUSSION

Diuretics relieve pulmonary congestion and peripheral edema and are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure (15). Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that the chloroform extract and methanol extract of *S. pinnata* significantly increased the

urinary out put as well as urinary electrolyte concentration at a dose of 300 mg/kg, p.o. but the effect was found to be the less potent in increasing the urinary out put when compared with the reference standard. Further, the chloroform and methanol extracts were found to be more effective in enhancing urinary electrolyte concentration for all the three ions tested (Na⁺, K⁺, Cl⁻). Petroleum ether extract on the other hand did not increase urinary electrolyte concentration. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extracts increase sodium ion excretion to a greater extent than potassium, which is a very essential requirement of an ideal diuretic with lesser hyperkalaemic side effect.

The laxative activity study revealed significant activity of the chloroform and methanol extracts up to 8 h of drug administration. The methanol extract was found to be superior to that of the standard drug and petroleum ether extract was found to be least active.

Presence of phytoconstituents like flavonoids, terpenoids, saponins, have been previously found to be responsible for diuretic and laxative activities in plants (16–18). The presence of the said constituents in different extracts of *S. pinnata* may be responsible for the observed diuretic and laxative activities. The exact mechanism exhibited by the extracts can only be established after further investigation.

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