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Research Article

Studies on diuretic and laxative activity of bark extracts of *Neolamarckia cadamba* (Roxb.) Bosser

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ABSTRACT

The diuretic and laxative activity of various extracts of the barks of *Neolamarckia cadamba* (Roxb.) Bosser (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 methanol extract significantly increased the urinary out put as well as urinary electrolyte concentration at the tested dosage regimen that is comparable with the reference standard except in increasing the urinary out put. The chloroform extract produced significant laxative activity. Presence of different phytoconstituents in various extracts of *N. cadamba* may be responsible for the specific activities

Keywords: **A**cute toxicity, diuretic, laxative, *Neolamarckia cadamba*.

INTRODUCTION

Neolamarckia cadamba (Roxb.) Bosser, syn. *Anthocephalus cadamba* var *A. chinensis* (Family: Rubiaceae) commonly known as Kadam is a large tree up to 45 m high, frequently found in moist deciduous evergreen forests and widely distributed through out the greater part of India. The bark is gray, smooth in young trees, rough and longitudinally fissured in old trees[1]. The dried stem bark is used as folk medicine in the treatment of anemia, uterine complaints and for improvement of semen quality[2]. The stem bark is also reported to possess astringent, febrifuge and antiseptic properties and is given in cough[3-5]. Chlorogenic acid isolated from the leaves has been reported to possess hepatoprotective activity *in vitro* and lipid peroxidation in liver microsomes *in vivo*[6]. In our present study, we report diuretic and laxative activity of different extracts from the bark.

MATERIALS AND METHODS

Plant material

The plant material was collected from the herbal garden of Regional Plant Research Centre, Bhubaneswar in July 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 bark material was collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extract

The powdered barks (500 g) was extracted successively with 2 lit each of petroleum ether (40-60^{0}C), chloroform, methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them[7,8]. The results are shown in Table-I.

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 ^{0}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).

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Acute toxicity study

The test was carried out as suggested by *Ganapaty et al*.,[9]. Selected animals were divided into different groups of six in each. The control group received 1% Tween-80 in 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 was employed for the assessment of diuretic activity [10, 11]. 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[12]; Group-III, IV, V and VI 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 faces, kept at $20^0 \pm 0.5^0$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 [13-15]. The results are depicted in Table II.

Laxative activity

The test was performed according to method of *Bose et al* [16] 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 [9] 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 faces. After 8 h drug administration, the faces 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 III).

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 Dunnet's t- test. A p-value less than <0.05 were considered to be significant. All the values were expressed as Mean ± SEM.

RESULTS

The results of the preliminary phytochemical screening of different extracts revealed presence of steroids, terpenoids, flavonoids, tannins, alkaloids, saponins and sugars in the test extracts (Table-I). In acute toxicity study, it was found that the chloroform and methanol extract induced sedation, diuresis, purgation and temporary postural defect at all tested doses. However, there was no mortality in any of the extracts at tested doses till the end of 14 days of observation.

Diuretic activity

Data represented in Table-II reveal that the methanol extracts were produced significant increase in excretion of sodium, potassium and chloride ions at the tested dose level (300 mg/kg, p.o.), when compared to other extracts. The order of increased urinary output was found to be methanol extract > aqueous extract > chloroform extract > petroleum ether extract. However, the order of increased urinary electrolytes excretion was found to be higher in the methanol extract.

Laxative activity

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

DISCUSSION

Diuretics are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea by decreasing plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and blood pressure [17]. Thus, diuretics play an important role in hypertensive patients. In present study, the methanol extract of *N. cadamba* significantly increased the urinary out put as well as urinary electrolyte concentration at the tested dosage regimen that is comparable with the reference standard except in increasing the urinary out put. 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

Table I: Phytochemical screening of different extract of the bark of *Neolamarckia cadamba* (Roxb.) Bosser

Extract	Phytoconstituents present
Pet ether extract	Lipids, Steroids, Terpenoids.
Chloroform extract	Lipids, Steroids, Terpenoids, Flavonoids, Tannins, Saponins, Alkaloids.
Methanol extract	Flavonoids, Tannins, Saponins, Sugars
Aqueous Extract	Flavonoids, Tannins, Saponins, Sugars

Table II: Diuretic Activity of Different Extracts of *Neolamarckia cadamba* Barks.

Group	Treatment	Dose	Urine Volume(ml)	Concentration of ions (mEq/l) Na^+	K^+	Cl^-	Na^+/K^+ ratio
I	Control	25 ml/kg	3.05 ± 079	52.12 ± 2.86	141.72 ± 2.68	87.85 ± 3.88	0.36
II	Furosemide	10mg/kg	12.1± 2.28**	108.13 ± 3.71**	187.55 ± 1.98**	164.83 ± 6.89**	0.58
III	Pet-Ether extract	300 mg/kg	4.06±0.57	54.30 ± 2.09	143.32 ± 1.83	85.70 ± 2.59	0.37
IV	Chloroform extract	300 mg/kg	5.26± 0.92	72.13 ± 1.47**	151.07 ± 3.35	108.87 ± 2.96**	0.48
V	Methanol extract	300 mg/kg	10.45± 1.55**	101.05 ± 2.42**	182.55 ± 3.05**	130.61 ± 3.69**	0.55
VI	Aqueous extract	300 mg/kg	8.3± 1.61**	67.85 ± 4.22	147.50 ± 2.49	104.95 ± 1.69*	0.46

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 t-test.

Table III: Laxative activity of Different Extracts of *Neolamarckia cadamba* Barks.

Group	Treatment	Dose	Faecal Output (g) 8h	8-16h
I	Control	___	0.652±0.097	0.385 ± 0.055
II	Agar-agar	300mg/kg	1.053±0.046**	0.360 ± 0.041
III	Pet-Ether extract	300 mg/kg	0.747±0.075	0.321±0.028
IV	Chloroform extract	300 mg/kg	1.035±0.072**	0.336±0.034
V	Methanol extract	300 mg/kg	0.909±0.105	0.232±0.031*
VI	Aqueous extract	300 mg/kg	0.859±0.029	0.189±0.021**

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 t-test.

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 extract up to 8 h of observation. Simultaneously, the methanol and aqueous extracts were found to be the less potent 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 [18-20]. The presence of the said constituents in different extracts of *N. cadamba* may be responsible for the observed activities. The exact mechanism exhibited by the extracts can only be established after further investigation.

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