

STUDIES ON DIURETIC AND LAXATIVE ACTIVITY OF THE BARK OF *SALIX TETRASPERMA* ROXBURGH

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Article Received on: 07/11/10 Revised on: 25/11/10 Approved for publication: 04/12/10

ABSTRACT

Aqueous extract of *Salix tetrasperma* Roxburgh (Family: Salicaceae) was investigated for diuretic and laxative activity in albino rats that was compared with standard drugs Furosemide (10mg/kg, p.o.) and Agar agar (300mg/kg, p.o.), respectively. The extract was found to produce significant diuretic as well as laxative activity in dose dependant manner. The activities may be contributed to the phytoconstituents present.

KEYWORDS: *Salix tetrasperma*, acute toxicity study, agar-agar, diuretic activity, furosemide, laxative activity.

INTRODUCTION

The extracts of many plants used in traditional medicine contain curative agents that are used in many modern medicines. As part of the quest for potentially valuable plants of medicinal value.

Salix tetrasperma Roxburgh (Family: Salicaceae), commonly called Indian Willow, It is a medium sized tree of wet and swampy places, shedding the leaves at the end of monsoon season. The bark is rough, with deep, vertical fissures and the young shoots leaves are silky¹⁻⁴.

The dried leaves are reported to possess cardiogenic and neurotonic activity^{5,6}. The leaves and bark of the willow tree have been mentioned in ancient texts from Assyria, Sumer and Egypt⁷ as a remedy for aches and fever⁸. The decoction of both leaf and root is used for treating whooping cough in children⁹. The paste of both leaf and root is applied externally in scorpion stings, bug bites, for sores and warts¹⁰. The decoction of the dried root is taken orally for the treatment of hepatitis¹⁰. The sap of the stem is used orally by females for treating dysmenorrhea¹. The hot water extract of the entire plant is used in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum¹.

The aqueous extract of the stem bark has been reported to increase testosterone level in rats at 500.0 mg/kg, p.o.¹¹ and also accelerates semen coagulation in rats at a concentration of 2% w/v¹². A dose of 0.094 mg/kg of aerial parts shows hypothermic activity in mice¹³. Aqueous extract of dried leaf reported to possess cardiogenic activity and the methanol extract of the dried leaf possess reverse transcriptase inhibition effect¹⁴. Ethanolic extract of the aerial parts is reported to be inactive against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhosa*, *Escherichia coli*, *Candida albicans*, *Trichophyton mentagrophytes* etc^{15,16}.

However, only a few phytochemical have been reported on this plant in the literature like various types of sapogenins such as quinovic acid, salicortin, saligenin, phenolic glycosides and pyrocatechol was isolated from the barks and leaves¹⁷. The active extract of the bark, called salicin¹⁸ was isolated to its crystalline form. The entire plant is reported contain tannins, triterpenes, viz. β -amyryn, lupeol⁵ and chalcinasterol⁴, steroids viz. β -sitosterol and stigmasterol^{19, 20}. Whilst salicortin, saligenin and pyrocatechol can occur in quite large quantities in intact plant material^{21, 22} free salicylaldehyde seems to occur only in very low concentrations²³. However, salicylaldehyde may be formed from saligenin^{24, 25} by

the action of an oxidase once plant material is damaged²⁶⁻²⁸ both 6-HCH and catechol are potential contact allergens but do not appear to have yet been investigated for such activity in the context of sensitisation to *S. tetrasperma*.

Present study aims at exploring the details of diuretic and laxative action of aqueous extract of *S. tetrasperma* barks.

MATERIALS AND METHODS

Plant material

The plant material was collected from the herbal garden of Regional Plant Research Centre, Bhubaneswar in July 2009 and authenticated by the by the taxonomist Prof. Dr. S. K. Dash, Head of the Department of P.G. department of Biosciences. College of Pharmaceutical Sciences, Mohuda, Berhampur, Ganjam dist., Orissa-760002. A voucher specimen 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 the extract

The dried powdered material was macerated in distilled water: chloroform (9:1) to form an aqueous extract. The extract was concentrated to a small residue (10 gm) and the phytoconstituents in the extract were identified to be saponins, flavonoides, tannins and phenolic compounds by using standard methods^{29,30}. Furosemide (10mg/kg, p.o.) and agar-agar (300 mg/kg, p.o.) were used as reference standards where applicable.

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 diuretic and laxative 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.

Acute toxicity study

The acute toxicity of aqueous extract of *S. tetrasperma* barks was determined as per the CPCSEA guideline no. 420 (fixed dose method). It was observed that the test extract was not mortal even at 2000 mg/kg dose hence, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose was selected for further study.

Evaluation of diuretic activity

The method of *Lipschitz et al.*, 1943 was employed for the assessment of diuretic activity^{31,32}. In this method, albino rats of either sex weighing 150 to 200 gm were divided into four groups of six animals each. The animals were fasted for 24 hrs and water was given *ad libitum* during fasting. On the day of experiment 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 saline³³; Group-III and IV treated with aqueous extract (200 and 400 mg/kg) through oral route 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 ± 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 indicator³⁴. The results are depicted in **Table 1**.

Evaluation of laxative activity

The laxative activity was performed according to *Bose et al.* 2006³⁴ on rats of either sex, fasted for 12 hours before the experiment, but with water provided *ad libitum*. The animals were divided into four groups, each group consisting of six rats. The first group of animals, serving as control, received normal saline (25 ml/kg, oral); second group, serving as reference, received agar-agar (300 mg/kg, p.o.) in saline; the third and fourth groups received orally the test extract at doses 200 and 400 mg/kg respectively in a similar manner. Immediately after dosing, the animals were separately placed in specially designed plastic

containers suitable for collection of faeces. After 8 hours of 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 2**).

Statistical analysis

All the results were statistically analysed using one way ANOVA followed by Dunnet's t-test. *P<0.05 were considered significant.

RESULTS

In acute toxicity study, it was found that the aqueous extract of *S. tetrasperma* barks 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.

The treatment with aqueous extract (200 and 400mg/kg, p.o.) has significantly enhanced the volume of urine in a dose dependant manner that is comparable with response of the standard drug furosemide (10 mg/kg, p.o.). Simultaneously the urinary levels of Na⁺, K⁺ and Cl⁻ ions were significantly increased by the test extract but lesser than the standard drug. The results are compiled in the table-1.

In the evaluation of laxative activity, the aqueous extract was found to produce significant dose dependant activity at both the tested level of doses (200 and 400 mg/kg, p.o.). The effect was superior to that of standard drug (**Table-2**).

DISCUSSION

The present study revealed that, aqueous extract of *S. tetrasperma* barks significantly increased the urinary output as well as urinary electrolyte concentration in a dose dependant manner. Determination of urinary electrolyte concentration revealed that, aqueous extract was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na⁺, K⁺, Cl⁻). The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect. Similarly the aqueous extract of *S. tetrasperma* barks was found to produce significant laxative activity, in a dose dependent manner up to 8h of drug administration. The effect was found to be superior to that of the standard drug.

Presence of phytoconstituents like saponins, flavonoids have been previously found to be responsible for diuretic and laxative activities in plants³⁴. The presence of the said constituents in aqueous extract of *S. tetrasperma* barks 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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Table 1: Diuretic activity of aqueous extract of *S. tetrasperma* bark

Group	Treatment	Dose	Urine Volume (ml)	Concentration of ions (mEq / l)			Na ⁺ / K ⁺ ratio
				Na ⁺	K ⁺	Cl ⁻	
I	Control	25 ml/kg	2.85 ± 0.14	52.12 ± 2.11	141.72±2.68	87.85±3.71	0.36
II	Furosemide	10mg/kg	10.5± 0.27**	108.21± 4.11**	187.51 ±2.51**	130.61±4.33**	0.57
III	Aqueous extract	200 mg/kg	6.22±0.75**	72.11±1.98**	151.08±2.12*	108.87±3.25**	0.47
IV	Aqueous extract	400 mg/kg	9.14±0.85**	101.65±2.58**	182.55±2.85**	123.05±2.56**	0.55

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 2: Laxative activity of aqueous extract of *S. tetrasperma* barks

Group	Treatment	Dose	Faecal Output (g)	
			8h	8-16h
I	Control	25 ml/kg	0.86±0.014	0.313±0.060
II	Agar-agar	300 mg/kg	1.087±0.07**	0.303±0.025
III	Aqueous extract	200 mg/kg	1.148±0.158**	0.295±0.17
IV	Aqueous extract	400 mg/kg	1.285 ±0.179**	0.400±0.61

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

Source of support: Nil, Conflict of interest: None Declared