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Antiurolithiatic Activity of *Salix taxifolia* Aqueous Extract

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Abstract

The aqueous extract of the bark of *Salix taxifolia* was tested for antilithiatic and diuretic activities. Urolithiasis was experimentally induced by implantation of a zinc disc in the urinary bladder of rats. A significant decrease in the weight of the stones was observed after treatment in animals with the aqueous extract. This extract caused an increase in the 24 h urine volume.

Keywords: *Salix taxifolia*, antilithiatic activity, diuretic activity.

Introduction

Salix taxifolia H.B.K, commonly known as "taray", belongs to the Salicaceae. This plant is a popular folk remedy for the treatment of urolithiasis and a wide variety of urinary disorders in many areas of the Mexican states, such as Durango, Chihuahua and Nuevo Leon (Villamar, 1994). Previous investigation showed that no systematic study has been done for the above mentioned activity. The present study was undertaken to verify the efficacy of the aqueous extract of this plant as a diuretic and antilithiatic drug.

Materials and methods

Plant material

Fresh leaves of *Salix taxifolia* were used in this study. The plant material, collected in the state of Durango, was taxonomically identified at the Department of Botany of Universidad Autonoma de Mexico, Xochimilco, and a voucher specimen of the plant is deposited at the herbarium for reference.

Preparation of extract

The fresh leaves were dried in the shade and the completely dried leaves were powdered with an electric grinder. Powder (1 kg) was extracted for 6 h with 8 L of distilled water. The extract was then filtered, and evaporated to dryness *in vacuo* to give a brown residue (yield 90 g, 9%).

Animals

Wistar rats of either sex with body weight of 180–250 g were used. They were housed under standard animal husbandry conditions and had free access to pellet diet and tap water during the study.

Antilithiatic activity

Insertion of zinc disc

Rats were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.). A suprapubic incision was made and the urinary bladder was exposed. A small cut was made at the top of the bladder. The urine was then aspirated asptically into a sterile vial for bacteriological examination and pH determination (using narrow range pH paper BDH) (Sheth et al., 1972). Previously weighed sterile zinc discs were inserted into bladders (Vermeulen, 1962; Komer et al., 1951), and the incision was closed with a single suture using absorbable 4-0 chromic catgut (Ethicon). The abdomen was closed in layers and the rats were allowed to recover for one week (Ghosh et al., 2000). Food and water were given *ad libitum*.

The rats were divided into seven groups of ten animals each and placed on different treatment schedules: Group I = Normal untreated and unoperated control and were given water (5 ml/kg/day) for 60 days. Group II = Sham operated. Group III = Control (only foreign body implanted). Group

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IV = Foreign body implanted and treatment with the plant extract (10 mg/kg, p.o.). Group V = Foreign body implanted and treatment with 20 mg/kg p.o. plant extract. VI = Foreign body implanted and treatment with 40 mg/kg p.o. plant extract, VI = Foreign body implanted and treatment with 60 mg/kg p.o. plant extract. The rats were housed in metabolic cages (Nalgene) with food and water *ad libitum*. These groups of animals were treated with *S. taxifolia* aqueous extract for 60 days to asses the curative activity of the extract.

Diuretic activity

For the evaluation of diuretic activity, male albino rats weighing 200–250 g were selected; the tail base was pressed to empty the bladder of remaining urine. The control rats received 10 mg/kg saline by gastric gavage, while the treated groups received doses of 20, 40 and 60 mg/kg of herbal drug. Rats were kept separately in metabolic cages (Nalgene) and had free access to water but not to food. The urine was measured at 5 h and 24 h. All experiments were performed under standard conditions of temperature, light, humidity and noise (Anand et al., 1994). The results were statistically analysed by Student's *t*-test.

Examination of stones

Radiographical studies

Radiographical examinations were done before sacrificing the animals to confirm the formation of stones. The animal was kept under light ether anaesthesia in the anteroposterior position to expose the pelvic region, on a Pleodor 3 X-ray machine (Siemens India Ltd). The film was kept 100 cm away from the tube.

Weight of stones

The difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of 8 week period indicated the amount of deposited stone.

Statistical analysis

Results are expressed as mean ± SEM. Each parameter was analysed by one way analysis of variance considering the homogeneity of variance (Student's *t*-test) (Wayne, 1992).

Results

Implantantion of zinc foreign bodies into the urinary bladder induced growth of urinary stones and hypertrophy of the organ smooth musculature that were greater in males than in females. From 4 and 8 weeks after surgery, the weight of formed calculi in female rats increased by 1.31- (from 35.21 ± 2.91 mg to 46.34 ± 4.61) and 2.12-times (from 40.23 ± 1.4 mg to 85.6 ± 24.57 mg), while the organ weight increased by 2.1- and 4.5-fold (intact: 63.71 ± 3.98 mg), respectively. In males, the weight of the formed stones increased by 1.77- (from 42.81 ± 2.50 mg to 76.12 ± 29.84 mg) and 3.52-times (from 44.61 ± 1.09 to 157.46 ± 58.40 mg) after 4 and 8 weeks surgery, respectively. The weight of the urinary bladder in the latter group increased by 2.4- and 4.6-fold (Intact: 74.21 ± 3.98 mg), respectively. Urolith formation was confirmed by X-ray examination. All the animals produced stones on the inserted zinc disc. Qualitative analysis of the stone material formed in all the groups indicated it to be magnesium ammonium phosphate type with traces of calcium oxalate (Dietrich et al., 1990).

Oral treatment of rats with the extract of *S. taxifolia* during 8 weeks showed a dose-dependent reduction in the size of the stones was observed with 10, 20, 40 and 60 mg/kg doses. The 10 mg/kg treated group showed very little deposition of stone in comparison to the untreated control group. The aqueous extract of *S. taxifolia* (60 mg/kg p.o.) showed 90.63% reduction in the stone deposition as compared to the control group. With 20 and 40 mg/kg doses the reduction was found to be 42.5 and 66.0%, respectively (Table 1). These animals presented a smaller hypertrophy of the urinary bladder the organ weight than that determined in sham-operated animals.

The results of diuretic activity are presented in Table 2. The same dose given for antilithiatic study was tried for diuretic activity too. The dose of 60 mg/kg of the aqueous extract produced maximum volume of urine (13.51 ml). The pH of urine was found to be between 6–7 before implantation and 7 to 9 four weeks after implantation in both the control groups. After the treatment with the aqueous extract of *S. taxifolia*, the urinary pH was found to be between 6.5–7.5. Some urine samples examined after the study contained Gram-positive and Gram-negative organisms. The results of such animals were discarded.

Discussion and conclusion

The results obtained in this study indicate that the model selected for inducing urolithiasis of foreign body insertion technique using zinc discs is suitable and reproducible. The

Table 1. Effect of aqueous extract of *Salix taxifolia* (ST) on the weight of bladder stones in rats.

Treatment mg/kg	Increase of the wt. of stone (mg)	% Reduction
Control	21.46 ± 1.11	–
ST 10	16.32 ± 2.35*	23.93
ST 20	12.34 ± 1.5**	42.49
ST 40	7.28 ± 0.99*	66.0
ST 60	2.01 ± 0.78**	90.63

Values are mean ± SE. * $p < 0.05$, ** $p < 0.001$ vs. the control group.

Table 2. Diuretic activity of aqueous extract of *Salix taxifolia* (ST) on rats.

Treatment mg/kg	Urine volume ml/24 h
Control	4.68 ± 1.98
ST 20	8.16 ± 0.74*
ST 40	10.32 ± 0.82*
ST 60	13.51 ± 1.78*

Each value is the mean ± SE. * $p < 0.05$ vs the control group.

aqueous extract of bark of *Salix taxifolia* significantly inhibited the formation of stone in rats in a dose-dependent manner. The extract was effective in both male and female rats, and at a dose that reduced growth of urinary stones, it did not produce signs of toxicity or change in the spontaneous motor activity with up to 8 weeks administration. The experiments carried out on rats with zinc disc implants showed that stones formed in females were smaller than those formed in males. These observation are in accordance with other studies reporting less stone depposition on zinc disc in female than in male rats of which the main component is magnesium ammonium phosphate (Prasad et al., 1994).

There was a considerable decrease in the weights of stones produced by groups V and VI which received aqueous extract compared with that of group III. This extract showed an increase in the 24 h urine volume as compared to the control and water intake was normal and there was no significant body weight decrease during the treatment. The diuretic activity can prevent the deposition of calculis (Davidsohn & Henry, 1969) due to increased diuresis and excretion of urinary salt forming stones.

The present investigation showed that the plant extract markedly prevents the formation of urolith. This comfirms the utility of use of the plant in folk medicine against urolithiasis. Further study is in progress for identification of the active constituents of the plant.

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