EVALUATION OF THE ANTIUROLITHIATIC ACTIVITY OF THE EXTRACT OF *BOSWELLIA SERRATA* ROXB IN RATS

Abstract

In rats' urinary bladders, the antiurolithiatic action of a methanolic extract of Boswellia serrata Roxb (root) was examined on the development of calculi on calcium oxalate crystal implants or zinc disc implants. The plant is a member of the Burseraceous family, and it has long been used as a folk remedy for urinary issues and stone removal. When a foreign body was present in the urinary bladder of adult rats, it caused urinary stones and smooth muscle hypertrophy. After 4 weeks after surgery, oral therapy with Boswellia serrata Roxb extract (0.25 and 0.5 g/kg per day) decreased calculi development but did not prevent smooth muscle growth. In the presence and absence of the extract (0.3–3 mg/ml) or atropine (0.3–3 nM), the contractile responses of isolated urinary bladder preparations to the muscarinic agonist bethanecol were not different among the experimental groups.

Key words: Antiurolithiatic effect, Methanolic extract, *Boswellia serrata* Roxb

Introduction

Nephrolithiasis is the formation of renal stones within the kidneys. Urolithiasis is a disorder in which stones leave the renal pelvis and move to the remainder of the urinary system via the ureters, bladder, and urethra. Many individuals with urolithiasis can benefit from routine care, analgesics, and anti-emetic drugs; however, stones that cause obstruction, renal failure, or infection require more aggressive therapy. [1]

Increased urine pH and reduced urinary acid excretion worsen nephrolithiasis/urolithiasis, according to a recent research of 5000 persons with a history of kidney stones and insulin resistance. Increased weight associated to adiposity in adulthood has a negative impact on health, according to a prospective, large study that monitored participants over time and examined baseline weight, weight gain, dietary exposure, BMI, and waist circumference [1-8]. It is becoming more common, and it primarily affects people in their working years. Men are more likely to be present than women (10.6 % vs. 7.1 %). Obese and overweight people have more kidney stones than normal-weight people, according to studies, and when men and women were compared, fat was an equaliser in the formation of kidney stones. [13-14]

Boswellia serrata is a medicinal plant found in the dry deciduous woods of Maharashtra, Andhra Pradesh, Gujarat, Madhya Pradesh, Jharkhand, and Chhattisgarh in India [10]. Indian Olibanum, Shallaki, Salai Guggul, Gajabhakshya, and other names have been given to it [9]. It belongs to the Burseraceous family. Serrata refers to the serrated leaf edges and is derived from the word "serra," which meaning "saw."

The gum-resin of Boswellia serrata contains essential oils (8-12%), terpenoids (25-35%), and a greater amount of carbohydrates (45-60 percent). [8]. Some terpenes are volatile oils, such as thujene, phellandrene, phellandrene, and terpineol [9]. The resin also contains diterpene alcohol, tetracyclic triterpenic acid, and pentacyclic triterpenes [9-12].

Material and methods

Plant material

The dried powder of Boswellia serrata Roxb roots had no visible extraneous particles. The loss on drying was less than 10% w/w for Boswellia serrata Roxb roots, indicating that the plant material was fully dried. Total ash readings approaching less than 5.0 percent w/w reflect the physiological and non-physiological ash levels in plant material. The acid insoluble ash values were less than 1.0 percent w/w, suggesting that no silicious material, such as sand or clay, was present in the roots of Boswellia serrata Roxb. The extractive values for both plants and Boswellia serrata Roxb roots were found to be greater than 3.0% w/w for polar solvents (such as alcohol and water) and less than 1.0 percent w/w for non-polar solvents.

A technique devised by Atmani F et al. was used to investigate antiurolithiatic activity in albino rats (2003). Rats developed experimentally induced hyperoxaluria after 14 days of continuous oral treatment with 0.75 percent v/v ethylene glycol in drinking water. Microscopic analysis of urine taken on the 14th day from randomly selected groups revealed typical calcium oxalate (CaOx) and calcium phosphate crystals under 50x, 100x, and 450x magnifications (CaPh). Curative therapy with crude extracts also caused significant alterations in serum and renal biochemistry, as well as renal histology markers.

Experimental groups

Antiurolithiatic activity was assessed in Wistar albino rats of either sexe weighing between 150 and 200 g. The rats were housed on a 12:12 h light:dark cycle and were acclimated to typical laboratory temperatures (22°C). They were provided free rat food and sanitised water from the Veterinary College Mahu in MP. After 8 weeks, all animals were euthanized under severe ether anaesthesia, and the weights of the urinary bladder and generated stones were measured. Fasting rats were kept in individual cages, administered with either tap water or the plant extract (0.5 g/kg, p.o.), and urine was collected every 2 hours for 12 hours to test diuretic activity. During the collecting interval, all animals were given free access to water.

Pharmacological studies

Following the removal of the calculi, longitudinal strips of the urinary bladder (2 to 3 mm wide) were placed in organ baths containing gassed Kreb's solution at 35°C with the following composition (mM): pH 7.4 after gassing with 95 percent O2; NaCl (117.9), NaHCO3 (25.0), Glucose (11.0), KCl (4.7), CaCl2 (1.30), MgSO4 (1.2), NaH2PO4 (1.2);

Cumulative concentration-response curves for bethanecol (Beth, 0.1 nM–0.1 mM) were produced before to and after 15–20 min incubation with atropine (0.1–3 nM).

The EC50 (mean effective concentration) value was determined for each concentration-response curve, and the dose-ratio (DR) was calculated as the relationship between EC50 in the presence and absence of the antagonist. A best-fit line was created using linear regression using the approach of least squares utilizing a logarithm plot of DR-1 against antagonist concentrations.

Drugs

Carbamyl-methyl-choline chloride, atropine sulfate (Sigma) and zinc granules (Labsynth-BR) were 99.8% pure. All other reagents were of analytical grade (Merck, India).

Statistics

Results were expressed as means 9 SEM. EC_{50} values were expressed as geometric means and 95% confidence limits (CL). Differences among data were determined using ANOVA followed by the Tukey– Kramer test. Differences between the data were considered significant at P B 0.05.

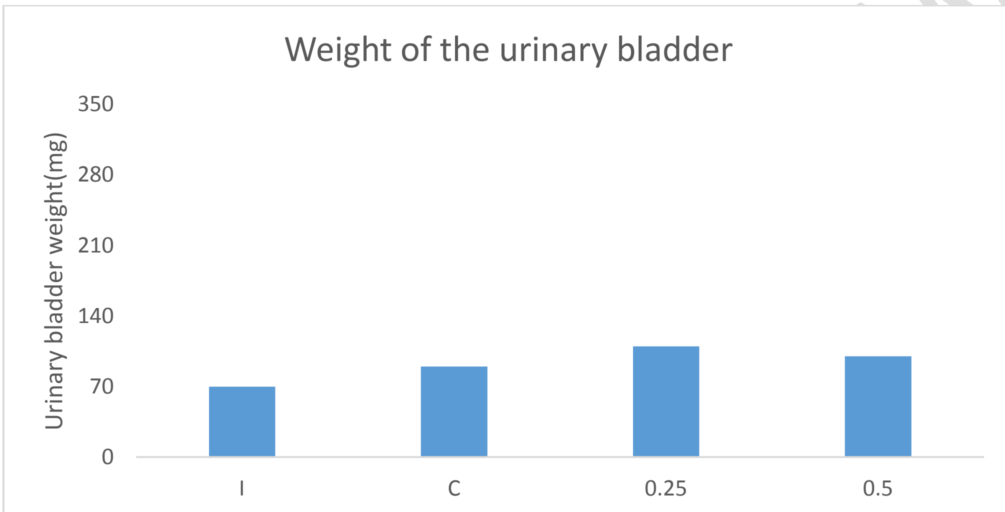
Results

Effects on stones growth and urinary bladder

Oral administration of the MEBS (0.5 g/kg per day) to intact, sham-operated, or operated rats for insertion of a foreign body for 8 weeks had no effect on their body weights when compared to the appropriate control value (Intact: 204 g; sham-operated: 210 g; operated: 250 g).

After 8 weeks of surgery, female rats with calcium oxalate crystals implanted in their urinary bladders developed urinary stones and had their smooth musculature enlarged. The weight of the urinary bladder rose 2.5-fold (from 83.545.14 mg to 211.7229.90 mg) in control mice, whereas the size of the produced stones increased 14 times (from 5.45 0.10 mg to 83.2643.47 mg) (Fig. 1).

Treatment of sham-operated rats with the plant extract (0.25 and 0.5 g/kg per day, p.o.) during 4 weeks did not change the weight of the urinary bladder (90.87±7.56 mg and 84.10±7.35 mg, respectively) compared to the control group (83.44±4.25 mg). Administration of the plant extract (0.25 and 0.5 g/kg per day, p.o) to rats with implants of calcium oxalate crystals, for 8 weeks.



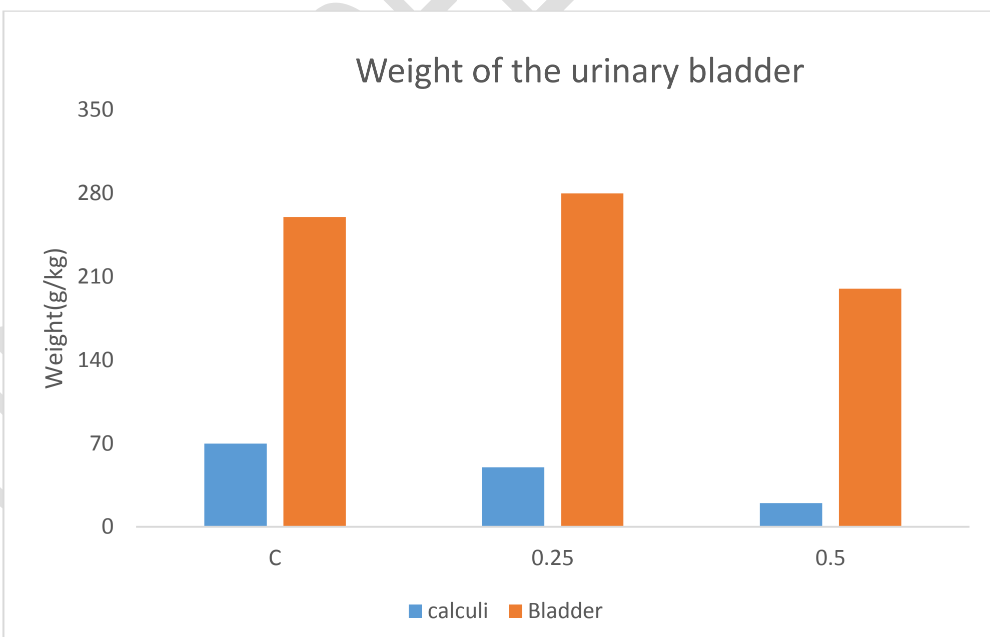


Fig. 1. (A) Weight of the urinary bladder of intact (I) and sham-operated rats given orally (p.o.) tap water (control, C) or MEBS (Methanolic extract of *Boswellia serrata*) (0.25 and 0.5 g/kg/day) during 8 weeks.

(B) Weight of the urinary bladder and calculi formed on calcium oxalate crystals implanted in rats given tap water or MEBS (Methanolic extract of Boswellia serrata) (0.25 and 0.5 g/kg/day), p.o., during 8 weeks. Columns and vertical bars are means 9 SEM of six to eight animals. * Different from Control (P B 0.05)

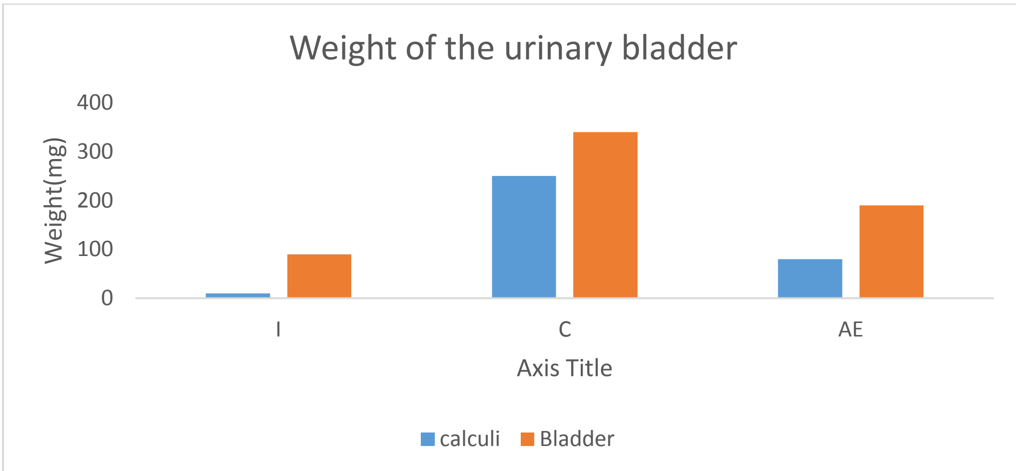


Figure 2: After 8 weeks of surgery, the weight of intact male rats' urinary bladders (I) and calculi developed on zinc disc foreign bodies implanted in animals fed tap water or MEBS (Methanolic extract of Boswellia serrata) (0.5 g/kg/day), p.o. The vertical bars and columns represent the 9 SEM of seven to fifteen animals. * Not to be confused with control (P B 0.05).

Prevented the formation of urinary stones, whose weights were lowered by 44 percent (47.40 33.63 mg) and 91 percent (7.37 2.20 mg) compared to control values, respectively (Fig. 1). These animals' urine bladder weights (251.60 90.86 mg and 151.509 3.01 mg, respectively) did not alter when compared to control values (Fig. 1).

The introduction of zinc foreign bodies into the urinary bladders of control rats resulted in the formation of urinary stones and enlargement of the smooth muscle of the organ, with males experiencing more of these effects than females. The weight of developed calculi in female rats grew by 1.2 (from 37.37 2.08 mg to 46.70 4.51 mg, n = 6) and 2.3 times (from 42.20 0.67 mg to 95.17 32.52 mg, n = 6), respectively, after 8 weeks after surgery, whereas the organ weight increased by 2.4 and 4.3 times (intact: 63.41 3.10 mg). In males, the weight of the formed stones increased by 1.8 (from 39.40+_0.32 mg to 73.61 ±31.71 mg, n = 6) and 3.7 times (from 44.00 ±0.90 to 159.20 ±54.02 mg, n = 6) after 4 and 8 weeks' surgery, respectively (Fig. 2). The weight of urinary bladder in the latter group increased by 2.5 and 4.5 fold (Intact: 78.30 ±4.40 mg), respectively.

Oral administration of Boswellia serrata extract (0.5 g/kg per day, p.o.) to male rats for four weeks had no effect on urinary bladder weights when compared to control values (intact: 75.3 4.4 mg; sham-operated: 91.4 3.5 mg). Administration of the plant extract (0.5 g/kg per day, p.o.) for four weeks decreased the weight of calculi produced by 76% (from 239.3 46.8 mg to 55.50 8.81 mg) in mice implanted with zinc discs (Fig. 2). These mice had a smaller hypertrophied urinary bladder, with organ weights that were 47 percent lower (from 323.1 44.8 mg to 164.4 18.0 mg) than sham-operated animals (Fig. 2).

Effects on diuresis

At a doses that reduced growth of urinary stones, the MEBS (Methanolic extract of *Boswellia serrata*) (0.5 g/kg, p.o.) did not change the urinary volume of rats deter- mined after 12 h administration when compared to control values (1.4 ±0.6 ml) (Fig. 3).

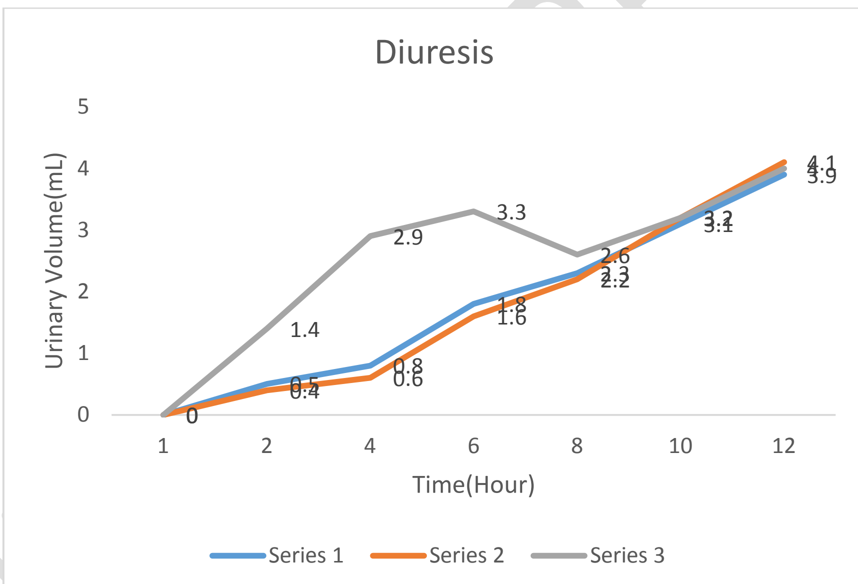


Fig. 3. Diuresis determined in intact rats given orally tap water (control), MEBS (Methanolic extract of *Boswellia serrata*) (0.5 g/kg) or furosemide (20 mg/kg). The urinary volumes were collected every 2 h during 12 h. Symbols are means 9 SEM of eight to 15 animals. * Different from control (P B 0.05).

Table 1: Antagonist affinities (pA2) and slopes from Arunlakshana– Schild plots at muscarinic receptors of the rat urinary bladder

Group	PA2	SLOPE

Intact	8.48(8.30-8.71)	0.70(0.86-0.89)
Control	8.36(8.31-8.44)	0.87(0.83-0.91))
Control Sham-Operated	9.83(8.53-8.61)	0.86(0.75-0.88)
Sham Added (MEBS)0.5g/kg	6.47(8.29-8.47)	0.77(0.78-0.81)
(MEBS)0.5g/kg	8.40(8.27-8.74)	0.79(0.66-0.87)

Data are geometric means and 95% confidence limits of six to 13 preparations. Uretic furosemide (20 mg/kg, p.o.), used for positive control, the urinary volume was increased to 3.2 ±0.6 ml after 6 h (Fig. 3).

Effects on the urinary bladder smooth musculature

Increased bethanecol concentrations caused contractions in the isolated rat urine bladder that were proportionate to the concentrations. All groups had similar mean EC50 values for bethanecol (sham-operated: 1.51 105 M, CL: 1.27 – 1.80 105 M; control operated: 2.33 105 M, CL: 1.84 – 2.95 105 M). Atropine (0.3–3.0 nM) incubation resulted in a proportionate and parallel rightward shift of the concentration-response curves to the agonist without affecting the peak response. The experimental groups did not differ in their pA2 values for atropine in the identical preparations (Table 1).

In a few experiments, exposure of the isolated rat urinary bladder of intact animals to the plant extract (0.3–3 mg/ml) did not affect the concentration-response curves to bethanecol. A high concentration of the extract (10 mg/ml), however, depressed the agonist contractile effect on the urinary bladder smooth musculature in a noncompetitive manner.

Discussion

The MEBS (Methanolic extract of Boswellia serrata) prevented the development of stones in rats generated by calcium oxalate crystal or zinc disc implants in the urinary bladder, according to the findings. Up to 8 weeks after treatment, the extract was beneficial in both male and female rats, and at a level that decreased the formation of urinary stones, it produced no evidence of toxicity or changes in spontaneous motor activity.

Experiments on rats implanted with zinc discs revealed that stones created in females were smaller than those formed in males, which is why the extract was examined in males. These observations are in accordance with other studies reporting less stone deposition on zinc disc in female than in male rats of which the main component is magnesium ammonium phosphate [12-13].

At a dose that reduced stone formation the extract of MEBS (Methanolic extract of *Boswellia serrata*) did not affect the urinary volume indicating that the antiurolithiatic effect was apparently unrelated to increased diuresis and excretion of stones forming salt. In fact, increase of diuresis could reduce super saturation of the urine with precipitating substances which is normally associated with formation of urinary calculi [13-14].

In both control and treated animals with the plant extract, formation of urinary stones was accompanied by a proportional hypertrophy of the urinary bladder smooth musculature. Such effect indicates increased contraction of the musculature probably to overcome obstruction of the bladder outlet by the formed calculi. Partial obstruction of the urinary bladder outlet leads to a compensatory growth of the detrusor smooth muscle cells, and occurs as a response to the increased intravesical pressure required to empty the bladder [11-14]. Partial obstruction of the urinary bladder was also shown to induce a decrease in the density of autonomic innervation and sensitivity to the muscarinic agonist bethanecol [10-14], the effect being related to the degree of muscle hypertrophy [12]. The presented data, however, did not show significant changes of either EC_{50} values of bethanecol or pA_2 values of atropine in both control and extract treated animals with zinc disc implants. These observations indicate that the calculi developed and consequent hypertrophy of the bladder smooth musculature was not great enough to affect the muscarinic receptor affinity for both agonist and antagonist.

Conclusion

The presented data indicate that administration of the MEBS (Methanolic extract of *Boswellia serrata*) to rats with experimentally-induced urolithiasis reduced growth of urinary stones, supporting folk information regarding the plant antiurolithiatic activity. The mechanisms underlying this effect are still unknown, but are apparently unrelated to increased diuresis and excretion of urinary salt forming stones. Despite hypertrophy of the urinary bladder associated with the calculi formed, the muscarinic receptor affinity for cholinergic ligands remained unchanged.

Ethical approval

The experimental methods and animal care were in accordance with the guidelines set out by the committee for the regulation and supervision of animal studies (CPCSEA). The study was authorised by the university's animal ethics committee (IAEC).

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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