

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

### Abstract

The antiurolithiatic effect of a methanolic extract of *Boswellia serrata* Roxb (root) on the formation of calculi on calcium oxalate crystal implants or zinc disc implants was studied in rats' urinary bladders. The plant belongs to the Burseraceae family and has long been used as a folk treatment for urinary problems and the removal of stones. Urine stones and smooth muscle hypertrophy were produced in adult rats when a foreign body was present in their urinary bladder. Oral treatment with *Boswellia serrata* Roxb extract (0.25 and 0.5 g/kg per day) after 4 weeks of surgery reduced the production of calculi but did not prevent hypertrophy of the smooth muscle of the organ. 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

The production of renal stones within the kidneys is known as nephrolithiasis. Urolithiasis is a condition that occurs when stones leave the renal pelvis and travel via the ureters, bladder, and urethra to reach the rest of the urinary system. Expectant care, analgesics, and anti-emetic medications can help many patients with urolithiasis; however, stones that cause blockage, renal failure, or infection require more severe treatment. [1]

Renal stones occur in the kidneys, which is known as nephrolithiasis. Urolithiasis is a condition in which stones leave the renal pelvis and migrate through the ureters, bladder, and urethra to the remainder of the urinary system. Expectant management, analgesics, and anti-emetic medications can help many people with urolithiasis, but stones that cause blockage, renal failure, or infection require more active therapy.

A recent study of 5000 people with a history of kidney stones and insulin resistance found that increased urine pH and impaired urinary acid excretion enhance nephrolithiasis/urolithiasis. According to a prospective, large study that tracked people over time and measured baseline weight, weight gain, dietary exposure, BMI, and waist circumference, increased weight related to adiposity in adulthood has a crucial role in symptomatic stone development. [2-7]

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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]

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The medicinal plant *Boswellia serrata* is found in India's dry deciduous forests of Maharashtra, Andhra Pradesh, Gujarat, Madhya Pradesh, Jharkhand, and Chhattisgarh (Upaganlawar & Ghule 2009). It's also known as Indian Olibanum, Shallaki, Salai Guggul, Gajabhakshya, and other names (Shah et al. 2009b). It is a member of the Burseraceae family. Dr. James Boswell of the Edinburgh Botanical Garden and William Roxburgh, Director of the Indian Botanical Garden in Calcutta are given the genus name "Boswellia." The term "serrata" derives from the word "serra," which means "saw," and refers to the toothed leaf margins.

*Boswellia serrata*'s gum-resin comprises essential oils (8-12%), terpenoids (25-35%), and a higher percentage of polysaccharides (45-60%). [8]. Some terpenes, such as thujene, phellandrene, phellandrene, and terpineol, are volatile oils [9]. Diterpene alcohol, tetracyclic triterpene acid, and pentacyclic triterpenes are also found in the resin [9-12].

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## Material and methods

### Plant material

There were no obvious foreign particles in the dried powder of *Boswellia serrata* Roxb roots. The loss on drying for *Boswellia serrata* Roxb roots was less than 10% w/w, indicating that the plant material was thoroughly dried. The physiological and non-physiological ash content in the plant material (was ) is indicated by total ash values nearly less than 5.0 % w/w. The acid insoluble ash values were less than 1.0 % w/w, indicating that the roots of *Boswellia serrata* Roxb do not include any silicious material such as sand or clay. The extractive values for both plants and *Boswellia serrata* Roxb roots with comparatively non-polar solvents were found to be more than 3.0 % w/w for polar solvents (such alcohol and water) and less than 1.0 % w/w for non-polar solvents.

Antiuro lithiatic activity in albino rats was assessed using a method developed by Atmani F et al. (2003). After 14 days of continuous oral treatment of 0.75 % v/v ethylene glycol in drinking water, rats developed experimentally induced hyperoxaluria. Under 50x, 100x, and 450x magnifications, microscopic examination of urine collected on the 14th day from randomly

selected groups revealed typical crystals of calcium oxalate (CaOx) and calcium phosphate (CaPh). Furthermore, curative treatment with crude extracts resulted in notable changes in serum and renal biochemistry, as well as renal histology parameters. **NOT SURE THIS IS NECESSARY HERE PLEASE**

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### **Experimental groups**

For the acute oral toxicity investigation, healthy Wistar albino rats of either sex weighing between 150 and 200 g were employed, while male Wistar albino rats of either sex weighing between 150 and 200 g were used for antiurolithiatic activity evaluation. The rats were acclimated to regular laboratory temperatures (22°C) and were kept on a 12:12 h light:dark cycle. They were given free access to rat food (Veterinary College Mahu, MP) and purified water. The experimental techniques and animal care were in conformity with the committee for the control and supervision of animal studies (CPCSEA). The study was approved by the animal ethics committee at the university (IAEC).

All animals were killed by deep ether anesthesia after 8 weeks after surgery, and the weights of the urinary bladder and produced stones were determined. 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**

After the calculi were removed, longitudinal strips (2 to 3 mm wide) of the urinary bladder were mounted in organ baths containing gassed Kreb's solution at 35°C with the following composition (mM): NaCl (117.9), NaHCO<sub>3</sub> (25.0), Glucose (11.0), KCl (4.7), CaCl<sub>2</sub> (1.30), MgSO<sub>4</sub> (1.2), NaH<sub>2</sub>PO<sub>4</sub> (1.2); pH 7.4 after gassing with 95 percent O<sub>2</sub>

Prior to and after 15 – 20 min incubation of atropine (0.1 – 3 nM), cumulative concentration-response curves [13] were built to bethanecol (Beth, 0.1 nM–0.1 mM).

For each concentration-response curve, the EC<sub>50</sub> (mean effective concentration) value was measured, and the dose-ratio (DR) was computed as the relationship between EC<sub>50</sub> 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. According to Arunlakshana and Schild, the abscissae intercept (pA<sub>2</sub> value) and slopes were calculated.

## **Drugs**

Carbamyl-b-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  $\pm$  SEM. EC<sub>50</sub> 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  $\leq$  0.05.

## **Results**

### ***Effects on stones growth and urinary bladder***

Oral administration of the MEBS (0.5 g/kg per day), during 8 weeks, to intact, sham-operated or operated rats for insertion of foreign body did not affect the body weights of either animal group compared to the respective control value (Intact: 204 g; sham-operated: 210 g; operated: 250 g).

Implantation of calcium oxalate crystals in the urinary bladder of female rats induced growth of urinary stones and hypertrophy of the organ smooth musculature after 8 weeks of surgery. In control animals, the formed stones increased by 14 times (from 5.45  $\pm$  0.10 mg to 83.26  $\pm$  43.47 mg), while the urinary bladder weight increased by 2.5-fold (from 83.54  $\pm$  5.14 mg to 211.72  $\pm$  29.90 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  $\pm$  7.56 mg and 84.10  $\pm$  7.35 mg, respectively) compared to the control group (83.44  $\pm$  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.

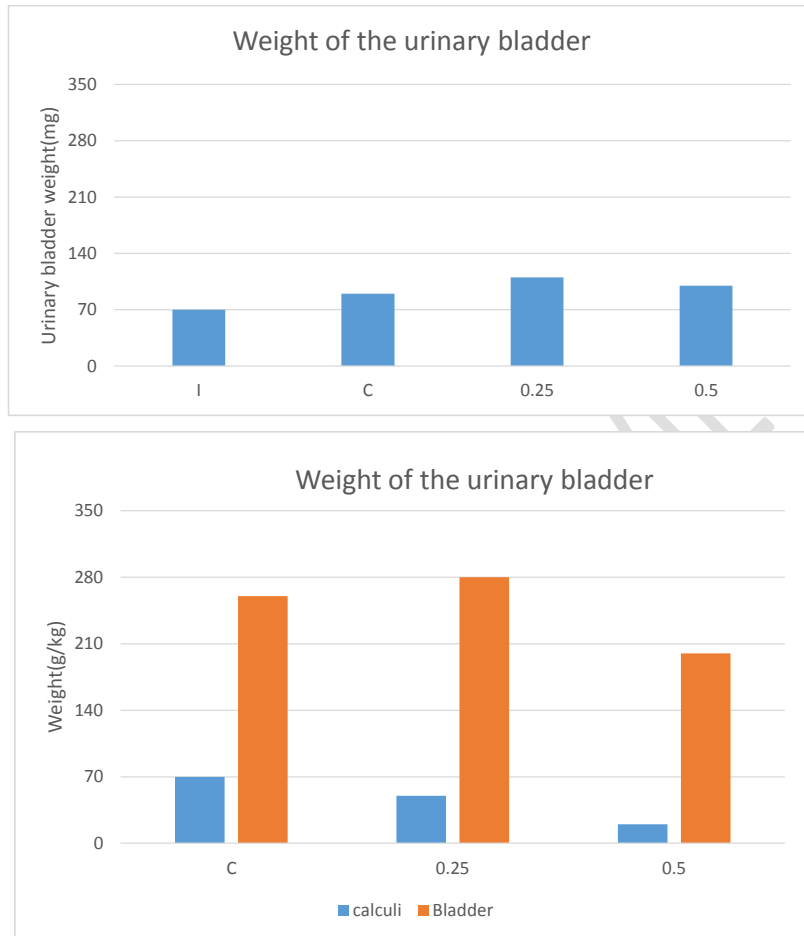


Fig. 1. (A) Weight of the urinary bladder of intact (I) and sham-operated rats given orally (p.o.) tap water (control, C) or MEBS (Methanolic extract of *Boswellia serrata*) (0.25 and 0.5 g/kg/day) during 8 weeks.

(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  $\pm$  SEM of six to eight animals. \* Different from Control (P < 0.05)

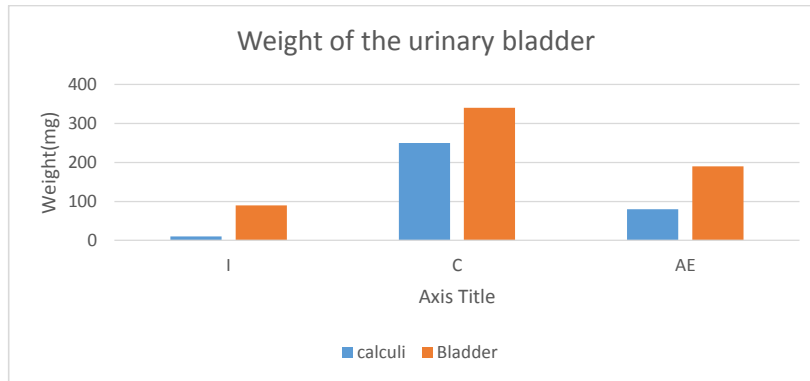


Fig. 2. Weight of the urinary bladder of intact male rats (I) and calculi formed on zinc disc foreign bodies implanted in animals given tap water or MEBS (Methanolic extract of *Boswellia serrata*) (0.5 g/kg/day), p.o., after 8 weeks' surgery. Columns and vertical bars are means  $\pm$  SEM of seven to 15 animals. \* Different from control (P < 0.05).

Prevented the growth of urinary stones, which weights were reduced by respectively, 44% ( $47.40 \pm 33.63$  mg) and 91% ( $7.37 \pm 2.20$  mg) of control values (Fig. 1). The urinary bladder weights ( $251.60 \pm 90.86$  mg and  $151.509 \pm 3.01$  mg, respectively) of these animals, however, did not change compared to control values (Fig. 1).

Implantation of zinc foreign bodies into the urinary bladder of control rats also induced growth of urinary stones and hypertrophy of the organ smooth musculature that were greater in males than in females. After 8 weeks' surgery, the weight of formed calculi in female rats increased by 1.2 (from  $37.37 \pm 2.08$  mg to  $46.70 \pm 4.51$  mg, n = 6) and 2.3 times (from  $42.20 \pm 0.67$  mg to  $95.17 \pm 32.52$  mg, n = 6), while the organ weight increased by 2.4- and 4.3-fold (intact:  $63.41 \pm 3.10$  mg), respectively. In males, the weight of the formed stones increased by 1.8 (from  $39.40 \pm 0.32$  mg to  $73.61 \pm 31.71$  mg, n = 6) and 3.7 times (from  $44.00 \pm 0.90$  to  $159.20 \pm 54.02$  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:  $78.30 \pm 4.40$  mg), respectively.

Oral treatment of male rats with the extract of *Boswellia serrata* (0.5 g/kg per day, p.o.) during 4 weeks did not alter the urinary bladder weights compared to control values (intact:  $75.3 \pm 4.4$  mg; sham-operated:  $91.4 \pm 3.5$  mg). In those animals submitted to zinc disc implants, administration of the plant extract (0.5 g/kg per day, p.o.) during 4 weeks, reduced the weight of calculi formed by 76% (from  $239.3 \pm 46.8$  mg to  $55.50 \pm 8.81$  mg) (Fig. 2). These animals

presented a smaller hypertrophy of the urinary bladder the organ weight being 47% lower than that determined in sham-operated animals (from 323.1 ±44.8 mg to 164.4 ±18.0 mg) (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 determined after 12 h administration when compared to control values (1.4 ±0.6 ml) (Fig. 3).

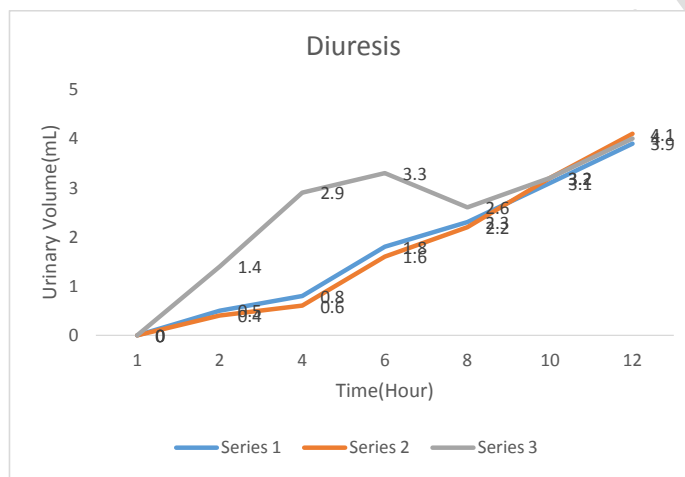


Fig. 3. Diuresis determined in intact rats given orally tap water (control), MEBS (Methanolic extract of *Boswellia serrata*) (0.5 g/kg) or furosemide (20 mg/kg). The urinary volumes were collected every 2 h during 12 h. Symbols are means  $\pm$  SEM of eight to 15 animals. \* Different from control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

**Table 1: Antagonist affinities (pA<sub>2</sub>) and slopes from Arunlakshana– Schild plots at muscarinic receptors of the rat urinary bladder**

Group	PA <sub>2</sub>	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  $3.2 \pm 0.6$  ml after 6 h (Fig. 3).

#### **Effects on the urinary bladder smooth musculature**

Addition of increasing concentrations of bethanecol ( $10^{-8}$ – $10^{-2}$  M) produced contractions of the isolated rat urinary bladder proportional to the concentrations. The mean  $EC_{50}$  values of bethanecol determined in those preparations did not significantly change among all groups (sham-operated:  $1.51 \times 10^{-5}$  M, CL:  $1.27 - 1.80 \times 10^{-5}$  M; control operated:  $2.33 \times 10^{-5}$  M, CL:  $1.84 - 2.95 \times 10^{-5}$  M). Incubation of atropine (0.3–3.0 nM) caused a proportional and parallel rightward shift of the concentration-response curves to the agonist without changing the maximal response. The  $pA_2$  values determined for atropine in the same preparations did not differ among the experimental groups (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 present study showed that the MEBS (Methanolic extract of *Boswellia serrata*) reduced formation of stones in rats induced by either calcium oxalate crystal or zinc disc implants in the urinary bladder. 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 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, the reason why the extract was tested in the latter animals. 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<sub>50</sub> values of bethanecol or pA<sub>2</sub> 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 water 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.

## COMPETING INTERESTS 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.

**Comment [17]:** THE DISCUSSION SHOULD BE DONE STEP BY STEP WISE, PROVIDING MORE ROBUST EVIDENCE BASED REASONS FOR DISAGREEMENT OR AGREEMENT WITH THE PREVIOUS WORKS.. IT IS PROBABLY POORLY DISCUSSED

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**Comment [18]:** If this is not the referencing style of your journal, I strongly suggest the author should look at the referencing again and make corrections

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