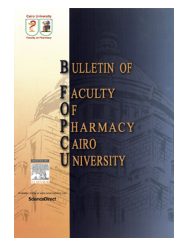




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ORIGINAL ARTICLE

Protective effect of standardized extract of *Biophytum sensitivum* against calcium oxalate urolithiasis in rats



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KEYWORDS

Biophytum sensitivum;
Calcium oxalate;
Ethylene glycol;
Urolithiasis

Abstract *Objective:* The present study investigated the antiurolithiatic effect of standardized methanolic extract of *Biophytum sensitivum* (MBS) against calcium oxalate urolithiasis in rats.

Methods: The MBS was standardized for amentoflavone content by HPLC method. Calcium oxalate urolithiasis was induced in rats by ethylene glycol–ammonium chloride feeding in drinking water. Antiurolithiatic activity of MBS was evaluated at three doses (100, 200 and 400 mg/kg) in curative and preventive regimen by estimating histological changes in kidney and biochemical changes in urine, serum and kidney homogenate. Cystone (500 mg/kg, p.o.) was used as reference standard drug.

Results: Ethylene glycol–ammonium chloride feeding caused an increase in urinary volume, oxalate, total protein, phosphate and uric acid levels, along with a decrease in urinary excretion of calcium, magnesium and citrate. Supplementation with MBS significantly prevented change in the urinary excretion of calcium, oxalate, phosphate, total protein, uric acid, magnesium and citrate. The MBS supplementation prevented the elevation of serum creatinine, uric acid and blood urea nitrogen levels. The increased calcium, oxalate and phosphate levels in the kidney of lithiatic control rats were significantly reduced by the MBS supplementation. The MBS supplementation caused a significant decrease in the lipid peroxidation of kidney tissue. Histological study revealed minimum damage and less number of calcium oxalate deposits in the kidneys of MBS-treated rats. *Conclusions:* These results indicated that the MBS reduced and prevented the growth of urinary stones. However, cystone (500 mg/kg) is more effective than the MBS (400 mg/kg) in alleviating the urolithiasis. This finding supports the traditional use of *B. sensitivum* for urolithiasis.

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1. Introduction

The incidences of urolithiasis have increased across the globe in the last five decades and now urolithiasis has been rated as third prevalent disorder in the urinary system. Urinary calculi may cause serious medical consequences such as obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system if left untreated.¹ Approximately 80% of these calculi are composed of calcium oxalate and phosphate.² In modern medicine, surgical operation, lithotripsy and local calculus disruption using high-power laser are common treatment options to remove the calculi. However, these procedures are associated with serious complications such as acute renal injury, decreased renal function, increased stone recurrence, etc.³ The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years which is the most important aspect to be addressed.⁴

Medicinal plants remain an important alternative source of new drugs. In the indigenous systems of medicine, a number of plants have been claimed to be efficient to cure and correct urinary stones.⁵ These plants and their products are also reported to be effective in the treatment as well as prevention of recurrence of renal calculi with minimal or no side effects.⁶ As per the Indian traditional system of medicine, Ayurveda, the whole plant of *Biophytum sensitivum* is claimed for its usefulness in the treatment of various health ailments including urinary stones.^{7,8} *B. sensitivum* (Family: Oxalidaceae) is distributed in tropical Asia, Africa, America and Philippines. It is found up to an altitude of 1800 m.⁸ It has been extensively studied for its biological activities and therapeutic potentials such as analgesic, anti-pyretic, anti-inflammatory, immunomodulatory, antitumor, antidiabetic, antioxidant, antibacterial, anti-hypertensive, chemoprotective, radioprotective, antifertility, etc.⁹ However, so far no scientific study has been reported regarding the antiurolithiatic potential of the plant. In this study, we investigated antiurolithiatic activity of standardized whole plant extract of *B. sensitivum* against calcium oxalate urolithiasis and extrapolated its possible underlying mechanisms using male Wistar albino rats.

2. Materials and methods

2.1. Animals

Male Wistar albino rats weighing between 150 and 250 g were used for the study. They were procured from National Institute of Biosciences, Pune, India. The animals were allowed for acclimatization for ten days under standard conditions in the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India) approved animal house of MAEER's Maharashtra Institute of Pharmacy, Pune, India. The animals were given standard diet supplied by Nutrivet Life Sciences, Pune, India. The study protocol was approved by the Institutional Animal Ethics Committee (Ref. No.: MIP/IAEC/2013-14/M1/Appr/003) of MAEER's Maharashtra Institute of Pharmacy, Pune, India.

2.2. Chemicals and apparatus

Amentoflavone (Sigma-Aldrich, Germany; B. No.: BCBD3849V), ethylene glycol (Qualigens Fine Chemicals,

India; B. No.: 75316806-2), ammonium chloride (Analab Fine Chemicals, India; B. No.: RKC221713), and cystone (The Himalaya Drug Company, India; B. No.: 19300259F) were used for the study. All other chemicals and reagents used were of analytical grade and procured from approved vendors. Apparatus such as the metabolic cages (New Neeta Chemicals, India), cold centrifuge (BioEra, India) and UV-spectrophotometer (LabIndia, India) were used in the study.

2.3. Collection of plant material and preparation of extract

The whole plant material of *B. sensitivum* was collected from the local region of Pune, India. It was authenticated by Dr. J. Jayanthi, Scientist, Botanical Survey of India, Pune, India. Thereafter, the plant material was washed and shade dried at room temperature. The dried whole plant was coarsely powdered, packed into soxhlet column and extracted with 70% v/v methanol in water at 65–70 °C for 22 h. This methanolic extract of *B. sensitivum* (MBS) was then evaporated at 45 °C and then dried in oven. The dried extract was stored in an airtight container.

2.4. Standardization of MBS by HPLC

The MBS was standardized for the content of marker compound, amentoflavone by HPLC method.¹⁰ Standard solution of amentoflavone and sample solution of MBS were prepared in DMSO at the concentration of 10 and 100 µg/ml respectively. Separate chromatograms were obtained for standard and MBS solution using an HPLC system consisting a binary pump (Model: Waters 515 HPLC pump), auto sample (Model: 717 plus auto-sampler), column heater and PDA detector (Waters 2998). Separation was achieved on Kromasil C-18 (250 mm × 4.6 mm, 5.0 µm) column maintained at 25 °C using column oven. Isocratic elution with acetonitrile: phosphate buffer (30:70) mobile phase at the flow rate of 0.7 ml/min was carried out. The detection was monitored at 330 nm. Data collection and analysis were performed using Empower-version 2 software.

2.5. Experimental design

Ethylene glycol and ammonium chloride-induced hyperoxaluria model was used to induce urolithiasis in rats.¹¹ Three dose levels of MBS (100, 200 and 400 mg/kg) were used for the evaluation of its antiurolithiatic effect.¹² Animals were randomly divided into ten groups each containing six animals. Group I served as vehicle control, animals of this group were maintained on standard rat food and drinking water *ad libitum* and received vehicle, i.e. 5% w/v gum acacia solution (5 ml/kg, p.o.). All the remaining groups received calculi inducing treatment for the period of 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for the period of 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for next 25 days. Group II served as lithiatic control and received 5% w/v gum acacia solution (5 ml/kg, p.o.). Group III served as the preventive standard treatment group and received cystone (500 mg/kg) from 1st day to 28th day of calculi induction. Group IV served as the curative standard treatment group and received cystone (500 mg/kg) from 14th day to 28th day

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