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Original Article

Protective effect of ethyl acetate fraction of *Biophytum sensitivum* extract against sodium oxalate-induced urolithiasis in rats

Anil T. Pawar [a, b, *], Niraj S. Vyawahare [c]

[a] *Centre for Research and Development, PRIST University, Thanjavur 613403, India*
[b] *Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune 411038, India*
[c] *Department of Pharmacology, Dr. D. Y. Patil Pratishthan's, Padmashree Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune 411044, India*

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ABSTRACT

The methanolic whole plant extract of *Biophytum sensitivum* (感应草gǎnyìng cǎo) has been found to possess antiurolithiatic effect. The present study was undertaken to evaluate the antiurolithiatic effect of some fractions of methanolic whole plant extract of *B. sensitivum* (MBS) in rats as a step toward activity-directed isolation of antiurolithiatic component. The MBS was successively extracted with dichloromethane, ethyl acetate, ethanol and water to obtain fractions. Sodium oxalate (70 mg/kg, i.p.) was administered to rats for seven days to develop calcium oxalate urolithiasis. These rats were treated with two doses (20 and 40 mg/kg, p.o.) of the fractions, 1 h before sodium oxalate injections. Antiurolithiatic activity was assessed by estimating biochemical changes in urine, serum and kidney homogenate along with histological changes in kidney tissue. Sodium oxalate administration caused biochemical alterations in urine which was found to be prevented significantly by the ethyl acetate fraction. Supplementation with ethyl acetate fraction prevented the elevation of serum creatinine, uric acid and blood urea nitrogen levels. The elevated calcium, oxalate and phosphate levels in the kidney tissue homogenate of lithiatic rats were significantly reduced by the treatment with ethyl acetate fraction. The ethyl acetate fraction also caused significant decrease in lipid peroxidation activity, accumulation of calcium oxalate deposits and histological changes in the kidney tissue. The results showed that the antiurolithiatic component of the methanolic whole plant extract of the plant is contained in the ethyl acetate fraction. The effect is attributed to its diuretic, antioxidant, nephroprotective properties and effect on lowering the concentration of urinary stone-forming constituents.

1. Introduction

Urolithiasis i.e. formation of urinary calculi (stone) anywhere in the urinary tract is one of the most painful and third prevalent ailments, has beset humans from centuries.[1] It is reported as complaint with an increasing incidence and prevalence worldwide. It is estimated that it affect about 12% of world population and expected to rise further with the advancement in the industrialization.[2–4] This increased incidence of urinary stones over the last few years, are associated with decrease in age of onset has an important effect on the healthcare system.[5] Recurrence is another major factor that makes it more serious issue to address. On recurrence, the subsequent relapse risk is raised and the interval between recurrences is shortened.[6] Common features associated with recurrence include a young age of onset, family history, frequent infections and underlying medical conditions.[7]

The current medical management of urolithiasis involves administration of symptomatic drugs like diuretics, alkanizers, anti-inflammatory etc., and other techniques like extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy.[7–9] However, these treatment options have certain hurdles such as limited therapeutic outcome, comparatively high cost and chances of frequent recurrence.[10] Furthermore, they also causes wide range of undesirable effects such as hemorrhage, hypertension, tubular necrosis followed by subsequent fibrosis of the kidney leading to cell injury.[11] The continuous research is going on to overcome these

* Corresponding author. Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Paud Road, Kothrud, Pune, India. Fax: +91 020 25460616.
E-mail address: anil.pawar@mippune.edu.in (A.T. Pawar).
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drawbacks and to develop more suitable and safe treatment options, however till today no satisfactory therapy has been developed that indicates need for effective alternative remedy for the prevention and treatment of urolithiasis.

In the Indian traditional systems of medicine i.e. Ayurveda, a number of plants have been claimed to be efficient to cure and correct urinary stones.[12] 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.[10] In Ayurveda, the whole plant of *Biophytum sensitivum* (感应草gǎnyìng cǎo) is claimed for its usefulness in the treatment of various health aliments including urinary stones.[13,14] *B. sensitivum* (感应草gǎnyìng cǎo) belonging to family oxalidaceae is distributed in tropical Asia, Africa, America and Philippines. It has been extensively studied for its analgesic, anti-pyretic, anti-inflammatory, immunomodulatory, antitumor, antidiabetic, antioxidant, antibacterial, antihypertensive, chemoprotective, radioprotective and antifertility effects.[15] We have previously reported antiurolithiatic potential of *B. sensitivum* (感应草gǎnyìng cǎo) against zinc disc implantation-induced lithiasis and ethylene glycol and ammonium chloride-induced lithiasis in rats.[16] The antiurolithiatic constituent of the plant is yet to be isolated. The present study was carried out to evaluate antiurolithiatic activity of fractions of *B. sensitivum* (感应草gǎnyìng cǎo) against sodium oxalate-induced calcium oxalate urolithiasis in rats in order to determine the fraction containing antiurolithiatic component of the plant.

2. Materials and methods

2.1. Collection and extraction of plant materials

The plant material was identified, collected and thereafter extracted as previously reported.[16] Briefly, whole plant material of *B. sensitivum* (感应草gǎnyìng cǎo) was collected from the local region of Pune, India. It was authenticated by Dr. J. Jayanthi, Scientist, Botanical Survey of India, Pune, India. The plant material i.e. whole plant of *B. sensitivum* (感应草gǎnyìng cǎo) was washed and dried in shade for 10–15 days. The dried whole plant was coarsely powdered, packed into soxhlet column and extracted with 70% v/v methanol in water for 22 h. This methanolic extracts of plant (MBS) was then evaporated at 45 °C and then dried in oven. The dried extract was stored in airtight container.

2.2. Fractionation of extract

The MBS was subjected to fractionation by earlier reported method.[17] In brief, 5 g of MBS was fractioned by filter column chromatography over 100 g silica gel 60–120 (S), and eluted with approximately 3 L of solvents dichloromethane, ethyl acetate, ethanol, and water, in the order of increasing polarity, until a clear elute was obtained at the end of the elution. The collected elutes were subjected to evaporation at 45 °C to obtain dichloromethane (DCM-MBS), ethyl acetate (EA-MBS), ethanol (E-MBS), and water (AQ-MBS) fractions of MBS. Fractions were stored at 4 °C until assayed.

2.3. Evaluation of antiurolithiatic activity of fractions

All fractions (i.e. DCM-MBS, EA-MBS, E-MBS and AQ-MBS) were evaluated for antiurolithiatic activity at the dose levels of 20 and 40 mg/kg (p.o.),[16] for seven days treatment,[18,19] against sodium oxalate-induced calcium oxalate urolithiasis in rats.

2.4. Animals

Male Wistar albino rats weighing between 150–200 g were used for the study. They were procured from National Institute of Biosciences, Pune, India. The rats were allowed for acclimatization for ten days under standard conditions in the CPCSEA 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/2014-15/M1/Appr/004) of MAEER's Maharashtra Institute of Pharmacy, Pune, India.

2.5. Chemicals and apparatus

Sodium oxalate (Qualigens Fine Chemicals, India) was 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), semi-automated clinical analyzer (Avantor Performance Materials, India) and UV-spectrophotometer (LabIndia, India) were used in the study.

2.6. Experimental design

Sodium oxalate-induced calcium oxalate urolithiasis model was used to evaluate antiurolithiatic effect of fractions of MBS in rats.[18,19] 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 were received vehicle, i.e. 0.5% w/v gum acacia solution (5 ml/kg, p.o.). All remaining groups received calculi inducing treatment, comprised of sodium oxalate (70 mg/kg, i.p.) administration for seven days. Group II served as lithiatic control and received 0.5% w/v gum acacia solution (5 ml/kg, p.o.). Groups III and IV served as DCM-MBS treatment groups and received DCM-MBS at doses of 20 and 40 mg/kg respectively for seven days. Groups V and VI served as EA-MBS treatment groups and received EA-MBS at doses of 20 and 40 mg/kg respectively for seven days. Groups VII and VIII served as E-MBS treatment groups and received E-MBS at doses of 20 and 40 mg/kg respectively for seven days. Groups IX and X served as AQ-MBS treatment groups and received AQ-MBS at doses of 20 and 40 mg/kg respectively for seven days. The fractions were suspended in distilled water using 0.5% w/v gum acacia solution and given once daily by oral route (5 ml/kg body weight) 1 h prior to sodium oxalate challenge.

2.7. Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 0 and 7th day of experimental period. The pH of freshly collected urine sample was measured and 24 h urine samples were analyzed for its volume, calcium, oxalate,[20] phosphate, magnesium,[21,22] uric acid, citrate,[23] and total protein contents. Commercial kits for estimating urinary levels of calcium (Transasia Bio-medicals Ltd., India), phosphate (Coral Clinical Systems, India), uric acid (Transasia Bio-medicals Ltd., India) and total protein (Transasia Bio-medicals Ltd., India) were used according to the manufacturer's protocol.

2.8. Serum analysis

After urine collection, blood was collected from retro-orbital sinus under ether anesthesia. Serum was separated by centrifugation at 10,000 $\times g$ for 10 min and analyzed for creatinine, uric acid