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Efficacy of *Adiantum capillus veneris* Linn in chemically induced urolithiasis in rats

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ABSTRACT

Ethnopharmacological relevance: *Adiantum capillus veneris* Linn has been recommended in ancient literature of Unani system of medicine as an important ingredient of many formulations for the treatment of urolithiasis. Its decoction has long been used for the same purpose by several Unani physicians.

Aim of study: To investigate the antiurolithiasic effect of the hydro alcoholic extract of *Adiantum capillus veneris* Linn in male Sprague Dawley rats.

Material and methods: The effects of oral administration of hydro alcoholic extract of test drug were studied on calcium oxalate urolithiasis. A total of 48 rats were used for the study. The animals were divided into six groups of eight animals each. Plain control rats were treated with distilled water only, throughout the study period, whereas in other groups nephrolithiasis was induced by providing drinking water containing 0.75% ethylene glycol and 1% ammonium chloride for 7 days. Thereafter, urine was examined for the presence of crystals. Negative control group A rats were sacrificed after 7 days, whereas negative control group B was left untreated up to the end of study. Test groups were treated with 127.6 mg/kg and 255.2 mg/kg of test drug and standard control with Cystone (750 mg/kg) for 21 days. At the end of experiment, number of crystals in urine and levels of calcium, phosphorus, urea and creatinine in serum were observed. Histopathological study of the kidney was done by light microscopy.

Results: Urine microscopy showed significant reduction ($p < 0.001$ and $p < 0.01$) in the number of crystals in test groups A and B respectively. Serum levels of calcium, phosphorous, and blood urea were found to be decreased significantly in all the groups. In both the test groups, serum creatinine level was found to be similar as in plain control. The animals treated with test drug showed much improvement in body weight. Histopathology of kidney showed almost normal kidney architecture in treated groups.

Conclusion: The above findings indicate the antiurolithic activity of *Adiantum capillus veneris* Linn, and thus, validate the claims of Unani physicians for its medicinal use in urolithiasis.

1. Introduction

Urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules (Bouanani et al., 2010). Levels of urinary supersaturation co-relate with the type of stone formed. Any cellular dysfunction that can affect various urinary ions and other substances can also influence calcium oxalate supersaturation and crystallization in the kidney. Formation of renal stone starts with the transient supersaturation that occurs within kidneys while excretion of millions of urinary crystals through them. However, supersaturation is only one step in the process of stone formation. It further needs crystals to be retained and cause ulceration within the kidneys. Renal injury in its turn, promotes crystal retention and development of a stone nidus on the renal papillary surface and further supports crystal nucleation at lower supersaturation. Reactive oxygen species (ROS_S) also seem to be responsible for cellular injury, therefore a reduction of renal oxidative stress could also be an effective therapeutic approach (Butterweck and Khan, 2009). Till date there is no satisfactory drug to be used for clinical therapy. A number of vegetable drugs are being used in many parts of the world for the treatment of urolithiasis (Bouanani et al., 2010). *Adiantum capillus veneris* Linn is traditionally used in Unani system of medicine for

Abbreviations: AC, Ammonium chloride; ACV, *Adiantum capillus veneris* Linn; BUN, Blood urea nitrogen; CaOx, Calcium oxalate; CPCSEA, Committee for the purpose of control and supervision of experiment on animals; EG, Ethylene glycol; GFR, Glomerular filtration rate; HPF, High power field; LPO, Lipid peroxidation

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the treatment of inflammatory diseases (Haider et al., 2011). It is an important drug, widely used in patients of urolithiasis and is included as an important ingredient in many formulations, used for litholytic activity. Its decoction is frequently used for its lithotriptic effect and considered to be capable of expelling stones from kidney and bladder (Ibn Sina, 2007; Ghani, 2011). Moreover, during chemical analysis, it is observed that beside tritepenes, phenylpropanoids and carotenoids (Haider et al., 2011; Ibraheim et al., 2011) *Adiantum capillus veneris* Linn, also possesses flavonoid (Takahisa et al., 1999; Pourmorad et al., 2006) which has been documented by Yadav et al. (2011) to be responsible for litholytic activity. Some of the studies carried out on *Adiantum capillus veneris* Linn include evaluation of antifungal (Guha et al., 2005), antibacterial (Parihar et al., 2010), antiviral (Abbasi et al., 2009) and antioxidant activities (Pourmorad et al., 2006). There seems to be no report on the antiurolithiasic activity of this drug. Therefore, this study was undertaken to investigate the anti urolithiasic effect of the hydro alcoholic extract of *Adiantum capillus veneris* Linn on calcium oxalate urolithiasis, induced by ethylene glycol and ammonium chloride in male Sprague Dawley (SD) rats.

2. Material and methods

2.1. Animals

The study was carried out on healthy male Sprague Dawley rats weighing 180–210 g. The animals were procured from registered breeder and allowed to get acclimatized for 1 week. They were housed in clean polypropylene cages at room temperature (25 ± 2 °C), humidity 45–55% with 12 h light–12 h dark cycle throughout the experimental period and were provided with standard diet and water ad libitum unless stated otherwise. The animal care procedures and experimental protocol were in accord with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). Study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine (NIUM), Bangalore, India. (Reg. no. IAEC/VII/01/IA).

2.2. Chemicals and reagents

All the chemicals used were of analytical grade. Ethylene glycol and ammonium chloride were obtained from Sigma Aldrich Chemicals Pvt. Limited, Bangalore, India. Cystone® manufactured by the Himalaya Drug Company, Bangalore, was purchased from the market of Bangalore. Kits used in this study for the determination of calcium, blood urea nitrogen (BUN), creatinine, and phosphorus were purchased from Lab Care Diagnostics (India) Pvt. Ltd., Bangalore.

2.3. Plant materials and preparation of extract

The fronds of *Adiantum capillus veneris* Linn were purchased from an authentic herb supplier in the local market of Bangalore, India, identified by Dr. Siddamallayya N.A. at National Ayurveda Dietetics Research Institute, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Ashoka Pillar, Jayanagar, Bangalore. A voucher specimen (Ref. no. Drug Authentication/SMPU/NADRI/BNG/2010-11/45a.) was deposited in the Department of Ilmul Advia, NIUM, Bangalore for future reference. The dried plant was made free of dirt and ground to powder using commercial mill, 100 g of which was then extracted in solvent (50% distilled water and 50% ethanol) for 6 h in a Soxhlet apparatus at 80 °C. The extract was filtered by filter paper (Whatman no. 40) and evaporated on water bath till it dried completely (Karim et al., 2011). The yield of the hydro alcoholic extract was found to be 11% *w*/*w*. The extract was stored in a refrigerator pending the time of biological investigations.

2.3.1. Dosage of the drug

The human therapeutic dose of *Adiantum capillus veneris* Linn as mentioned in Unani classical literature is 10 g (Ghani, 2011). The dose for rats was calculated by dividing it by adult human weight of 60 kg and multiplying it with the conversion factor of 7 to accommodate the surface area of animal (Freirich et al., 1968) and found to be 1.16 gm/kg which was lesser than the safe dose, 3 gm/kg, as indicated by acute toxicity study carried out by Haider et al., (2011). The dose of the extract was determined with reference to the yield% of crude drug and found to be 127.6 mg/kg. Another higher dose was also calculated that is just double of the first dose (255.2 mg/kg) to evaluate the efficacy of test drug in dose-dependent manner. Fresh aqueous suspension (in 1 ml distilled water) was prepared daily before each administration.

2.4. Ethylene glycol and ammonium chloride induced urolithiasis

This test was carried out by the method of Fan et al. (1999) with some modification in the treatment schedule. All the animals were weighed and divided into six groups of eight animals each. Group I served as plain control and received regular rat food and drinking water ad libitum. The animals of groups II–VI were treated with ethylene glycol (EG) 0.75% (*V*/*V*) and ammonium chloride (AC) 1% (*W*/*V*) by adding in their drinking water for 7 days for induction of urolithiasis. On the 8th day, they were again weighed and kept in individual metabolic cages for 3 h to collect fresh urine samples. Animals had free access to drinking water during the urine collection period. Urine was analyzed for crystalluria through microscopy, for which 1 ml of the fresh urine sample was centrifuged at 3000 rpm and then 950 µl of the supernatant was discarded. Ten microlitres of the vortex mixed sediment was then transferred to slide. The number of the crystals were identified and counted using light microscope ($45 \times$) (Fan et al., 1999). Thereafter, the animals of group IV were treated with Cystone with a dose of 750 mg/kg (Mitra et al., 1998) and served as standard control. The animals of group V and group VI were treated with the hydro alcoholic extract of test drug with a doses of 127.6 mg/kg and 255.2 mg/ kg and served as Test groups A and B, respectively. The treatment was continued for 21 days. The animals of group II were sacrificed on the 8th day just after urine collection and served as negative control A while the animals in group III were left untreated for next 21 days and served as negative control B. On 21st day of treatment the animals of plain control, negative control B, standard and test groups were again weighed and kept in metabolic cages for 3 h urine collection. Crystalluria was analyzed by the previous method.

2.5. Serum analysis

After collection of urine, rats were sacrificed under theopentone anesthesia (50 mg/kg IP). Blood samples were collected by cardiac puncture and serum was separated by centrifugation at 10,000 rpm for 10 min. Creatinine, urea nitrogen, calcium and phosphorus were assessed by using auto-analyzer and specific kits, namely serum creatinine estimating kit, serum urea nitrogen estimating kit, serum calcium estimating kit and serum phosphorus estimating kit, manufactured by Lab Care Diagnostics (India) Pvt. Ltd., Bangalore. One kidney from one animal of each group was dissected out,