Antilithiatic Activity of Poly-Herbal Extracts on Ethylene glycol-Induced Lithiasis in Rats

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Abstract: The aqueous and alcoholic extracts of Aerva Lanata (Amaranthaceae), Dolichos Biflorus (Fabaceae) and Musa (Musaceae) were evaluated for its antilithiatic activity in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%v/v) in adult male albino Wistar rats for 28 days and also it increased renal excretion of calcium and phosphate. Supplementation with aqueous and alcoholic extracts significantly reduced the elevated urinary oxalate. The increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly lowered by curative and preventive treatment using alcoholic and aqueous extract. The results indicated that the poly-herbal mixture is capable with antilithiatic activity at lower dose.

Key words: Ethylene glycol · Hyperoxaturia · Nephrolithiasis · Aerva Lanata · Dolichos biflorus · Renal calculi

INTRODUCTION

Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in men, while phosphate stones formation is more in women. The pathogenesis of lithiasis seems to be multifactorial and complicated [1]. Most calculi in the urinary system arise from a common compartment of urine, e.g., calcium oxalate (CaOx), representing up to 80% of analyzed stones [2]. A number of medicinal plants have been used in India and elsewhere which claim efficient cure of urinary stones [3]. Unfortunately, the underlying risk factors are not corrected by these techniques; hence there is a need to continue medical supervision and therapy to prevent stone recurrence [4].

Aerva lanata (Amaranthaceae) found in open forests on mountain slopes, on waste and disturbed ground, deserted cultivation and coastal scrub [5] and at altitudes from sea level to 900 meters (3,000 ft) [6]. It gives protection against evil spirits, is a good-luck talisman for hunters and safeguards the well-being of widows [7].

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a reduced risk of colorectal cancer [13] and in women, breast cancer [14] and renal cell carcinoma [15]. Individuals with a latex allergy may experience a reaction to bananas [16]. Dolichos biflorus seeds belong to the family Fabaceae; it's a pulse crop, particularly in Madras, Mysore, Mumbai and Hyderabad. Entire plant used in measles, small pox, adenitis, burns, sores, seeds are mainly used as astringent, antipyretic, diuretic and menstrual disorders. It’s a valuable protein supplement [17]. Our present study was carried out to observe antilithiatic activity of Polyherbal extracts (a mixture of Aerva Lanata leaves, Dolichos seeds, Musa trunk) has been investigated scientifically to validate its folkloric use for lithiasis in South India.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: All these three plant materials were identified by the taxonomist of botany department of Regional Forest Research Center (RFRC), Rajahmundry, East Godavari District andhra Pradesh. Subsequently they were dried under shade and powdered.

Preparation of Extract: The course powder of the Aerva Lanata leaves and Dolichos seed were subjected to simple maceration process separately in order to get extract. After 24 hours of maceration it was filtered dried to get a crude extract. Fresh Musa trunk was sliced and juice was extracted and it is used for the treatment. Alcoholic extracts are prepared for the leaves and seeds by Soxhlet extraction process by 70% (v/v) alcohol at temperature 60-70°C, then filtered and concentrated.

Animal Selection: In the current study, male rats body weight ranged between 150-200g were selected to induce urolithiasis and were housed in standard environmental conditions (22±1°C, humidity 60±5% and light 12 h dark 12 h light cycle) with free access to a standard commercial diet and water. Experiment was performed according to the guidelines and the study was approved by the Institutional Animal Ethics Committee (IAEC-CPCSEA registration No- 1069/AC/07/CPCSEA). Only male rats are selected because the urinary system of male rats resembles that of humans and earlier studies showed that the amount of stone deposition in female rats was significantly less [18].

Induction of Experimental Urolithiasis: Ethylene glycol (0.75%) induced hyperoxaluria model [19] was used to assess the antilithiatic activity in rats.

Design of Work: The rats were housed in polyethylene cages and divided into seven groups of six animals each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Group II to VII for induction of renal calculi till 28th day. Group III received standard antilithiatic drug, cystine (750 mg/kg b.w) from 15th day till 28th day [20] Groups IV & V received as curative regimen(CR). Group IV received aqueous extract (250 mg/kg b.w) and Group V received alcoholic extract (250 mg/kg b.w) from 15th day till 28th day, Group VI received aqueous extract (250 mg/kg b.w) and Group VII received alcoholic extract (250 mg/kg b.w) from 1st day till 28th day and served as preventive regimen (PR). All extracts were given once daily by oral route.

Collection and Analysis of Urine: All animals were kept individually in metallic cages and urine samples of 24 hour were collected on 28th day. Animals had free access to drinking water during the urine collection period. The collected urine was analyzed for calcium (using calcium liquid kit: Raichem method [21], oxalates (Hodgkinson and Williams procedure) [22] and inorganic phosphates (using phosphorous reagent kit: Raichem method) [23].

Kidney Homogenate Analysis: The abdomen was opened to remove both kidneys from each animal. Isolated kidneys were cleaned of extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2,000× g for 10 min and supernatant was separated [24]. The calcium, phosphate and oxalate content in kidney homogenate were determined.

Statistical Analysis: The results were expressed as mean ±S.D. Differences among data were determined using one-way ANOVA (p<0.05) [25].

RESULTS AND DISCUSSION

In this study, chronic administration of ethylene glycol aqueous solution (0.75%v/v) to male Wistar albino rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals. However, supplementation with aqueous and alcohol extracts of these plant materials significantly lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney in curative regimens and preventive regimens as compared to cystine-treated
animals. Although the reduction was non-significant (p>0.05) on inter-regimen comparison (curative regimen versus preventive regimens), the results were significantly (p<0.001) comparable with constipated.

Urinary separation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidences in previous studies indicated that in response to 14 days period of ethylene glycol (0.75% v/v) administration young male rats form renal calculi composed mainly of calcium oxalate [15, 26, 27]. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate [28, 29]. However, aqueous and alcoholic extracts of this mixture of extracts lower the levels of oxalates well of calcium excretion. An increase in urinary phosphate is observed in calculi induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium oxalate deposition [30]. Treatment with this mixture of extracts restores phosphate levels and reducing the risk of stone formation. Effect of poly-herbal extracts on urinary parameters in control and experimental animals (Table 1). Histopathology studies also produced significance results (Figure 1).

In conclusion, the present study indicates that the administration of the aqueous and alcoholic extracts to rat with ethylene glycol induced lithiasis reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plants. Accordingly, it can be concluded that the poly-herbal extracts has a beneficial effect on lithiasis induced by ethylene glycol.

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