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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 antiurolithiatic 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 antiurolithiatic activity at lower dose.

Key words: Ethylene glycol · Hyperoxaluria · 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 underling 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]. *Aerva lanata* is a woody, prostrate or succulent, perennial herb in the Amaranthaceae family of the genus Aerva that sometimes flowers in the first year [8]. The plant is said to be diuretic and demulcent. Its diuretic action is said to be very effective in the treatment of urethral discharges and gonorrhea and is of value in cases of lithiasis and as an anthelmintic. A trace of alkaloid has been detected in it. Musa is the common name for herbaceous plants of the genus Musa and for the fruit they produce. The banana plant is the largest herbaceous flowering plant. The Musa plant is the largest herbaceous flowering plant [9]. Plants are normally tall and fairly sturdy and are often mistaken for trees, but their main or upright stem is actually a pseudo stem that grows 6 to 7.6 meters (20 to 24.9 ft) tall, growing from a corm. Leaves are spirally arranged and may grow 2.7 meters (8.9 ft) long and 60 cm (2.0 ft) wide [10]. They are easily torn by the wind, resulting in the familiar frond look [11]. The genus Musa is in the family Musaceae. The APG (Angiosperm Phylogeny Group) II system, of 2003 (unchanged from 1998), assigns Musaceae to the order Zingiberales in the clade commelinids in the monocotyledonous flowering plants. Some sources assert that the banana's genus, Musa, is named for Antonio Musa, physician to the Emperor Augustus [12]. Health benefits of musais associated with

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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 papilionaceae; 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, cystone (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 28 thday, 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 off 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 ($p<0.001$) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney in curative regimens and preventive regimens as compared to cystone-treated

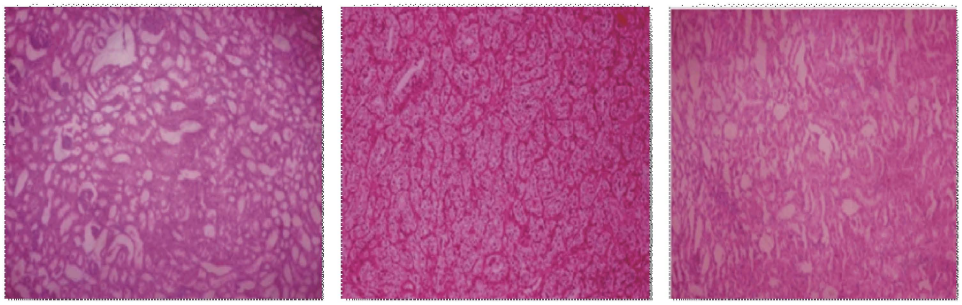

Fig. 1: Histopathological studies

Table 1: Effect of poly-herbal extracts on urinary parameters in control and experimental animals

	Urine Analysis			Kidney Analysis		
Group	Oxalates	Calcium	Phosphates	Oxalates	Calcium	Phosphates
I	0.36±0.03	0.99±0.07	5.35±0.04	1.39±0.06	3.22±0.04	2.25±0.03
II	3.95±0.11*	4.25±0.10*	7.09±0.06*	6.01±0.06*	4.68±0.16*	3.98±0.10*
III	0.61±0.04***	1.32±0.06***	3.65±0.09***	1.51±0.06***	3.13±0.07***	2.13±0.07***
IV	0.59±0.04***	1.16±0.12***	3.98±0.08***	1.42±0.04***	3.05±0.09***	2.20±0.08***
V	1.01±0.06**	1.72±0.06**	4.12±0.10**	1.98±0.07**	3.95±0.05**	2.90±0.10**
VI	0.53±0.03***	1.19±0.06***	3.12±0.04***	1.37±0.04***	2.98±0.08***	2.01±0.07***
VII	0.87±0.08**	0.98±0.08**	4.09±0.09**	1.42±0.07**	3.15±0.08**	2.15±0.07**

Values are expressed as Mean ± SEM of six observations; Statistical comparisons are made between Group I with Group I and Group II (*p<0.001); Group I with Group VI and Group VII (**p<10.1); Group II with Group IV and Group V (***p<0.01)

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 cystone-treated.

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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REFERENCES

1. Lee, Y., W. Huang, J. Huang and L. Chang, 1991. Testosterone Enhances whereas Estrogen Inhibits Calcium Oxalate Stone Formation in Ethylene Glycol Treated Rats. J. Urol., 156: 502-505.
2. Prien, E.L. and E. L.J. Prien, 1968. Composition and structure of urinary stone. Am. J. Med., 45: 654-72.

3. Mukharjee, T., N. Bhalla, G.S. Aulakh and H.C. Jain, 1984. Herbal drugs for urinary stones literature appraisal. Indian Drugs, 21: 224-228.
4. Halabe, A. and O. Sperling, 1994. Uric acid nephrolithiasis. Miner Electrolyte Metab., 20: 424-431.
5. Townsend, C.C., 1974. Notes on Amaranthaceae: Kew Bulletin, 29: 461-475.
6. Upwta, A.L. and A.P. Aluka, XXXX. Entry for *Aerva lanata (Linn.)* Juss. (Family *Amaranthaceae*"). African Plants. Ithaka Harbors, 130 Inc. doi:10.5555/1.
7. *Aerva lanata* (L.) Juss. ex Schult. record n° 177 (HTML). *African Plants Database*. South African National Biodiversity Institute, the Conservatoire et Jardin botaniques de la Ville de Genève and Tela Botanica. Retrieved on 2008-04-27. (http://www.enotes.com/topic/Aerva_lanata).
8. We have more bananas published in the Royal Horticultural Society Journals, May 2002. Banana from "Fruits of Warm Climates" by Julia Morton. Hort.purdue.edu. http:// www.hort.purdue.edu/ newcrop/ morton/banana.html. Retrieved 2009-04-16.
9. Gold, C.S., G.H. Kagezi, G. Night and P.E. Ragama, 2004. The effects of banana weevil, Cosmopolites sordidus, damage on highland banana growth, yield and stand duration in Uganda. Annals of Applied Biology, 145: 263-269. doi: 10.1111/j.1744-7348.2004.tb00382.x
10. Greenearth, Inc., Banana Plant Growing Info, 1916. Liberty Hyde Bailey, the Standard Cyclopedia of Horticulture, 2076.
11. Pellegrini, H.D, E.D. Stefani and A. Ronco, 1996. Vegetables, fruits and risk of colorectal cancer: a case-control study from Uruguay. Nutrition & Cancer., 25: 297-304.
12. Zhang, E.X. *et al*, 2009. Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. Int. J. Cancer, 125: 181-188.
13. Rashidkhani, B., P. Lindblad and A. Wolk, 2005. Fruits, vegetables and risk of renal cell carcinoma: A prospective study of Swedish women. Int. J. Cancer, 113: 451-455.
14. Taylor, J.S. and E. Erkek, 2004. Latex allergy: Diagnosis and management. Dermatological Therapy, 17: 289-301.
15. Khare, Indian Medicinal Plants; An illustrated Dictionary, pp: 11-12.
16. Prasad, K.V., K. Bharathi and K.K. Srinivasan, 1993. Evaluation of (*Musa parasidica Linn* Cultivar)-"Puttubale" stem juice for antilithiatic activity in albino rats. Indian J. Physiol Pharmacol., 37: 337-341.
17. Atmani, F., Y. Slimani, M. Mimouni and B. Hacht, 2003. Prophylaxis of calcium oxalate stones by Herniariahirsuta on experimentally induced nephrolithiasis in rats. BJU Int., 92: 137-140.
18. Mitra, S.K., S. Gopumadhavan, M.V. Venkataranganna and R. Sundaram, 1998. Effect of Cystone, a herbal formulation, on glycolic acid-induced urolithiasis. Phytother Res., 12: 372-374.
19. Mustafa, M.A. and D.M. Mederios, 1985. Proximate Composition, Mineral Content and Fatty Acids of Catfish (Ictaluruspunctatus Rafin). For Different Seasons and Cooking Methods, J. Food Sci., 50: 585-588.
20. Hodgkinson, A. and A. Williams, 1972. Improved colorimetric procedure for urine oxalate. Clin Chem. Acta, 36: 127-132.
21. Fiske, C. H. and Y. Subbarow the coorimetric determination of phosphorus, 1925. J. Biol. Chem., 66: 375-381.
22. Chow, F.C., M.I. Dysart, D.W. Hamar and R.H. Udall, 1975. Control of oxalate urolithiasis by D L-alanine. Invest Urol., 13: 113-116.
23. Kulkarni, S.K., 1993. Handbook of experimental pharmacology. 2nd ed., Mumbai: VallabhPrakashan, pp: 172-189.
24. Selvam, R., P. Kalaiselvi, A. Govindaraj, V. Balamurugan and A.S. Sathish kumar, 2001. Effect of *Aerva lanata* flowers extract and Vediuppuchunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res., 43: 89-93.
25. Huang, H.S., M.C. Ma, J. Chen and C.F. Chen, 2002. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J. Urol., 167: 2584-93.
26. Selvam, R. and M. Adhirai, 1997. E. Vitamin, pretreatment prevents cyclosporin A-induced crystal deposition in hyperoxaluric rats. Nephron, 75: 77-81.
27. Muthukumar, A. and R. Selvam, 1997. Effect of depletion of reduced glutathione and its supplementation by glutathione monoester on renal oxalate retention in hyperoxaluria. J. Nutritional Biochem., 8: 445-450.
28. Low R.K. and R.K. Stoller, 1997. Uric acid nephrolithiasis. Urol Clin North Am., 24: 135-148.
29. Viel, T.A., C.D. Domingos, A.P. Da silva *et al*., 1999. Evaluation of the antiurolithiatic activity of the extract of Costus spiralis Roscoe in rats. J. Ethnopharmaol., 66: 193-198.
30. Soundararajan, P., R. Mahesh, T. Ramesh and V.H. Begum, 2006. Effect of Aerva lanata on calcium oxalate urolithiasis in rats. Indian. J. Experimental Biol., 44: 981-986.