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RESEARCH ARTICLE

AMELIORATIVE ANTIUROLITHIATIC EFFECT OF A POLYHERBAL SUSPENSION

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ABSTRACT

A polyherbal suspension was prepared for use in urolithiasis. Three drugs namely *Cyperus rotundus* roots, *Azadirachta indica* leaves and *Bryophyllum pinnatum* leaves were used in the formulation. Antiurolithiatic activity was studied in the ethylene glycol induced urolithiasis model at a dose of 100, 200 and 300mg/kg polyherbal suspension in wistar rats. Cystone was used as the standard drug. All the doses were found to be effective in a dose related manner. There was an increase in the urinary excretion of calcium, oxalate, phosphate, urea, uric acid and creatinine significantly in the suspension treated group. P<0.001was obtained when compared to control. The results show that the polyherbal suspension has prevented or decreased the supersaturation of urine leading to prevention of stone formation and agglomeration. The excretion of these ions was much lesser when compared to the negative control group. The dose of 300mg/kg of polyherbal suspension had activity quite similar to that of the standard drug cystone. Thus we conclude that the formulated polyherbal suspension has potential antiurolithiatic activity.

Keywords: Antiurolithiatic activity, *Cyperus rotundus*, *Azadirachta indica*, *Bryophyllum pinnatum*, Polyherbal suspension.

INTRODUCTION

Urolithiasis is the process of formation of stones in kidney, urethra or bladder. It is associated with pain in abdomen, flank or groin. The stones are formed due to decreased urine output or increased excretion of constituents like calcium, oxalate, urate and phosphate which are responsible for the formation of stone. It occurs in people with frequent urinary tract infections or using antacid in excessive amount, has hypophosphatemia and hypercalciuria[1]. Herbs have been used traditionally for the treatment of urolithiasis[2] and pain[3]. Many experimental studies have been done on combination of herbs[4,5]. Therefore an oral suspension containing three medicinal herbs was prepared for the study of antiurolithiatic activity. These drugs were *Cyperus rotundus* L. (Cyperaceae), *Azadirachta indica* A. Juss (Meliaceae) and *Bryophyllum pinnatum* Lam., (Crassulaceae).

Cyperus rotundus has antidiarrhoeal[6,7], analgesic, antipyretic and anti-inflammatory[8,9] diuretic, antispasmodic and litholytic[10,11] activity. It is used in stomach pain[12]. *Azadirachta indica* is reported to have hepatoprotective[13], antioxidant[14], anti-inflammatory[15], immunomodulatory[16] and antiurolithiatic activity[17]. Studies show that *Bryophyllum pinnatum* have good antiurolithiatic[18-21], antimicrobial[22,23] and nephroprotective[24] activity. Considering the presence of antiurolithiatic activity, antimicrobial, analgesic and anti-inflammatory activities in these drugs a polyherbal formulation was prepared and evaluated for antiurolithiatic activity.

MATERIALS AND METHOD

Plant material

Collection of the roots of *Cyperus rotundus* (CR), leaves of *Azadirachta indica* (AI) and *Bryophyllum pinnatum* (BP) was done locally and authenticated from the Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.). The voucher specimen number is JC/B/PAN/054a-c. They were shade dried, grinded to get their coarse powders and stored in well closed containers.

Preparation of polyherbal suspension (PHS)

The coarsely powdered plant material was macerated separately with alcohol for 7 days, filtered and concentrated to get the extracts of CR, BP and AI. Each extract was weighed individually and mixed in a ratio of 2:1:1. It was well triturated with Tween 80. Gradually distilled water was added to get a well dispersed suspension.

Animals

Male wistar albino rats weighing between 140 to 180 gm were kept under standard laboratory conditions with 12 hrs. light and dark cycle, temperature 25±2°C, relative humidity 60±5% standard pellet diet and water ad libitum. The experiment was approved (approval no. 1413/PO/a/11/CPCSEA) by the Institutional Animal Ethics Committee as per CPCSEA guidelines (protocol approval no. SBRL/IAEC/2013/03).

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Acute toxicity

Acute toxicity studies were done as per OECD guidelines. A dose of 250, 500, 750, 1000, 1500 and 2000mg/kg was given to the rats. They were observed for any physiological or behavioural changes.

Antiurolithiatic activity

Ethylene Glycol induced urolithiasis[25-27]

The activity was studied in Ethylene glycol induced hyperoxaluria model. Six group were prepared having six rats in each group. They were given their doses orally. Group I- Served as normal control. GroupII- was treated as negative control and was given ethylene glycol (0.75%) in drinking water for 28 days, to induce renal calculi. Group III to VI were administered ethylene glycol (0.75%) in drinking water for 28 days. In addition they were given their respective doses orally from 15th to 28th day. Group III was given Standard Cystone 500mg/kg, Group IV - PHS 100mg/kg, Group V - PHS 200mg/kg and Group VI - PHS 300mg/kg.

Collection and analysis of urine[28-30]

After completion of dosing the animals were transferred to metabolic cages for collection of urine samples which were estimated for calcium, oxalate and phosphate. Table 1, Fig. 1

Collection of blood and serum analysis[31]

Retro-orbital puncture was done to collect the blood samples from rats. The blood sample was kept aside for 30 minutes at room temperature. It was than centrifuged at 3000 rpm, 20°C for 15 minutes and analyzed for urea, uric acid and creatinine. Table 2, Fig 2. The rats were sacrificed, kidney was removed and used for histopathological examination. Fig 3.

Table 1: Effect of polyherbal suspension on urinary parameters

S. no.	Group	Treatment	Urinary Parameters (mg/dl)		
			Calcium	Oxalate	Phosphate
1	I	Normal control	2.52±0.18	0.87±0.02	3.42±0.11
2	II	Negative control	4.48±0.1a***	2.72±0.15a***	7.37±0.16a***
3	III	Cystone 500mg/kg	2.91±0.08 b***	1.69±0.04a***, b***	4.19±0.24a*, b***
4	IV	PHS 100mg/kg	3.45±0.03 a***,b***	1.94±0.06 a***, b***	5.95±0.19 a***, b***
5	V	PHS 200mg/kg	3.52±0.13 a***,b***	1.9±0.16 a***, b***	5.46±0.13 a***, b***
6	VI	PHS 300mg/kg	3.04±0.12 b***	1.75±0.05a***, b***	5.26±0.04 a***,b***

All values are mean ± SEM, n = 6, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$
a- significant difference as compared to Normal control (Group-I)
b- Significant difference as compared to Negative control (Group-II)

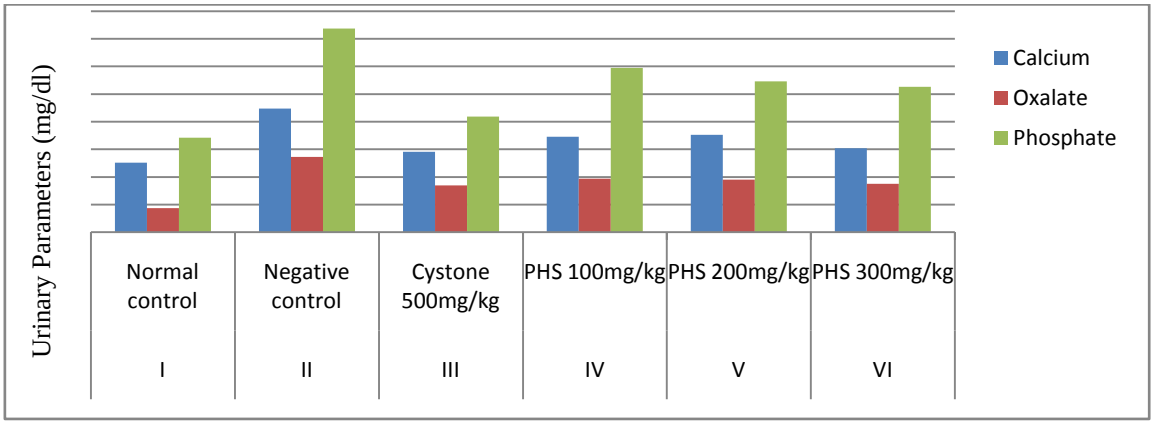


Figure 1: Effect of Polyherbal suspension on urinary parameters

Table 2: Effect of polyherbal suspension on serum parameters.

S. no.	Group	Treatment	Serum Parameter (mg/dl)		
			Creatinine	Uric acid	Urea
1	I	Normal control	0.65±0.06	1.29±0.09	36.91±0.19
2	II	Negative control	1.57±0.07 a***	3.42±0.11 a***	71.79±1.95 a***
3	III	Cystone 500mg/kg	1.16±0.04 a***,b***	1.85±0.12 b***	54.16±1.55 a***,b***
4	IV	PHS 100mg/kg	1.45±0.03a***	2.61±0.17 a***,b***	61.47±2.06 a***,b***
5	V	PHS 200mg/kg	1.35±0.04a***, b*	2.51±0.2 a***,b***	58.74±0.17 a***,b***
6	VI	PHS 300mg/kg	1.37±0.02a***	2.23±0.05 a***,b***	53.98±0.13 a***,b***

All values are mean ± SEM, n = 6, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$
a- significant difference as compared to Normal control (Group-I)
b- Significant difference as compared to Negative control (Group-II)

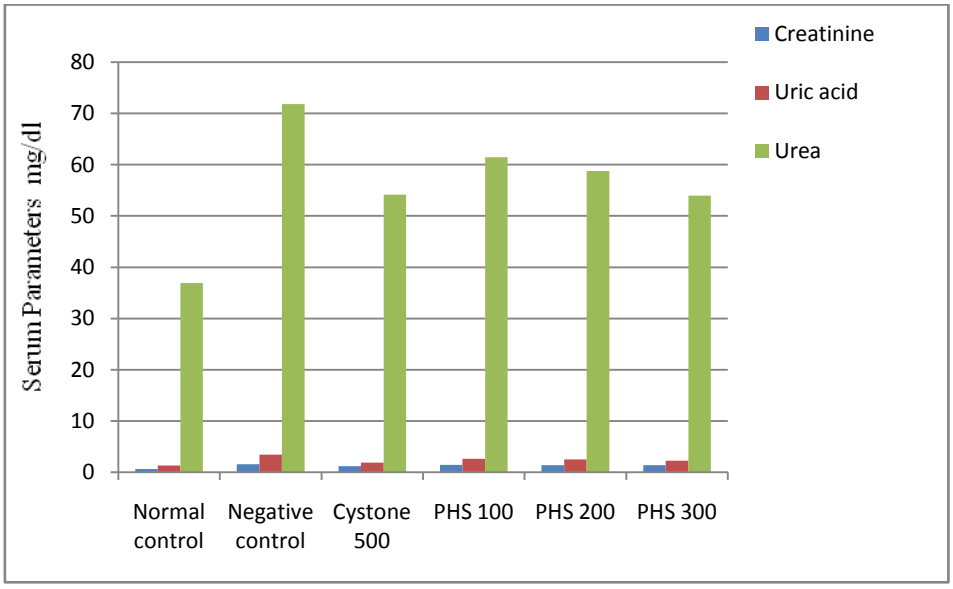
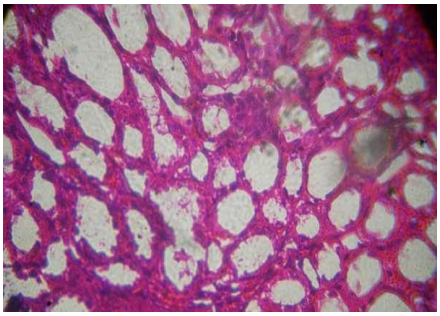
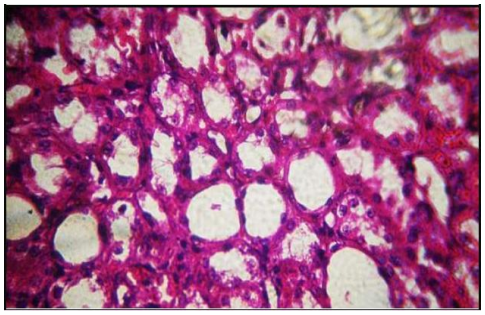


Figure 2: Effect of Polyherbal suspension on serum parameters

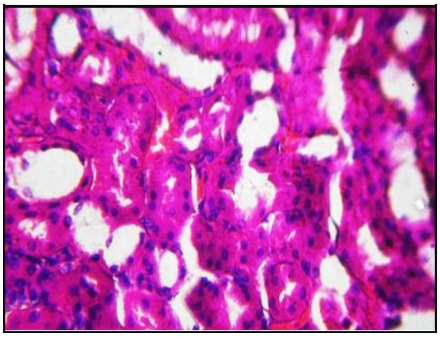
Histopathology



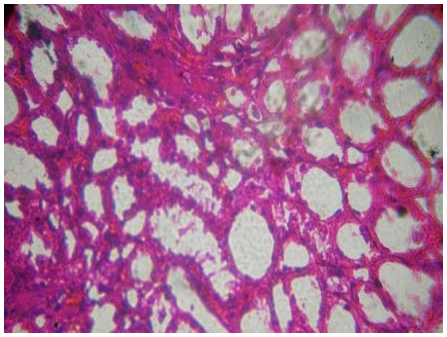
(a) Normal Control



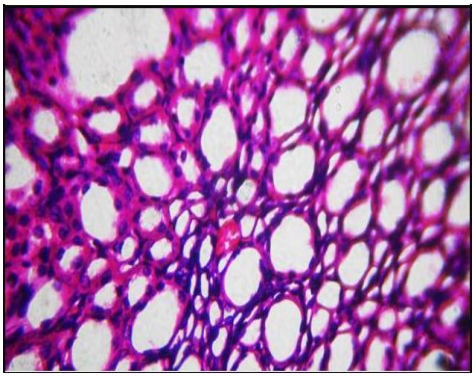
(b) STZ Negative control



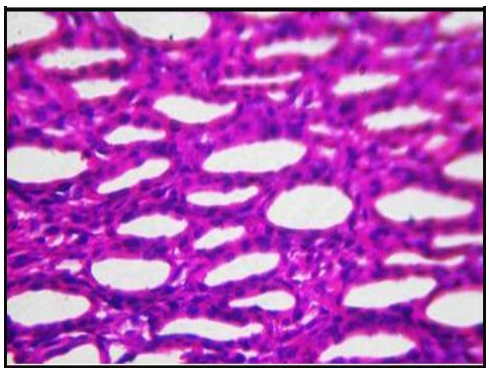
(c) Standard – Cystone



(d) PHS (100 mg/kg)



(e) PHS (200 mg/kg)



(f) PHS (300 mg/kg)

Figure 3: Histology of sections of kidney for antiurolithatic activity of PHS.

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Statistical analysis

The data was analysed by ANOVA and posthoc Tukey-Kramer Multiple Comparisons Test by employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at $P < 0.05$. All the values are expressed as mean±standard error of mean (S.E.M.).

Results

The formulation did not produce any abnormal behavioural changes or toxicity till the dose of 2000mg/kg. The data in Table 1 reveals an increase in the excretion of calcium, oxalate and phosphate in the ethylene glycol treated groups especially in negative control group. The polyherbal suspension significantly ($P<0.001$) enhanced the excretion of these ions at all dose levels thereby reducing the formation of stone. A dose of PHS 300mg/kg and cystone were able to bring the level of calcium to almost normal. All the doses of PHS were able to lower creatine, uric acid and urea significantly ($P<0.001$). Histology of kidney shows that in all the ethylene glycol treated animals the tissues are degenerated having vacuoles and obstructions when compared to the normal group but cystone and PHS have protected and/or regenerated the tissues.

DISCUSSION AND CONCLUSION

The polyherbal formulation is found to be safe till a dose of 2000mg/kg. Excess calcium, oxalate and phosphate lead to supersaturation of urine and formation of stones. Lithiasis occurs because of the supersaturation of urine due to hyperoxaluria and hypercalciuria[32]. Oxalates play an important role in stone formation and have about 15-fold greater effect than urinary calcium[33]. Hyperoxaluria can lead to formation of calcium oxalate[34] and increase in urinary phosphorus cause calcium phosphate crystals[35].

As urolithiasis is associated with pain and inflammation, the polyherbal formulation was prepared with herbs having diuretic, antimicrobial, analgesic, anti-inflammatory and antiurolithiatic activity. The polyherbal suspension was found to be safe and effective in expelling excess urinary calcium, oxalate, phosphate, creatine, uric acid and urea resulting in antiurolithiatic effect. It is preventing and/or dissolving the formed stones which can be seen from the data when compared to the negative control group.

Thus it can be concluded that the prepared polyherbal suspension can be used as a safe and effective antiurolithiatic formulation. It can be a boon to people suffering from lithiasis who have to undergo lithotripsy or surgery.

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CONFLICT OF INTEREST: We declare that there is no conflict of interest between us.

REFERENCES

1. Anderson RA, A complementary approach to urolithiasis prevention, World Journal of Urology, 2002, 20, 294-301.
2. Galani VJ, Panchal RR, Antiurolithiatic activity of centratherum anthelminticum (l.) Kuntze seeds against ethylene glycol induced urolithiasis in rats, International Journal of Phytotherapy Research, 2014, 4(1), 29-38.
3. Pathak AK, Argal A, Analgesic activity of *Calotropis gigantea* flower, Fitoterapia 2007, 78, 40–42.
4. Jagtap AG, Shirke SS, Phadke ASJ, Effect of polyherbal formulation on experimental models of inflammatory bowel diseases, Journal of Ethnopharmacology, 2004, 90(2-3),195-204.
5. Tatiya AU, Surana SJ, Sutar MP, Gamit NH, Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats, Pharmacognosy Research, 2012, 4(1), 50-56.
6. Daswani PG, Brijesh S, Tetali P, Birdi TJ, Studies on the activity of *Cyperus rotundus* Linn. tubers against infectious diarrhea, Indian J Pharmacol, 2011, 43(3), 340–344.
7. Uddin SJ, Mondal K, Shilpi JA, Rahman MT, Antidiarrhoeal activity of *Cyperus rotundus*, Fitoterapia, 2006, 77, 134–136.
8. Gupta MB, Palit TK, Singh N, Bhargava KP, Pharmacological studies to isolate the active constituents from *Cyperus rotundus* possessing anti-inflammatory, anti-pyretic and analgesic activities, Indian Journal of Medical Research, 1971, 59, 76–82.
9. Imam MZ, Sumi CD, *BMC Complementary and Alternative Medicine*, Evaluation of antinociceptive activity of hydromethanol extract of *Cyperus rotundus* in mice, 2014, 14**,** 83.
10. Sivapalan SR, Medicinal uses and Pharmacological activities of *Cyperus rotundus* Linn – A Review, International Journal of Scientific and Research Publications, 2013, 3(5), 1-8.
11. Jadav PD, Zalavadiya SK, Ghodasara JV, Rachchh MA, Evaluation of antiurolithatic activity of *cyperus rotundus* linn. rhizomes in rats , Inventi Impact: Ethnopharmacology , 2013, Article ID- Inventi:pep/841/13
12. Meena AK, Yadav AK, Niranjan US, Singh B, Nagariya AK, Verma M, Review on *Cyperus rotundus* - A Potential Herb, International Journal of Pharmaceutical and Clinical Research, 2010, 2(1), 20-22.
13. Chattopadhyay RR**,** Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II, Journal of Ethnopharmacology**,** 2003, 89(2–3), 217–219.
14. Sultana B , Anwar F , Przybylski R, Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. Trees, Food Chemistry, 2007, 104(3), 1106–1114.
15. Okpanyi SN, Ezeukw GC, Anti-Inflammatory and Antipyretic Activities of *Azadirachta indica*, Planta Med, 1981, 41(1), 34-39.
16. Nat JM, Klerx JPAM, Dijk HV, De Silva KTD, Labadie RP, Immunomodulatory activity of an aqueous extract of *Azadirachta indica* stem bark, Journal of Ethnopharmacology**,** 1987, 19(2), 125-131.
17. Hwisa NT, Assaleh FH, Gindi S, Melad FL, Chandu BR, Katakam P, A Study on Antiurolithiatic Activity of *Melia Azadirachta* L. Aqueous Extract in Rats, American Journal of Pharmacological Sciences, 2(1), 27-31.
18. Prasad AK, Kumar S, Iyer SV, Sudani RJ, Vaidya SK, Pharmacognostical, Phytochemical and Pharmacological Review on *Bryophyllum Pinnata*, International Journal of Pharmaceutical & Biological Archives, 2012, 3, 423-433.
19. Patil R, Bhargava K, Patel P, Singh K, Surana J, Diuretic and anti-urolithiatic activity of hydroalcoholic extract of leaves of *Kalanchoe pinnata*, J Pharm Res, 2009, 7(2), 87-91.
20. Fahad J, Vijayalakshmi, Satish Kumar MC, Sanjeeva, Kodancha GP, Adarsh B, Udupa AL, Rathnakar UP,

Antiurolithiatic activity of aqueous extract of bark of *moringa oleifera* (lam.) in rats, Health, 2010, 2(4), 352-355.
21. Ahmad A, Garg R, Sharma S, Evaluation on antiurolithiatic activity of *Bryophyllum pinnatum* of rats, International Journal of Pharmaceutical Science and Health Care, 2013, 3(6) 1-33.
22. Akinsulire OR, Aibinu IE, Adenipekun T, Adelowotan T, Odugbemi T, In vitro antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*, Afr J Tradit Complement Altern Med, 2007, 16(4), 338-344.
23. Akinpelu DA, Antimicrobial activity of *Bryophyllum pinnatum* leaves, Fitoterapia, 2000, 71(2), 193–194.
24. Harlalka GV, Patil CR, Protective effect of *Kalanchoe pinnata* pers.(Crassulaceae) on Gentamicine induced nephrotoxicity in rats, Indian Journal of Pharmacology, 2007, 39(4), 201-205.
25. Ashok P, Koti BC, Vishwanathswamy AH, Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats, Indian J Pharmacol, 2010, 42(6), 380-383.
26. Atmani F, Slimani Y, Mimouni M, Hacht B, Prophylaxis of calcium oxalate stones by *Herniaria hirsuta* on experimentally induced nephrolithiasis in rats, BJU Inter, 2003, 92, 137-140.
27. Mitra SK, Gopumadhavan S, Venkataranganna MV, Sundaram R, Effect of Cystone, a herbal formulation, on glycolic acid-induced urolithiasis, Phytotherapy Res, 12, 372-374.
28. Medeiros DM, Mustafa MA, Proximate composition, mineral content and fatty acids of cat fish (Ictalurus punctatus rafinesque) for different seasons and cooking methods, J Food Sci, 1985, 50, 585-587.
29. Hodgkinson A, Williams A, An improved colorimetric procedure for urine oxalate, Clinica Chimica Acta, 1972, 36, 127-132.
30. Fiske CH, Subbarow Y, The colorimetric determination of phosphate, J Biol Chem, 1925, 66, 375-400.
31. Caraway WT. Uric acid. In: Seligson D. Standard methods of clinical chemistry. London: Academic Press; 1963. P. 239-47.
32. Michell AR, Urolithiasis-historical, comparative and pathophysiological aspects: a review, Journal of the Royal Society of Medicine, 1989, 82, 669.
33. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV, Effect of *Moringa oleifer*a Lam. root-wood on ethylene glycol induced urolithiasis in rats, Journal of Ethnopharmacology, 2006, 105, 1(2) 306-311.
34. Khan SR, Hackett RL, Calcium oxalate urolithiasis in the rats: Is it a model for human stone disease? A review of recent literature, Scanning Electron Microscopy, Pt 2, 1985, 759-774.
35. *Soundararajan P*, Mahesh R, Ramesh T, Begum H, Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats, *Indian Journal* of Experimental *Biology*, 2006, 44, 981-986.

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