

Effect of *Scoparia dulcis* (Linn.) and *Aerva lanata* (Linn.) Whole Plant and Fruit Part Extracts on Ethylene Glycol Induced Urolithiasis in Male Albino Rats

P. Pandi Lakshmi^{1*}, Dr. C. D. Lethi¹, Dr. P. Kokilavani²

¹Department of Zoology, Holy Cross College, Tiruchirapalli, Tamil Nadu, India

²Department of Environmental Biotechnology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

Correspondence Address:

Pandi Lakshmi .P,

Department of Zoology,

Holy Cross College, Tiruchirapalli-620002

TamilNadu, India.

Email: VKSPPandi@gmail.com

Abstract: The potential of two commonly used phytotherapeutic agents such as *Scoparia dulcis* (Linn.) and *Aerva lanata* (Linn.) in the management of Calcium oxalate urolithiasis has been studied in male albino rats. Oral feeding of Ethylene glycol resulted in hyperoxaluria, increased renal excretion of calcium, phosphate and decreased level of magnesium, uric acid, citrate, creatinine as well as increased level of serum uric acid and creatinine. The pH level also decreased in Ethylene glycol administered rat urine. Administration with aqueous extract of whole plant and fruit part of *Scoparia dulcis* and *Aerva lanata* gradually reduced the stone constituents and increased the stone inhibiting constituents. Comparatively animal group supplemented with fruit part of *Scoparia dulcis* in combination with *Aerva lanata* showed significantly reduced stone forming constituents and increased stone inhibiting constituents. This indicates that the fruit part of *Scoparia dulcis* in combination with *Aerva lanata* is endowed with higher antiurolithiatic activity than others.

Keywords: *Scoparia dulcis*, *Aerva lanata*, Hyperoxaluria, Urolithiasis, Ethylene glycol

1. Introduction

Kidney stone is a ubiquitous disease afflicting mankind and it continuous to pose a universal health problem. It is a complex multifactorial disease resulting from an interaction between environmental and genetic factors (Aggarwal *et al.*, 2000). Nearly 4-15% of human populations are suffering from urinary stone problem all over the globe and 80% of them are men between the ages of 20 and 50 years (Chauhan *et al.*, 2008). Areas of high incidence of urinary calculi include the British Isles, Scandinavian countries, Northern Australia, Central Europe, Northern India, Pakistan and Mediterranean countries. In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage (Mohamed *et al.*, 2009). Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analysed stones (Prien and Prien, 1968).

Currently surgical procedures and extra corporeal shock wave lithotripsy are commonly employed in the management of urinary stones. The major drawback of these procedures is the recurrence of stones. A number of plant extracts and their derivatives are also used in its management (Pharmacognosy reviews, 2007). Many

medicinal plants are evaluated to manage against calcium oxalate and magnesium ammonium phosphate types of kidney stones, employing various experimental models of urolithiasis. However, most of these studies are preliminary and are not sufficient for the development of pharmaceutical product (Pharmacognosy reviews, 2007).

Scoparia dulcis belonging to the Family Plantaginaceae is commonly known as Bitter broom, broom weed and licorice weed. It is an erect annual herb that grows to a height of 0.5 meters widely distributed in many tropical countries and is found abundance in South America and the Amazon rain forest. In many areas, the herb is considered as an invasive weed. Indigenous people in Ecuador consume tea of the entire plant to reduce swellings, aches and pains. The indigenous tribes in Nicaragua use a hot water infusion or decoction of the leaves or the whole plant for stomach pain, for menstrual disorder, as an aid in child birth, as a blood purifier, for insect bites, fever, heart problems, liver and stomach disorders, malaria, sexually transmitted diseases and as a general tonic (The healing power of Rain forest herbs. 2005). Extracts of the plant have been shown to have antihyperglycemic (Pari *et al.*, 2004), antioxidant (Latha *et al.*, 2004 and Coulibaly *et al.*, 2011) and antimicrobial activity (Zulfiker *et al.*, 2011). Scoparinol, an isolate of the

plant was shown to have analgesic, diuretic and anti-inflammatory activity (Ahmed *et al.*, 2001).

Aerva lanata belonging to the Family Amaranthaceae is commonly known as mountain knotgrass. It is a woody, prostrate or succulent, perennial herb, 30-60 cm in height, native to Asia, Africa, and Australia (Germplasm Resources Information Network (GRIN), 1987). It is common throughout the hotter parts of India. It is also found to be present in Sri Lanka, Arabia, Egypt, tropical Africa, Java and Phillipines (Annie *et al.*, 2004). It is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala. This plant is used as food for people and animals. The whole plant, especially the leaves, is edible. The leaves are put into soup or eaten as spinach or as a vegetable (Medicinal Plants Used For Snake Treatment, 2013). In the traditional system of medicine, the plant is being used as diuretic, antihelminthic, antidiabetic (Gupta and Neerai, 2004), for arresting haemorrhage during pregnancy, burn healing, as an anti-inflammatory, for head ache, skin disease, to dissolve kidney and gall bladder stones (Yoga *et al.*, 1979), to treat nasal bleeding, cough, scorpion stings, fractures and spermatorrhoea (Sikarwar and Kaushik, 1993), in rheumatism and bronchitis, as an antimalarial drug and in snakebite (Sing and Sing, 1992; Bedi, 1978). The active components present in *Aerva lanata* are flavonoids, glycosides, carbohydrates, alkaloids, tannins, saponins, terpenoids, phenols and phytosterols are responsible for its pharmacological activities (Devi Rajeswari *et al.*, 2012).

In the present study, an effort has been made to exploit the antiurolithiatic property of *Scoparia dulcis* and *Aerva lanata* whole plant and fruit part extracts using ethylene glycol induced hyperoxaluria model in rats.

2. Material and Methods

Plant Collection

The plant *Scoparia dulcis* was collected from Trichur District, Kerala, India and *Aerva lanata* from Tiruchirappalli, Tamil Nadu, India. The identification of both the plants was authenticated at The Rapinat Herbarium, St. Joseph's College (campus), Tiruchirappalli, Tamil Nadu, India by Dr.S.John Britto. A voucher specimen of the plants was deposited in the Rapinat herbarium under the number PPL001 for *Scoparia dulcis* and PPL002 for *Aerva lanata*. The whole plant and fruit part of both the plants were dried separately and were ground to get a coarse powder.

Preparation of Extract

Powder of whole plant and fruit part of *Scoparia dulcis* and *Aerva lanata* (200 mg / Kg of body weight) was suspended in distilled water just prior to oral administration.

Animal Selection

Healthy adult male Wister albino rats weighing between 150 and 200g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory

conditions. They were provided with regular rat chow and drinking water ad libitum and they were used as per the ethical committee recommendation.

Ethylene Glycol Induced Urolithiasis Model

Ethylene glycol induced hyperoxaluria model (Atmani *et al.*, 2003) was used to assess the antilithiatic activity in albino rats. Rats have been used for decades as a kidney stone model induced by the administration of oxalate precursors. Among many methods, free drinking of 1% ethylene glycol is the easiest method with advantages of the method being that animals did not need to be handled on a daily basis (Khan *et al.*, 2002; Harris and Richardson, 1980). Male rats were used to induce urolithiasis because the urinary system of male rats resembles that of humans (Vermeulen, 1962) and also earlier studies have shown that the amount of stone deposition in female rats was significantly less (Prasad *et al.*, 1993).

Animals were divided into eight groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (1%) in drinking water was fed to Group II, III, IV, V, VI and Group VII for induction of renal calculi till 60th day. Group III received *S. dulcis* whole plant extract (200mg/kg body weight), Group IV received *S. dulcis* fruit extract (200mg/kg body weight), Group V received *A. lanata* whole plant extract (200mg/kg body weight), Group VI received *A. lanata* fruit extract (200mg/kg body weight), Group VII received *S. dulcis* in combination with *A. lanata* fruit extract from first day till 60th day (After 60 days of Ethylene glycol induction) and Group VIII (Control without Ethylene glycol induction) received *S. dulcis* in combination with *A. lanata* fruit extract (200mg/kg body weight). All extracts were given once daily by oral route.

Assessment of Antiurolithiatic Activity

Collection and analysis of Urine and serum

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 60th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated HCL was added to the urine being stored at 4 °C. Urine was analysed for Calcium (Schwarzenbach G, 1985 and Kessler *et al.*, 1964), Phosphate (Fiske and Subbarow, 1925), Oxalate (Hodgekinson and Williams, 1972), Uric acid (Fossati *et al.*, 1980), Creatinine (Allen L.C, 1982 and Haeckel *et al.*, 1981), Magnesium (Sky-peck, 1964), Citrate (Petarulo *et al.*, 1995) and pH (Beckman pH meter). Blood was collected from the retro-orbital under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min and analysed for Creatinine (Allen L.C, 1982 and Haeckel *et al.*, 1981) and Uric acid (Fossati *et al.*, 1980).

3. Statistical Analysis

Results were expressed as mean ± SD. Significance among data were determined using Two – way ANOVA followed

by Duncan's post multiple comparison test (SPSS 20 Tool). Differences between the data were considered significant at $P < 0.05$.

4. Results

Administration of 1% Ethylene glycol aqueous solution to male albino rats resulted in increased level of urinary risk factors. Calcium, Oxalate, Phosphate excretion were grossly increased and the level of Creatinine, Uric acid, Magnesium, Citrate and pH decreased in calculi induced animals (Table 1, Group II and Fig.1 and 2) compared to that of control (Table 1, Group I and Fig.1 and 2).

Supplementation with whole plant and fruit part extract of *S. dulcis* and *A. lanata* significantly ($P < 0.05$) lowered the elevated levels of Calcium, Oxalate, Phosphate and increased the level of Creatinine, Uric acid, Magnesium, Citrate and pH in urine (Table 1, Group III, IV, V and VI and Fig. 1 and 2).

However, fruit part of *S. dulcis* in combination with *A. lanata* more significantly ($P < 0.05$) reduced the levels of

stone forming constituents and increased the stone inhibiting constituents in urine (Table 1, Group VII and Fig.1 and 2). In Group VIII (fruit part of *S. dulcis* in combination with *A. lanata* – control), the level of Calcium, Oxalate and Phosphate were highly decreased, whereas Magnesium, Citrate and pH level were increased and the excretion of Uric acid and Creatinine in urine were also increased even than that of normal control.

The serum Uric acid and Creatinine were dramatically increased in calculi induced animals indicating marked renal damage (Table 1, Group II and Fig. 3). Administration of *S. dulcis* and *A. lanata* whole plant and fruit part extracts lowered the elevated serum levels of uric acid and creatinine (Table 1, Group III, IV, V and VI and Fig. 3). However, fruit part of *S. dulcis* in combination with *A. lanata* remarkably decreased the serum levels of uric acid and creatinine (Table 1, Group VII and Fig. 3). In Group VIII (fruit part of *S. dulcis* in combination with *A. lanata* – control), the serum level of Uric acid and Creatinine significantly increased even than that of normal control.

Table 1: Effect of *S. dulcis* and *A. lanata* whole plant and fruit part extracts on urinary and serum parameters in control and experimental animals

Parameter (Unit)	Group I- Normal Control	Group II- Calculi induced	Group III- Calculi induced + <i>S. dulcis</i> whole plant treated	Group IV- Calculi induced + <i>S. dulcis</i> fruit part treated	Group V- Calculi induced + <i>A. lanata</i> whole plant treated	Group VI- Calculi induced + <i>A. lanata</i> fruit part treated	Group VII- Calculi induced + <i>S. dulcis</i> + <i>A. lanata</i> fruit part treated	Group VIII- <i>S. dulcis</i> + <i>A. lanata</i> fruit part treated control	P-Values
Urine(mg/dl)									
Calcium	47.03 ± 0.91	77.24 ± 1.26	44.78 ± 0.51	38.87 ± 0.46	37.07 ± 0.30	35.35 ± 0.76	33.63 ± 0.41	31.01 ± 0.53	<0.05
Oxalate	0.60 ± 0.01	5.31 ± 0.15	1.01 ± 0.05	0.88 ± 0.006	0.89 ± 0.04	0.70 ± 0.01	0.67 ± 0.008	0.48 ± 0.009	<0.05
Phosphate	3.94 ± 0.05	8.24 ± 0.12	4.23 ± 0.03	4.12 ± 0.02	4.12 ± 0.01	3.99 ± 0.11	3.68 ± 0.04	3.04 ± 0.02	<0.05
Magnesium	53.19 ± 1.83	30.10 ± 0.11	66.91 ± 0.21	69.91 ± 0.19	68.99 ± 0.07	73.08 ± 0.10	74.94 ± 0.09	75.92 ± 0.21	<0.05
Citrate	3.65 ± 0.008	0.98 ± 0.008	3.99 ± 0.008	4.48 ± 0.02	4.15 ± 0.01	4.70 ± 0.008	4.70 ± 0.02	4.75 ± 0.007	<0.05
Uric acid	47.66 ± 0.42	22.22 ± 1.27	47.55 ± 0.52	47.93 ± 0.09	47.78 ± 0.43	50.78 ± 0.43	53.24 ± 0.77	54.86 ± 0.22	<0.05
Creatinine	1.01 ± 0.01	0.55 ± 0.009	1.65 ± 0.01	1.69 ± 0.008	1.67 ± 0.008	1.73 ± 0.007	1.74 ± 0.005	1.75 ± 0.006	<0.05
pH	7.03 ± 0.01	6.02 ± 0.013	7.09 ± 0.012	7.15 ± 0.008	7.14 ± 0.008	7.16 ± 0.005	7.19 ± 0.008	7.20 ± 0.008	<0.05
Serum(mg/dl)									
Uric acid	1.57 ± 0.31	4.02 ± 0.31	2.00 ± 0.008	1.78 ± 0.01	1.83 ± 0.02	1.69 ± 0.02	1.60 ± 0.008	1.16 ± 0.01	<0.05
Creatinine	0.74 ± 0.01	0.95 ± 0.03	0.86 ± 0.01	0.80 ± 0.02	0.85 ± 0.01	0.78 ± 0.02	0.75 ± 0.01	0.73 ± 0.01	<0.05

Values for urinary parameters are assessed in 24 h urine sample. All values are expressed as mean ± S.D for six animals in each group

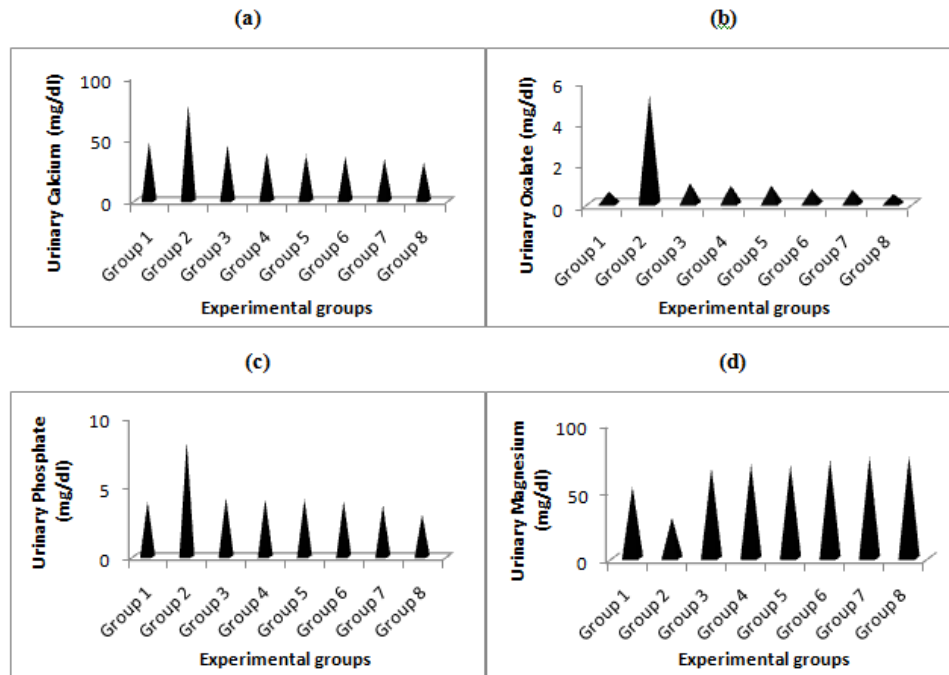


Figure 1- Effect of *S. dulcis L.* and *A. lanata L.* whole plant and fruit part extracts on Urinary parameters of Ethylene glycol induced male albino rats (a) Calcium, (b) Oxalate, (c) Phosphate and (d) Magnesium. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* - Fruit, Group 5 - EG induced + *A. lanata* - whole plant, Group 6 - EG induced + *A. lanata* - Fruit, Group 7 - EG induced + *S. dulcis* - Fruit + *A. lanata* - Fruit, Group 8 - *S. dulcis* fruit + *A. lanata* fruit - control

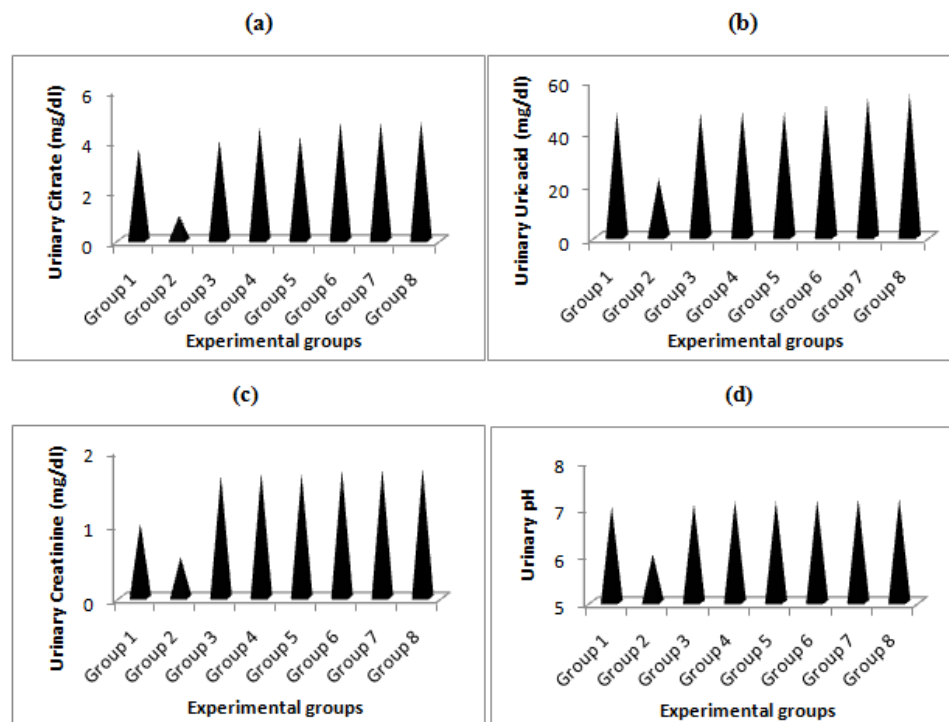


Figure 2- Effect of *S. dulcis L.* and *A. lanata L.* whole plant and fruit part extracts on Urinary parameters of Ethylene glycol induced male albino rats. (a) Citrate, (b) Uric acid, (c) Creatinine and (d) pH. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* - Fruit, Group 5 - EG induced + *A. lanata* - whole plant, Group 6 - EG induced + *A. lanata* - Fruit, Group 7 - EG induced + *S. dulcis* - Fruit + *A. lanata* - Fruit, Group 8 - *S. dulcis* fruit + *A. lanata* fruit - control

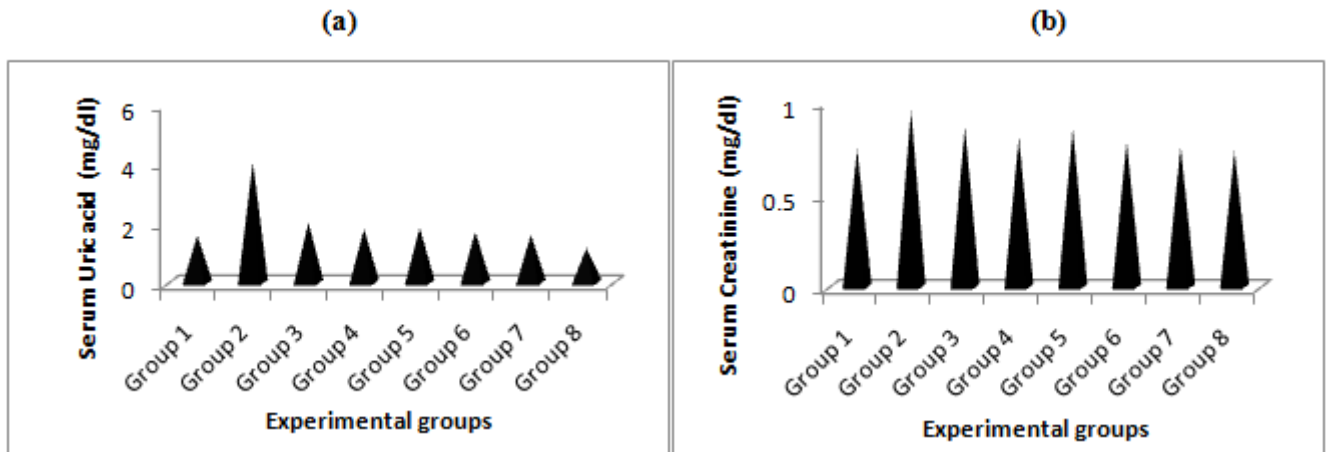


Figure 3- Effect of *S. dulcis* L. and *A. lanata* L. whole plant and fruit part extracts on Serum parameters of Ethylene glycol induced male albino rats. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* - Fruit, Group 5 - EG induced + *A. lanata* - whole plant, Group 6 - EG induced + *A. lanata* - Fruit, Group 7 - EG induced + *S. dulcis* - Fruit + *A. lanata* - Fruit, Group 8 - *S. dulcis* fruit + *A. lanata* fruit - control

5. Discussion

Administration of Ethylene glycol induced kidney stones in rats. This finding is in corroboration with many earlier research evidences. Treatment with ethylene glycol (0.75%) for 14 days is reported to develop renal calculi composed mainly of calcium oxalate in young male albino rats (Selvam *et al.*, 2001; Huang *et al.*, 2002; Atmani *et al.*, 2003). Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate (Selvam *et al.*, 2001). Similar studies have also been obtained when rats were treated with ethylene glycol and ammonium oxalate (Adhirai and Selvam 1997; Muthukumar and Selvam 1997; Kavadi *et al.*, 2006). Formation of renal calculi depends on the nucleation of stone forming constituents principally calcium oxalate in the urinary tract (Ryall, 2011).

In this study, Calcium, Oxalate and Phosphate excretion were progressively increased in calculi-induced animals (Group II). Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or calcium phosphate from urine and subsequent crystal growth (Lemann *et al.*, 1991). Hyperoxaluria is known as a far more significant factor in the pathogenesis of renal stones than hypercalciuria (Tisselius, 1996) and the changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Roger *et al.*, 1997). Treatment with *S. dulcis* and *A. lanata* whole plant and fruit part lowered the levels of Calcium, Oxalate as well as Phosphate excretion.

Magnesium is suggested to be an important inhibitor of calcium oxalate stone formation. Magnesium may reduce oxalate absorption and urinary excretion nearly as effectively as calcium by binding oxalate in the gut

(Liebman and Costa, 2000). A high urinary excretion and concentration of magnesium has been shown to decrease both nucleation and growth rates of calcium oxalate crystals, due to the higher solubility of magnesium oxalate compared with calcium oxalate (Kohri *et al.*, 1988 and Li *et al.*, 1985). Magnesium is a divalent cation, which can complex with oxalate and reduces its urinary station. Calcium oxalate stone formation may thereby be inhibited. Urinary citrate has been identified as a potent inhibitor of Calcium stone formation (Pak, 1994). Evidence over many years has now established hypocitraturia as a key contributor to the formation of CaOx stones (Ryall, 2011). Citrate inhibits stone formation by forming soluble complexes with calcium which inhibit crystal nucleation and growth (Heilberg and Schor, 2006). During the stone formation period urinary pH decreased to a significantly lower level (pH 6.2) (Okada *et al.*, 2007). Our studies also correlates with the above evidences that low urine Magnesium, Citrate and pH may also promote stone formation. However administration of whole plant and fruit part extract of *S. dulcis* and *A. lanata* increased the urinary level of Magnesium, Citrate and pH. Phytochemical studies suggest that *S. dulcis* and *A. lanata* are rich sources of Magnesium (Muthumani *et al.*, 2010; Omoyeni and Adeyeye, 2009).

One of our outstanding findings is that the urinary excretion of Uric acid and Creatinine has been lowered in calculi induced animals due to renal damage. However treatment with whole plant and fruit part extract of *S. dulcis* and *A. lanata* highly increased urinary excretion of Uric acid and Creatinine.

In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the outflow of urine by stone in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood (Ghodkar, 1994). Increased lipid peroxidation and decreased levels of antioxidant potential have also been reported in the kidneys of rats supplemented with a calculi producing diet (Sumathi *et al.*, 1993; Saravanan *et al.*, 1995). Oxalate has

been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with poly saturated fatty acids in cell membrane (Ernster and Nordenbrand, 1967). However treatment with whole plant and fruit part extract of *S. dulcis* and *A. lanata* highly reduced the serum levels of Uric acid and Creatinine.

Another important finding in our study is that in Group VIII ((fruit part of *S. dulcis* in combination with *A. lanata* – control), the level of stone forming constituents were highly decreased and stone inhibiting constituents were remarkably increased than that of normal control, this is because even in normal control the stone forming constituents can rise to fairly high levels due to some factors such as drinking water, diet and aging.

In conclusion, the presented data indicate that administration of *S. dulcis* and *A. lanata* whole plant and fruit part extract to rats with ethylene glycol induced lithiasis reduced the levels of stone forming constituents and thereby prevents the formation of kidney stones. However, administration of fruit part of *S. dulcis* in combination with *A. lanata* highly reduces the risk of stone formation than others. The fruit juice and seed extract of the medicinal plants is moderate to good inhibitor of calcium oxalate, calcium carbonate and calcium phosphate mineralization. Sequestering of this insoluble calcium salts by the fruit juice might be due to effective single and mixed ligand chelation by the hydroxyl acids present in them (Mohamed Farook *et al.*, 2009)

References

- [1] Adhirai, M., Selvam, R., 1997. Vitamin E pretreatment prevents cyclosporineA – induced crystal deposition in hyperoxaluric rats. *Nephron*. 75, 77-81.
- [2] *Aervalanata. Medicinal Plants Used For Snake Treatment*. ToxicologyCentre.com. Retrieved 2013-12-10.
- [3] Aggarwal, S., Tandon, C.D., Forouzandez, N., Single, K., Kiran, R., Jethi, R.K., 2000. Role of Biomolecules from human renal stone matrix on COM crystal growth. *Molecular and cellular Biochemistry* 210: 109 – 119, 2000.
- [4] Ahmed, M., Shikha, H.A., Sadhu, S.K., Rahman, M.T., Datta, B.K., 2001. "Analgesic, diuretic, and anti-inflammatory principle from *Scopariadulcis*". *Die Pharmazie* 56 (8): 657–60. PMID 11534346.
- [5] Allen, L.C., 1982. *Clin. Chem.* Vol. 28 No. 3, 555.
- [6] Annie Shirwaikar., Deepti Issac., Malini, S., 2004. Effect of *Aervalanata* on cisplatin, gentamicin models of acute renal failure. *J. Ethnopharmacol* ; 90:81-86.
- [7] Atmani., 2000. Prophylaxis of calcium oxalate stones by *Herniaria hirsute* on experimentally induced nephrolithiasis in rats. *British Journal of Urology International* 92, 137-140.
- [8] Bedi, SJ (1978). *Ethnobotany of the Ratanmahal Hills, Gujarat, India*, *Econ Bot*, 32, 278-284.
- [9] Chauhan C.K., Joshi, M.J., Vaidhya, A.D.B., 2008. Growth inhibition of struvite crystals in the presence of herbal extract *Commiphora wightii*, Springer science + Business Media, LLC.
- [10] Coulibaly, Ahmed, Y., Kiendrebeogo, Martin, Kehoe, Patrick, G., Sombie, Pierre, A.E.D., Lamien, Charles, E., Millogo, Jeanne, F., Nacoulma, Odile, G., 2011. "Antioxidant and Anti-Inflammatory Effects of *Scopariadulcis* L". *Journal of Medicinal Food* 14 (12): 1576–82. Doi:10.1089/jmf.2010.0191. PMID 21870938.
- [11] Devi Rajeswari, V., Gajalakshmi, S., Vijayalakshmi, S., 2013. Pharmacological activities of *Aerva lanata*: A perspective review. *International Research Journal of Pharmacy*, 3(1) ISSN 2230 – 8407.
- [12] Ernster, L., Nordenbrand, K., 1967. Oxidation and phosphorylation In. Ronald WE, Maynard EP (Eds.). *Methods in enzymology*, vol. 10, Academic press, New York, pp574-580.
- [13] Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphate, *Journal of Biological Chemistry* 66, 375-381.
- [14] Fossati, P., Prencipe, L., Berti, G., 1980. Use of 3,5-dichloro-2-hydroxybenzene sulfonic acid /4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine, *Clin Chem* 1980; 26:227-231.
- [15] Germplasm Resources Information Network (GRIN) (1987-04-28). "Taxon : *Aervalanata* (L.) Juss. ExSchult. *Taxonomy for Plants*. USDA, ARS, National Genetic Resources Program, National Germplasm Resources Laboratory, Beltsville, Maryland. Retrieved 2008-04-27.
- [16] Ghodkar, P.B., 1994. Chemical tests in kidney disease. In. *Textbook of medical laboratory technology*, first ed. Bhalanipublishing house. Mumbai. pp 118-132.
- [17] Gupta, A.K., Neeraj, T., 2004. Reviews on Indian medicinal plants, Vol. I, ICMR; New Delhi, pp.338-340.
- [18] Haeckel *et al.*, 1981. *Clin. Chem.* 27/1, 179-183.
- [19] Harris, K.S., Richardson, K.E., 1980. Glycolate in the diet and its conversion to urinary oxalate in the rat. *Invest Urol* 18: 106-109.
- [20] Heilberg, I.P., Schor, N., 2006. Renal stone disease: causes, evaluation, and medical treatment. *Arq Bras Endocrinol Metab.* ;50:823.
- [21] Hodgkinson, A., Williams, A., 1972. An improved colorimetric procedure for urine oxalate. *Clinica Chimica Acta* 36, 127-132.
- [22] Huang, H.S., Ma, M.C., Chen, J. Chen. C.F., 2002. Changes in the oxidant – antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. *Journal of Urology* 167, 2584-2593.
- [23] Karadi, R.V., Gadge, N.B., Alagawadi, K.R., Savadi, R.V., 2005. Effect of *Moringa oleifera* Lam. Root on ethylene glycol induced urolithiasis in rats. *Journal of Ethnopharmacology* 105, 306-311.
- [24] Kessler, G., Wolfman, M., 1964. *Clin. Chem.* 10, 686-703.
- [25] Khan, S.R., Johnson, J.M., Peck, A.B., Cornelius, J.G., Glenton, P.A., 2002. Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. *J Urol* 168: 1173-1181.

- [26] Kohri, K., Garside, J., Blacklock, N.J., 1988. The role of magnesium in calcium oxalate urolithiasis. *Br J Urol.*; 61:107-115.
- [27] Latha, Muniappan, Pari, Leelavinothan, Sitasawad, Sandhya, Bhonde and Ramesh, 2004. "Scopariadulcis, a traditional antidiabetic plant, protects against streptozotocin induced oxidative stress and apoptosis in vitro and in vivo". *Journal of Biochemical and Molecular Toxicology* 18 (5): 261-72. Doi:10.1002/jbt.20035. PMID 15549711.
- [28] Lemann, Jr. J., Worcester, E.M., Gray, R.W., 1991. Hypercalciuria and stones. *American journal of kidney diseases* 27, 386-391.
- [29] Li, M.K., Blacklock, N.J., Garside, J., 1985. Effects of magnesium on calcium oxalate crystallization. *J Urol.*; 133:123-125.
- [30] Liebman, M., Costa, G., 2000. Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *J Urol.*; 163:1565-1569.
- [31] Mohamed Farook, N.A., Rajesh, S., Jamuna, M., 2009. Inhibition of Mineralization of Urinary Stone Forming Minerals by Medicinal Plnts. E- Journal of chemistry, 6(3), 938-942.
- [32] Muthukumar, A., Selvam, R., 1997. Effect of deposition of reduced glutathione and its supplementation by glutathione monoester on renal oxalate retention in hyperoxaluria. *Journal of nutrition and Biochemistry* 8, 445-150.
- [33] Muthumani, P., Christina, A.J.M., Venkataraman, S., Meera, R., Jiju Abraham, Devi, P., Kameswari, B., Eswarapriya B., 2010. Preliminary phytochemical screening, chemical investigation, enzyme inhibiting activity and atomic absorption spectrophotometric determination of minerals of plant extracts of *Scopariadulcis*. *International Journal of Pharmaceutical Sciences Review and Research*, Volume 2, issue 2, Article 011, ISSN 0976 – 044x.
- [34] Okada, A., Nomura, S., Higashibata, Y., Hirose, M., Gao, B., Yoshimura, M., Itoh, Y., Yasui, T., Tozawa, K., Kohri, 2007. Successful formation calcium oxalate crystal deposition in mouse kidney by intrabdominal glyoxylate injection. *UrolRes* 35: 89-99.
- [35] Omoyeni, O.A., Adeyeye, E.I., 2009. Chemical composition, calcium, zinc and phytochemical relationships in *Aerva lanata* (Linn) Juss. *exschult* leaves. *Oriental journal of chemistry*, vol. 25(3), 485-488.
- [36] Pak, C.Y.C., 1994. Citrate and renal calculi: an update. *Min Electrolyte Metab.* 20:371-377.E
- [37] Pari, Leelavinothan, Latha and Muniappan, 2004. Protective role of *Scopariadulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complementary and Alternative medicine* 4: 16. Doi:10.1186/1472-6882-4-16. PMC 533881. PMID 15522116.
- [38] Petrarulo, M., Facchini, P., Cerelli, E., Marangella, M., Linari, F., 1995. Citrate lyase method, *Clin. Chem.*, 41/10, 1518-1521.
- [39] *Pharmacognosy Reviews*, 2007. Vol 1, Issue 1.
- [40] Prasad, K.V.S.R.G., Bharathi, K., Srinivasan, K.K., 1993. Evaluation of *Musa (Parasidica Linn Cultivar)* – "Pettubale" stem juice for antilithiatic activity in albino rats. *Indian journal of physiology and pharmacology.* 37: 337-341.
- [41] Prien, E.L., Prien, E.L.J., 1968. Composition and structure of urinary stones. *American Journal of Medicine* 45, 654-672.
- [42] Robertson, W.G., Peacock, M., 1980. The course of idiopathic calcium disease: hypercalciuria and hyperoxaluria? *Nephron* 26, 105-110.
- [43] Roger, K., Low, M.D., Stoller, M.L., 1997. Uric acid nephrolithiasis. *Urologic clinics of North America* 24, 135-148.
- [44] Ryall, R.L., 2011. The possible roles of inhibitors, promoters and macromolecules in the formation of calcium kidney stones. P.N. Rao et al. (eds.), *Urinary Tract Stone Disease*, 31, DOI 10.1007/978-1-84800-362-0_4, © Springer-Verlag London.
- [45] Saravanan, N., Senthil, D., Varalakshmi, P., 1995. Effect of L- cysteine on lipid peroxidation in experimental urolithiatic rats. *Pharmacological Research* 32, 165-169.
- [46] Schwarzenbach, G., 1955. *Analyst*, 80, 713-729.
- [47] Selvam, P., Kalaiselvi, P., Govindaraj, A., Murugan, V.B., Sathiskumar, A.S., 2001. Effect of *A. lanata* leaf extract and *Vediuppuchunnam* on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. *Pharmacological Research* 43, 89-93.
- [48] Sikarwar, R.L.S., Kaushik, J.P., 1993. Folk medicines of the morena district, Madhya Pradesh, India, *Int J pharmacgn*, 31, 283-287.
- [49] Sing, L.B., Sing, C.L., 1992. An ethno-medico-botanical study of Deoghar district, Bihar, *Bio Journal*, 4, 83-86.
- [50] Sky-peck, H.H., 1964. *Clinical Chemistry*, 10/5/391.
- [51] Sumathi, R., Jayanthi, S., Kalpanadevi, V., Varalakshmi, P., 1993. Effect of α - lipoic acid on tissue lipid peroxidation and antioxidant system in normal and glycolate treated rats. *Pharmacological Research* 27, 1-10
- [52] *The healing power of Rain forest herbs*. 2005. Square One publishers. New York.
- [53] Tisselius, H.G., 1996. *Solution chemistry of supersaturation*. Int: Coe.F.L., Favus, M.J., Pak, C.Y.C., Parks, J.H., Preninger, G.M. (Eds.). *Kidney stones: Medical and Surgical Management*. Lippincott Reven. Philadelphia, p.33.
- [54] Vermeulen, C.W., *Experiments on causation of urinary calculi*. In: *Essays in Experimental Biology*. University of Chicago. 1962; pp.253-269.
- [55] Yoga Narasimhan, S.N., Bhat, A., Togunashi, V.S., 1979. *Medicinal plants from mysore district, Karnataka*, *Indian drug pharmaceut Ind*, 14, 7-22.
- [56] Zulfiker, Abu Hasanat, Siddiqua, Masuma, Nahar, Laizuman, Habib, Razibul, Uddin, Nizam, Hasan, Nahid, Rana and Sohel. 2011. "Invitro Antibacterial, antifungal and cytotoxic activity of *Scopariadulcis L*". *International Journal of Pharmacy and Pharmaceutical Sciences* 3 (Suppl 2): 198-203