International Journal of
Biological Chemistry

ISSN 1819-155X

Academic
Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 1 (4): 221-228, 2007
ISSN 1819-155X
© 2007 Academic Journals Inc.

Biopotency of *Aerva lanata* on Membrane Bound ATPases and Marker Enzymes in Urolithic Rats

P. Soundararajan, R. Mahesh, T. Ramesh and V. Hazeena begum
Department of Siddha Medicine, Faculty of Science, Tamil University,
Thanjavur, Tamilnadu-613 010, India

Abstract: *A. lanata* aqueous suspension was tested for its biopotency on membrane bound enzymes and marker enzymes in urolithiasis in male albino rats. Calcium oxalate urolithiasis was induced by 0.75% ethylene glycol in drinking water for 28 days. There was a significant decrease in membrane bound enzymes such as Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and marker enzymes Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) and Gamma-Glutamyl Transferase (GGT) in kidney and liver while Lactate Dehydrogenase (LDH) was increased. In serum electrolytes, sodium was reduced and K, Ca and Mg levels were elevated. The AST, ALT, ALP, LDH and GGT were increased in serum and urine of urolithic rats. Therapeutic treatment with *A. lanata* aqueous suspension (2 g kg^{-1} b.wt. $dose^{-1}$ day^{-1} $oral^{-1}$) has significantly ameliorated to near normalcy in the curative group. The results showed that *A. lanata* might represent an alternative form of treatment and/or prevention of urolithiasis.

Key words: Urolithiasis, membrane bound ATPases, marker enzymes, *A. lanata*

INTRODUCTION

Ethylene glycol was reported to induce calcium oxalate type of urolithiasis by the formation of oxalate, because of the intermediates of glycolate and glyoxylate in the rat liver (Halabe *et al*., 2003; Poore *et al*., 1997). Oxalate causes peroxidation of membrane lipids and oxidation of proteins, initiates the loss of membrane integrity and activities of membrane bound enzymes (Selvam, 2002). Membrane ATPases may play an important role in the maintenance of ionic gradients between the intracellular/ extracellular compartments of the cell. Membrane Na^+K^+ATPase plays an important role in active transport of Na^+ and K^+ ions across the plasma membrane similarly; Ca^{2+}ATPase is clearly linked with Ca^{2+} pump and transport of Ca^{2+} ions, while Mg^{2+} activated ATPase is distributed in all renal cell compartments. The Mg^{2+} ion forms Mg^{2+}ATP complex, which is the substrate for the enzyme. Mg^{2+}ATPase is to control the intracellular Mg^{2+} concentration, changes which can modulate the activity of Mg^{2+} dependent enzymes and regulate rates of protein synthesis and cell growth (Stekhoren and Bonting, 1981). Lipid peroxidation leads to cell damage leading to the release of marker enzymes namely Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH) and Gamma-Glutamyl Transferase (GGT) into blood circulation and urine. Most of the urinary enzymes originating in the kidneys are localized to specific regions and cellular components of the nephron, thereby studies pertaining to these enzymes will show the pathological status of the kidney (Srinivasan *et al*., 2004). Current-day medical management of urolithiasis mainly involves the surgical removal of stones by the techniques such as ESWL (Extracorporeal shock-wave lithotripsy), PCNL (Percutaneous nephrolithotomy), but they do not assure the prevention of recurrence of the stone. They cause side effects such

Corresponding Author: Dr. V. Hazeena Begum, Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur 613 010, Tamilnadu, India

as heamorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney. Hence, the search of effective herbal drugs for the treatment of urolithiasis with no side effects is necessary.

Aerva lanata is herbaceous weed, found throughout the hotter parts of the India, especially in the states of Tamilnadu, Andhra Pradesh and Karnataka. *A. lanata* is endowed with various chemical components such as flavonoids, alkaloids, steroids, polysaccharides, tannins, saponins, etc. (Chandra and Sastry, 1990; Afaq *et al*., 1991; Zapesochnaya *et al*., 1992), which possibly contribute to its diverse uses in folklore medicine. Also *A. lanata* was reported to have anti-inflammatory, diuretic (Vetrichelvan *et al*., 2000), antimicrobial, cytotoxicity (Chowdhury *et al*., 2002), antidiabetic (Vetrichelvan and Jegadeesan, 2002) and nephroprotective (Shirwaikar *et al*., 2004) activities. In our previous studies, *A. lanata* reduced the oxalate synthesizing enzymes, oxalate and urinary risk factors and lipids in ethylene glycol induced urolithiasis (Soundararajan *et al*., 2006, 2007). The present study was planned to evaluate the potential of *A. lanata* aqueous suspension on membrane bound ATPases in kidney and liver and marker enzymes in kidney, liver, serum and urine of ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS

Plant Material

Fresh *A. lanata* aerial parts were collected during the months of September to December 2003. The aerial parts were dried thoroughly under shade and powdered finely. The fine powder was suspended a concentration of 500 mg mL^{-1} with distilled water as aqueous suspension and used for the study.

Animals

Male albino rats of Wistar strains weighing approximately 140-150 g were used. All animal experiments and maintenance were carried out in our laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee after January 2004. Animals were housed in polypropylene cages under controlled conditions of 12 h light/12 h dark cycle and temperature 27±2°C. All of the rats received standard pellet diet (Amrut rat feed, Pune) and water *ad libitum*.

Stone Induction

Animals were fed with 0.75% ethylene glycol in drinking water for 28 days *ad libitum* to induce the urolithiasis. The urolithic rats were then used for the study.

Experimental Design

In the experiment a total of 24 rats (12 urolithic rats, 12 control rats) were used. The rats were divided in to 4 groups of 6 rats each. Group I served as control. Group II served as drug control which received *A. lanata* aqueous suspension alone (2 g kg^{-1} b.wt. $dose^{-1}$ day^{-1} $oral^{-1}$) for 28 days. Group III served as urolithic rats. Calcium oxalate urolithic rats were treated with *A. lanata* aqueous suspension (2 g kg^{-1} b.wt. $dose^{-1}$ day^{-1} $oral^{-1}$) for 28 days (Group IV).

At the end of 28 days, the animals were housed in metabolic cages and 24 h urine was collected. After the urine collection, the animals were anaesthetized with Pentobarbitol sodium (35 mg kg^{-1} bodyweight, ip). Blood was drawn from the external jugular vein and serum was separated by centrifugation at 3000 rpm. Kidneys and liver were excised and immersed in ice-cold physiological saline and blotted with filter paper. Known weight of tissues were homogenized in 0.1 M tris HCl buffer pH 7.4 containing 0.25 M sucrose and used for biochemical estimations.

Biochemical Assays

The total homogenate was used for assaying membrane bound enzymes like Na^+K^+ATPase (Bonting, 1970), Ca^{2+}ATPase (Hjerten and Pan, 1983), Mg^{2+} ATPase (Ohniski *et al*., 1982) activities. The amount of inorganic phosphorus was determined by the method of Fiske and Subbarow (1925). Serum sodium, potassium, calcium and magnesium were analysed by using the methods of Trinder (1951), Maruna (1958), Gitelman (1967) and Neill and Neely (1956), respectively. The activities of AST (Mohun and Cook, 1957), ALT (Mohun and Cook, 1957), ALP (Kind and King, 1954), LDH (King, 1965) and GGT (Persijin and vander Silk, 1976) in kidney, liver, serum and 24 h urine were assayed.

Statistical Analysis

The results were presented as the mean±SD. One-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons was used. Values of $p<0.05$ was considered to be significant. SPSS 7.5 version was used for this analysis.

RESULTS

The activity of Na^+K^+ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase were significantly diminished in ethylene glycol induced urolithic rats (Group III) when compared with control rats (Group I). The activity of these enzymes was reverted to near normal in *A. lanata* aqueous suspension administered rats (Group IV). No significant change was observed in *A. lanata* aqueous suspension treated drug control rats (Group II) as compared to control group (Group I) (Table 1).

The level of Na^+ was markedly lowered while K^+, Ca^{2+} and Mg^{2+} levels were elevated in ethylene glycol induced urolithic rats (Group III) when compared to control rats (Group I). Administration of *A. lanata* aqueous suspension brought the Na^+, K^+, Ca^{2+} and Mg^{2+} levels near to

Table 1: Effect of *A. lanata* on membrane bound ATPases in control and experimental groups

Experimental groups	Drug dosage ($g\ kg^{-1}$ b.wt.)	Na^+K^+ATPase	Ca^{2+}ATPase	Mg^{2+}ATPase
		(μmoles of inorganic phosphorus liberated h^{-1} mg^{-1} protein)		
Kidney				
I	-	3.52±0.14	2.22±0.04	1.32±0.10
II	2	3.51±0.10	2.27±0.09	1.34±0.07
III	-	2.89±0.09^{a*}	1.25±0.06^{a*}	0.93±0.04^{a*}
IV	2	3.54±0.12^{b*}	2.14±0.07^{b*}	1.29±0.11^{b*}
Liver				
I	-	2.42±0.11	1.79±0.07	1.35±0.09
II	2	2.44±0.12	1.82±0.06	1.35±0.04
III	-	1.62±0.07^{a*}	1.14±0.11^{a*}	0.95±0.06^{a*}
IV	2	2.27±0.09^{b*}	1.81±0.14^{b*}	1.32±0.03^{b*}

Values are expressed as mean±SD for 6 animals in each group. a: as compared with group I, b: as compared with group III. *: $p<0.001$

Table 2: Effect of *A. lanata* on serum sodium, potassium, calcium and magnesium in control and experimental groups

Groups	Drug dosage ($g\ kg^{-1}$ b.wt.)	Sodium	Potassium	Calcium	Magnesium
		($mg\ dL^{-1}$)			
I	-	322.12±6.21	14.14±0.62	6.82±0.74	2.62±0.17
II	2	326.32±6.43	14.07±0.70	6.80±0.53	2.57±0.19
III	-	262.41±5.47^{a*}	25.13±0.87^{a*}	10.74±0.57^{a*}	5.73±0.14^{a*}
IV	2	318.47±6.82^{b*}	15.22±0.65^{b*}	7.02±0.68^{b*}	2.76±0.21^{b*}

Values are expressed as mean±SD for 6 animals in each group. a: as compared with group I, b: as compared with group III. *: $p<0.001$

Table 3: Effect of *A. lanata* on liver and kidney marker enzymes in control and experimental groups

Experimental groups	Drug dosage (g kg^{-1} b.wt.)	AST	ALT	ALP	LDH	GGT
		(IU gm^{-1} protein)				
Kidney						
I	-	9.44±0.63	9.81±0.94	1.02±0.05	2.73±0.17	1.52±0.10
II	2	9.47±0.47	9.84±0.63	1.05±0.06	2.64±0.13	1.59±0.09
III	-	6.32±0.49^{a*}	3.76±0.60^{a*}	0.43±0.04^{a*}	4.92±0.18^{a*}	0.59±0.07^{b*}
IV	2	9.35±0.53^{b*}	8.40±0.72^{b*}	0.96±0.04^{b*}	3.02±0.10^{b*}	1.43±0.09^{b*}
Liver						
I	-	18.42±0.72	27.42±1.72	6.32±0.32	1.52±0.10	1.62±0.08
II	2	18.45±0.91	27.93±2.32	6.38±0.35	1.56±0.12	1.68±0.06
III	-	12.10±0.92^{a*}	16.24±1.43^{a*}	2.34±0.21^{a*}	4.07±0.17^{a*}	0.76±0.05^{a*}
IV	2	17.89±0.67^{b*}	25.63±1.64^{b*}	6.22±0.27^{b*}	1.47±0.13^{b*}	1.58±0.06^{b*}

Values are expressed as mean±SD for 6 animals in each group. [a]: as compared with group I, [b]: as compared with group III. *: $p<0.001$

Table 4: Effect of *A. lanata* on serum and urine marker enzymes in control and experimental groups

Experimental groups	Drug dosage (g kg^{-1} b.wt.)	AST	ALT	ALP	LDH	GGT
		(IU L^{-1} serum, IU/24 h urine)				
Serum						
I	-	30.14±1.76	33.16±2.12	34.02±1.74	378.43±18.12	3.84±0.24
II	2	30.13±1.55	33.12±2.01	34.02±1.52	377.54±18.03	3.83±0.26
III	-	62.47±1.67^{a*}	59.74±2.53^{a*}	55.18±1.84^{a*}	563.17±22.14^{a*}	7.07±0.32^{a*}
IV	2	32.73±1.82^{b*}	35.47±2.51^{b*}	35.15±1.63^{b*}	389.79±20.63^{b*}	3.95±0.28^{b*}
Urine						
I	-	2.14±0.10	2.06±0.12	0.14±0.01	122.17±6.15	2.54±0.17
II	2	2.12±0.07	2.02±0.06	0.13±0.02	120.11±6.22	2.54±0.24
III	-	3.42±0.14^{a*}	3.17±0.09^{a*}	0.32±0.04^{a*}	214.37±6.14^{a*}	3.47±0.16^{a*}
IV	2	2.24±0.12^{b*}	2.23±0.09^{b*}	0.17±0.02^{b*}	132.65±6.32^{b*}	2.72±0.18^{b*}

Values are expressed as mean±SD for 6 animals in each group. [a]: as compared with group I, [b]: as compared with group III. *: $p<0.001$

normal (Group IV). *A. lanata* aqueous suspension administered to drug control rats (Group II) did not show any significant changes as compared with control rats (Group I) (Table 2).

The activities of AST, ALT, ALP, LDH and GGT (Table 3 and 4) were significantly decreased in kidney and liver tissues except LDH which was found to increase. The activity of these enzymes increased in serum and urine of ethylene glycol induced urolithic rats (Group III). *A. lanata* aqueous suspension brought the enzyme activity to near normal in ethylene glycol induced urolithic rats (Group IV). Group II animals did not show any significant changes.

DISCUSSION

One of the major functions of the kidney is the transport of sodium and other solutes across the tubular epithelium. Generation of free radicals such as peroxyl, alkoxyl and aldehyde radicals can cause severe damage to the membrane bound enzymes such as Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase (Pragasam *et al*., 2004).

Membrane Na^+K^+ATPase plays an important role in active transport of Na^+ and K^+ ions across the plasma membrane (Vani and Reddy, 2000). The enzyme Na^+K^+ATPase utilizes the energy derived from ATP hydrolysis to pump out Na^+ from inside the cell to transfer K^+ from outside to cytosol. Its activity has been frequently used as a marker for plasma membranes and has been followed as a probe for monitoring membrane integrity following alterations in the physical state of various biological membranes (Stepherd, 1994). In the present study, Na^+K^+ATPase activity was markedly reduced in liver and kidney of ethylene glycol induced urolithic rats. This may be due to increased oxalate formation. Oxalate is known to interfere with a broad spectrum of solute transport processes in the renal tubule and the inhibition of Na^+K^+ATPase. Besides, the depression of Na^+K^+ATPase activity

may be associated with changes in the lipid profile (Tulenko *et al*., 1988). This membrane bound enzyme requires phospholipids for its activity and is highly vulnerable to oxidative insult and the mechanism of inactivation under such conditions involves disruption of phospholipids microenvironment of the enzyme or direct damage to enzyme protein by reactive oxygen radicals or lipid peroxidation products (Lehtosky *et al*., 1999; Morel *et al*., 1999). This observation is in conformity with the fact that reactive aldehydes such as 4-hydroxynonenal derived from lipid peroxidation can lead to formation of protein carbonyls and is also capable of inhibiting Na^+K^+ATPase activity (Mark *et al*., 1997). Due to decreased activity of Na^+K^+ATPase, Na^+ cannot be pumped out of the cell and K^+ cannot be pumped in to cytosol. K^+ efflux is also an important index of membrane damage. The elevated level of serum K^+ and reduced level of serum Na^+ were accompanied by diminished activity of Na^+K^+ATPase in ethylene glycol induced urolithic rats. On the therapeutic level of *A. lanata* aqueous suspension, Na^+K^+ATPase and their electrolytes were reverted to near normal in ethylene glycol induced urolithic rats by inhibiting lipid peroxidation process and reduces the protein carbonyl levels. This may be attributed by the flavonoids, aerva flavonoids, aervitrin, chrysin and narcission, which are present in *A. lanata* (Chandra and Sastry, 1990; Afaq *et al*., 1991; Zapesochnaya *et al*., 1992).

Ca^{2+}ATPase, the enzyme responsible for active calcium transport, is extremely sensitive to hydroperoxides and this may lead to its inhibition. In the present study, Ca^{2+}ATPase activity was significantly lowered in the liver and kidney of ethylene glycol induced urolithic rats. The decreased activity of this enzyme may be due to the peroxidative stress, which may act on the sulphydryl groups present in the active sites of the Ca^{2+}ATPase. The reduced activity of Ca^{2+}ATPase causes the increased serum calcium concentration (Srinivasan *et al*., 2004). The present study also observed higher concentration of serum calcium in ethylene glycol induced urolithic rats. This may be attributed to the increase of calcium mobilization from bones that has been reported in nephrolithic patients (Coe and Parks, 1997). In addition, reactive oxygen species produced by oxalate may have a significant role in the increased serum Ca^{2+} concentrations by altering the activities of membrane Ca ATPase. Administration of *A. lanata* aqueous suspension reverted the Ca^{2+}ATPase activity and calcium level to near normal in ethylene glycol induced urolithic rats. This may be due to sulfhydryl groups resulting in an activation of antioxidant enzymes and thus limiting the free radical actions.

Mg^{2+}ATPase is to control the intracellular Mg^{2+} concentration, changes which can modulate the activity of Mg^{2+} dependent enzymes and regulate rates of protein synthesis and cell growth (Sanui and Rubin, 1982). Mg^{2+}ATPase plays a role in endergonic process other than ion transport. It utilizes a pool of ATP that is not directly related to the change in free energy for sodium transport (Chohen and Kamma, 1981). In the present study, Mg^{2+}ATPase activity was significantly decreased in liver and kidney of ethylene glycol induced urolithic rats. This may be due to free radical induced cell damage by oxalate and their severe cytotoxic effect, such as lipid peroxidation and protein oxidation in cell membrane followed by the alteration of the membrane fluidity, enzyme properties and ion transport (Tappel, 1973; Hall and Braughler, 1989). In addition, the decreased activity of Mg^{2+}ATPase may be due to the depletion of Mg^{2+} ions resulting from the release of divalent cations from the membrane (Poddar and Ghosh, 1976). This may cause the elevation of serum Mg^{2+} concentration in ethylene glycol induced urolithic rats. Administration of *A. lanata* aqueous suspension to urolithic rats, the Mg^{2+}ATPAse activity and magnesium level was reverted to near normal. Reduced glutathione protect against the cytocoxic effects and that a cellular Mg^{2+} deprivation by inhibiting Mg^{2+} pump. Enzymes that are most commonly employed as indicators of kidney and liver damage are AST and ALT. Both enzymes are present in high concentrations in these organs (Raj, 1978). In the present study, increased activities of serum and urine AST and ALT in ethylene glycol induced urolithic rats were observed. This can be attributed to the damaged structural integrity of the renal and hepatic cells causing the enzymes which are located in the cytoplasm to be released into the circulation (Senthilkumar *et al*.,

2003). If membrane of other organelles such as mitochondria is damaged, soluble enzymes such as compartmentalized AST will also be released. The release of these enzymes into the circulation will indicate both the plasma and organelle membranes are damaged. Due to this cellular damage, AST and ALT levels in kidney and liver tissues of the ethylene glycol induced urolithic rats will decrease. On administration of *A. lanata* aqueous suspension, the activities of these enzymes were found to revert to near normal in the ethylene glycol induced urolithic rats.

In the present study, reduced ALP activity in renal and hepatic tissues of ethylene glycol induced urolithic rats was observed. This might be due to leakage of the enzyme into the general circulation from the collateral circulation. The stone formation may occlude the ureter, leading to an increase in back pressure in the renal pelvis and because of ischeamia, may ultimately damage the tubular cells (Thind and Nath, 1978). The cellular damage causes the activity of ALP in serum and urine of ethylene glycol induced urolithic rats to increase. Administration of *A. lanata* aqueous suspension causes the ALP activity to return to near normal in ethylene glycol induced urolithic rats.

An elevation in the activities of LDH (renal tubular cytoplasmic enzyme) and GGT (brush border membrane luminal surface enzyme) were observed in serum and urine, while GGT was reduced and LDH was elevated in kidney and liver tissues of ethylene glycol induced urolithic rats. LDH is an oxalate-synthesizing enzyme; its activity was increased on ethylene glycol administration. These enzymes were released into the blood serum and urine. This may be attributed to oxalate induced renal and hepatic cellular damage. Renal damage is particularly confined to the proximal tubule, a part of the nephron closely involved in handling urinary oxalate (Hackett *et al*., 1990). Further damage to proximal tubular epithelium is generally associated with the shedding of brush border membrane thereby causing crystal retention (Khan *et al*., 1989; Jayanthi and Varalakshmi, 1993). Crystals associate with cell debris and then attach to the surface of injured epithelial cells and denuded basement membrane (Grases *et al*., 1998). Therapeutic treatment of *A. lanata* aqueous suspension had minimized the stress and thus decreased the enzymes related to stone synthesis and protected the cell integrity.

In conclusion, biopotency of *A. lanata* aqueous suspension against urolithiasis might be attributed to the contents of flavonoids, triterpenes, tannins, etc., which may help in the cytoprotective mechanism in membrane damage by enhancing membrane bound ATPases, maintaining the marker enzymes through the reduction of free radicals formation.

REFERENCES

Afaq, S.H., S. Tajuddin and R. Afridi, 1991. Bisheri Booti (*Aerva lanata*) some lesser known uses and pharmacognosy. Ethnobotany, 5: 37-40.

Bonting, S.L., 1970. Sodium-Potassium Activated Adenosine Triphosphatase and Cation Transport. In: Membranes and Ion Transport. Bittar, E.E. (Ed.), Wiley Interscience, England, pp: 257-363.

Chandra, S. and M.S. Sastry, 1990. Chemical constituents of *Aerva lanata*. Fitoterapia, 61: 188.

Chohen, J.J. and D.E. Kamma, 1981. The Kidney. Brenner B.M. and F.C. Rector (Eds.), 2nd Edn., Saunders, Philadelphia, pp: 141-248.

Chowdhury, D., A. Sayeed, A. Islam, M.S.A. Bhuiyan and G.R.M.A.M. Khan, 2002. Antimicrobial activity and cytotoxicity of *Aerva lanata*. Fitoterapia, 73: 92-94.

Coe, F.L. and J.H. Parks, 1997. New insights into the pathophysiology and treatment of nephrolithiasis: New research renues. J. Bone Miner. Res., 12: 522-533.

Fiske, C.H. and Y. Subbarrow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.

Gitelman, H.J., 1967. An improved automated procedure for determination of calcium in biochemical specimen. Anal. Biochem., 18: 521-531.

Grases, F., L. Garcia-Ferragut and A. Costa-Bauza, 1998. Development of calcium oxalate crystals on urothelium: Effect of free radicals. Nephron, 78: 296-301.

Hackett, R.L., P.N. Shevock and S.R. Khan, 1990. Cell injury associated calcium oxalate crystalluria. J. Urol., 144: 1535-1538.

Halabe, A., R. Shor, N.L.M. Wong and R.A.L. Sutton, 2003. Effect of vitamin D3 on the conversion of ethylene glycol to glycolate and oxalate in ethylene glycol-fed rats. Clin. Chim. Acta, 330: 135-139.

Hall, E.D. and J.M. Braughler, 1989. Central nervous system trauma and stroke. II. Physiological and pharmacological evidence of oxygen radicals and lipid peroxidation. Free Radic. Biol. Med., 6: 303-313.

Hjerten, S. and H. Pan, 1983. Purification and characterization of two forms of a low affinity calcium ion ATPase from erythrocyte membranes. Biochim. Biophys. Acta, 728: 281-288.

Jayanthi, S. and P. Varalakshmi, 1993. Changes in urinary excretion pattern in experimental calcium oxalate lithiasis and the effects of DL-alpha-lipoic acid. Med. Sci. Res., 21: 37-39.

Khan, S.R., P.N. Shevock and R.L. Hackett, 1989. Urinary enzymes and calcium oxalate nephrolithiasis. J. Urol., 142: 846-849.

Kind, P.R.N. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-326.

King, J., 1965. Practical Clinical Enzymology. Van Nostrand, London, pp: 87.

Lehtosky, J., P. Kaplan, P. Racay, M. Matejovicova, A. Drgova and V. Mezesova, 1999. Membrane ion transport systems during oxidative stress in rodent brain: Protective effect of stobadine and other antioxidants. Life Sci., 65: 1951-1958.

Mark, R.J., M.A. Lovell, W.R. Markesbery, K. Uchida and M.P. Mattson, 1997. A role for 4-hydroxynonenal, an aldehyde product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. J. Neurochem., 68: 255-264.

Maruna, R.F.L., 1958. Beitrag zur serum-Natrium-Bestimmung. Eine kritische studie iiber kolorimetrische bestimmungen und angabe einer einfachen fotometrischen methode. Clin. Chem. Acta, 2: 581-585.

Mohun, A.F. and I.J.P. Cook, 1957. Simple methods for measuring serum levels of the glutamic-oxalacetic and glutamic pyruvic transaminases in routine laboratories. J. Clin. Pathol., 10: 394-399.

Morel, P.F., L. Barrier, B. Fauconneau, A. Piriou and F. Huguet, 1999. Origin of 4-hydroxynonenal incubation-induced inhibition of dopamine transporter and Na^+/K^+ adenosine triphosphatase in rat striatal synaptosomes. Neurosci. Lett., 277: 91-94.

Neill, D.W. and R.A. Neely, 1956. The estimation of magnesium in serum using titan yellow. J. Clin. Pathol., 9: 162-163.

Ohniski, T., T. Suzuki, Y. Suzuki and K. Ozawa, 1982. A comparative study of plasma membrane magnesium ion ATPase activities in normal, regenerating and malignant cells. Biochim. Biophys. Acta, 684: 67-74.

Persijin, J.P. and W. Vander Silk, 1976. A new method for the determination of gamma-glutamyl transferase in serum. J. Clin. Chem. Clin. Biochem., 14: 421-427.

Poddar, M.K. and J.J. Ghosh, 1976. Effect of cannabis extract and Delta-9-tetra hydrocannabinol on brain adenosine triphosphatase activity. Indian J. Biochem. Biophys., 13: 267-272.

Poore, R.P., C.H. Hurst, D.G. Assimos and R.P. Holmes, 1997. Pathways of hepatic oxalate synthesis and their regulation. Am. J. Physiol., 39: 289-294.

Pragasam, V., P. Kalaiselvi, K. Sumitra, S. Srinivasan and P. Varalakshmi, 2005. Counteraction of oxalate induced nitrosative stress by supplementation of L-arginine, a potent antilithic agent. Clin. Chim. Acta, 354: 159-166.

Raj, R., 1978. Aspartate aminotranferase activity and isoenzyme proportions in human liver tissues. Clin. Chem., 24: 1971.

Sanui, H. and H. Rubin, 1982. The Role of Magnesium in Cell Proliferation and Transformation. In: Ions, Cell Proliferation and Cancer. Boyton, A.L., W.L. Mc Keehan and J.P. Whitfield (Eds.), Academic Press, New York, pp: 517-537.

Selvam, R., 2002. Calcium oxalate stone disease: Role of lipid peroxidation and antioxidants. Urol. Res., 30: 35-47.

Senthilkumar, R., M. Ponmozhi, P. Viswanathan and N. Nalini, 2003. Activity of *Cassia auriculata* leaf extract in rats with alcoholic liver injury. J. Nutr. Biochem., 14: 452-458.

Shirwaikar, A., D. Issac and S. Malini, 2004. Effect of *Aerva lanata* on cisplatin and gentamicin models of acute renal failure. J. Ethnopharmacol., 90: 81-86.

Soundararajan, P., R. Mahesh, T. Ramesh and V. Hazeena Begum, 2006. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. Indian J. Exp. Biol., 44: 981-986.

Soundararajan, P., R. Mahesh, T. Ramesh and V. Hazeena Begum, 2007. Hypolipidemic activity of *Aerva lanata* on ethylene glycol induced calcium oxalate urolithiasis in rats. Pharmacologyonline, 1: 557-563.

Srinivasan, S., V. Pragasam, X. Jenita, P. Kalaiselvi, V. Muthu and P. Varalakshmi, 2004. Oxidative stress in urogenital tuberculosis patients: A predisposing factor for renal stone formation-amelioration by vitamin E supplementation. Clin. Chim. Acta, 350: 57-63.

Stekhoren, M.A. and S.L. Bonting, 1981. Transport ATPases: Properties and functions. Physiol. Rev., 61: 1-76.

Stepherd, G.M., 1994. Neurobiology. 3rd Edn., Oxford University Press, New York.

Tappel, A.L., 1973. Lipid peroxidation damage to cell components. Fed. Proc., 32: 1870-1874.

Thind, S.K. and R. Nath, 1978. Experimental Urolithiasis. Part III-A comparative kinetic study of glyoxalase I, glycolate oxidase, alkaline phosphatase and LDH in the normal rat kidney and bladder and its alterations in urolithiasis. Indian J. Exp. Biol., 16: 66-70.

Trinder, P., 1951. A rapid method for the determination of sodium in serum. Analyst, 76: 596-599.

Tulenko, T.N., J.L. Robinowitz, R.H. Cox and W.P. Santamore, 1988. Altered Na^+K^+ATPase, Cell Na^+ and lipid profiles in canine arterial wall with chronic cigarette smoking. Int. J. Biochem., 20: 285-289.

Vani, M.L. and K.P. Reddy, 2000. Effects of fluoride accumulation on some enzymes of brain and gastrochemius muscle of mice. Fluoride, 33: 17-26.

Vetrichelvan, T., M. Jagadeesan, M. Senthilpalaniappan, N.P. Murali and K. Sasikumar, 2000. Diuretic and anti-inflammatory activities of *A. lanata* in rats. Indian J. Pharm. Sci., 300-302.

Vetrichelvan, T. and M. Jegadeesan, 2002. Antidiabetic activity of alcoholic extract of *Aerva lanata* (L.) Juss. ex Schultes in rats. J. Ethnopharmacol., 80: 103-107.

Zapesochnaya, G.G., V. Okhanov, V. Kurkin and A. Kiroshnikov, 1992. Cathine-6-one and beta carboline alkaloids from *Aerva lanata*. Planta Med., 37: 192-196.