

## A study Antiuro lithiatic Activity of ethanolic extract of *Asparagus racemosus* in animal models

Jagannath N<sup>1</sup>, Asha P Dass <sup>\*2</sup> and Khasif Ahmed<sup>2</sup>

<sup>1</sup>Department of Pharmacology, SVS Medical College, Mehabubnagar, India

<sup>2</sup>Department of Pharmacology, KBNIMS, Gulbarga, India

### Corresponding author\*

Dr. Asha P. Dass

Department of Pharmacology,

KBNIMS, Gulbarga, India

E-mail: [ashapdass@gmail.com](mailto:ashapdass@gmail.com)

### Abstract

**Objective:** To investigate the Antiuro lithiatic Activity of ethanolic extract of *Asparagus racemosus* in animal models.

**Materials and Methods:** The study includes performing on healthy albino rats of either sex weighing 220 – 270gms and urolithiasis was induced by oral administration of ethylene glycol and ammonium chloride water. The parameters studied are serum analysis for Urea, Creatinine, Calcium and Phosphorus, Body Weight of animals included in the study group and Histopathological Study of kidney for the presences crystals.

**Results:** In our study the Ethanolic extract of *Asparagus Racemosus* with doses of 800mg/kg and 1600mg/kg per orally to rats showed significant reduction in serum urea, creatinine, calcium and phosphorus levels in urolithiatic rats when compared to the positive control rats (Group II). These results were found to be statistically significant (p<0.05).

**Conclusion:** Ethanol Extract of *Asparagus racemosus* has a significant antiuro lithiatic activity.

**Keywords:** *Asparagus racemosus*, urolithiasis, animal models, albino rats

### 1. Introduction

Urolithiasis affects 1% to 5% of the population in developed countries with a peak incidence between 20 and 50 years of age; men are three times more likely to be effected than women and the life time risk of developing a calculus in a Caucasian man is nearly 20 % (1-6). At present, it seems clear that renal epithelial cell injuries play a decisive role in such a type of renal calculi development [7,8], and in fact the lithogenic effect caused by Ethylene Glycol (EG) must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by EG. Thus, although EG rat model can be taken as a general model to study renal stone formation, it must be considered as an interesting model to evaluate renal papillary stone development, at least for those stones whose genesis is linked to oxidative cell damage Thus, the first studies on experimental EG renal lithiasis appeared in the 60' decade [9, 10] but the importance of the oxidative damage caused by hyperoxaluria was not clearly proposed until the end of the century [11]. From this last period it appeared several prophylaxis proposals on EG induced nephrolithiasis using herbal extracts and antioxidants [12]. In all these papers the positive effects on calcium oxalate lithiasis are most likely due to antioxidative effects. Since the *Asparagus racemosus* (A.R) having the antioxidant activity the present study is carried to examine the effect of A.R on experimentally EG and AC induced calcium oxalate (coax) urolithiasis in rats.

### 2. Materials and Methods

Ethylene Glycol & Ammonium Chloride Induced Urolithiasis Model (69): The thirty six were divided into six groups comprising six animals per group. Each group underwent a different treatment protocol for 10 days. Group 1: Negative Control, ad libitum access to regular food and drinking water, and administered 6 µl distilled water per 1 g of body weight by gavage (intra-gastric administration).

Groups II, III, IV, V and VI: ad libitum access to regular food, and ad libitum access to drinking water containing 0.75% [v/v] ethylene glycol (EG) and 2% [w/v] ammonium chloride (AC) in order to promote hyperoxaluria and CaOx deposition in the kidneys. Groups III, IV, V and VI were also administered test drug *Asparagus racemosus* by gavage at the following concentrations: Group II, 200mg/kg body weight; Group III, 400mg/kg body weight; Group IV, 800mg/kg body weight and Group V, 1600mg/kg body weight. Group II rats were also administered 6 µl distilled water/g body weight by gavage (positive control) Groups III, IV, V and VI served as test groups. All rats were weighed daily. Serum analysis: After the 10-day experimental period, rats were anaesthetized and blood was collected from the retro-orbital region, centrifuged at 10,000 × g for 10 min, and the serum collected and analyzed for level of calcium, phosphorus, urea

and creatinine using calcium (Coral Clinical Systems), phosphorus (Coral Clinical Systems), urea (Coral Clinical Systems), creatinine (Coral Clinical Systems) diagnostic kits. Histopathological studies: The rats were scarified by giving high doses of ether and abdomen is cut opened and kidneys were removed. The kidneys were stored in formalin (10%) and the right kidney was fixed in bouin liquid, soaked in paraffin, cut at 3–4 $\mu$ m intervals, and the slices stained using hematoxylin and eosin1. Tissue slices were photographed using optical microscopy under polarized light.

### 2.1 Statistical Analysis

The results were expressed as the mean  $\pm$  SEM and the data was analyzed using one way analysis of variance (ANOVA) followed by student's t test.  $P < 0.05$  was considered as significant. Conventional Windows software was used for statistical computations.

## 3. Results

Ethylene Glycol and Ammonium Chloride Induced Urolithiasis in rats: In the present study, ethanol extracts of the root of *Asparagus racemosus* were assessed for its antiurolithiatic activity in ethylene glycol and ammonium chloride induced urolithiasis in rats and the results obtained were recorded. Serum analysis showed that urea and creatinine levels were higher in Groups II, III, IV, V and VI compared to Group I (Table. 1). These data indicate marked renal damage in the EG/AC-treated rats. The data also showed that urea, creatinine, calcium and phosphorus levels were significantly retained near normal level in rats treated with test drug (Groups V (800mg/kg b.w) and VI (1600mg/kg b.w)) compared to rats treated with EG/AC alone (Group II, positive control). Body weight EG/AC-treated rats (Groups II, III, IV, V and VI) weighed less than the normal rats (Group I) at the completion of the experiment. Histopathological studies clearly revealed that the tissue samples from the normal group (Group I) shows tubules with single epithelial lining along the margin and were of normal size. In Group II (positive control), all the tubules showed the presence of crystals, there was marked dilatation of the tubules and total degeneration of the epithelial lining with infiltration of inflammatory cells into the Interstitial space. In Group V, VI (test group) the specimen showed characters similar to the normal group.

**Table No.1: Effect of *Asparagus racemosus* (AR) on Serum concentrations of urea, creatinine, calcium, phosphorus in urolithiatic rats**

Groups	Urea(mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
I. Negative control(6 $\mu$ l D.W/1g body weight)	13.83 $\pm$ 3.06	0.38 $\pm$ 0.11	8.63 $\pm$ 0.75	8.18 $\pm$ 0.61
II. Positive control(0.75% ethylene glycol + 2% ammonium chloride)	32 $\pm$ 4.47	0.63 $\pm$ 0.05	10.1 $\pm$ 0.55	9.43 $\pm$ 0.63
III. Test 1(0.75% ethylene glycol + 2% ammonium chloride) + (A.R 200mg/kg body weight)	31.33 $\pm$ 11.33	0.66 $\pm$ 0.05	9.28 $\pm$ 0.43	8.5 $\pm$ 1.16
IV. Test 2 (0.75% ethylene glycol + 2% ammonium chloride) + (A.R 400 mg/kg body weight)	31.83 $\pm$ 7.46	0.63 $\pm$ 0.11	9.45 $\pm$ 0.19	8.65 $\pm$ 1.89
V. Test3 (0.75% ethylene glycol + 2% ammonium chloride) +(A.R 800 mg/kg body weight)	26.8 $\pm$ 2.31*	0.53 $\pm$ 0.08*	8.86 $\pm$ 0.92*	7.86 $\pm$ 1.44*
VI. Test 4 (0.75% ethylene glycol + 2% ammonium chloride) + (A.R 1600 kg body weight)	25.33 $\pm$ 2.80*	0.51 $\pm$ 0.07*	8.7 $\pm$ 0.81*	7.73 $\pm$ 1.20*

**Table No.2: Effect of *Asparagus racemosus* (AR) on body weight in urolithiatic rat**

Groups	Day 1 (Average)	Day 10 (Average)
I. Normal(6 $\mu$ l D.W/1g body weight)	253.3g	259.1g
II. Positive control(0.75% ethylene glycol + 2% ammonium chloride)	265g	251.6g
III. Test 1(0.75% ethylene glycol + 2% ammonium chloride) + (A.R 200mg/kg body weight)	260.8g	252.5g
IV. Test 2 (0.75% ethylene glycol + 2% ammonium chloride) + (A.R 400 mg/kg body weight)	261g	255g
V. Test3 (0.75% ethylene glycol + 2% ammonium chloride) +(A.R 800 mg/kg body weight)	246.6g	243.3g
VI. Test 4 (0.75% ethylene glycol + 2% ammonium chloride) + (A.R 1600 kg body weight)	245g	240g

## 4. Discussion

Urolithiasis is generally the result of an imbalance between inhibitors and promoters of stone formation in the kidneys. Approximately 85% of the stones in humans are calcium stones comprising oxalate and phosphate, either alone or combined. Several studies have examined the effect of the citrus juices on calcium salt crystallization. However, the conclusions from those studies were not consistent. Many in vivo models have been developed to investigate the

mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease. Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Rat models of CaOx urolithiasis induced by either EG (Ethylene Glycol) alone or in combination with other drugs such as AC (Ammonium Chloride) are often used to study the pathogenesis of kidney crystal deposition. Using the accelerated model, in the present study rats were treated with 0.75% EG and 2% AC for 10 days. All positive control rats (Group II) developed CaOx depositions in kidney during that period. The present study examined the effect of ethanolic extract of *Asparagus racemosus* (AR) on the deposition of CaOx crystals within the rat kidney. In the current study, body weight, serum concentrations of calcium, phosphorus, urea, creatinine and the histopathology of the kidney were analyzed. We found that Group I rats (normal group) remained active and gained weight, while Group II, III, IV, V and VI rats lost weight over the 10 days of treatment. Microscopic examination using polarized light of urolithiatic kidney sections of rats showed intra-tubular and interstitial crystal deposits. These crystal deposits were observed in the kidneys of all Group II rats. Rats treated with ethanolic extracts of *Asparagus racemosus* had far less kidney calcification and lower renal tissue calcium levels than the positive control rats (Group II) (Table 1).

This study of antiurolithiatic activity of AR showed significant decrease in serum concentration of urea, creatinine, calcium, phosphorus levels which are similar to that of other studies of antiurolithiatic activity of *Helianthus Annuus* Linn. Leaf Extract, Earlier studies by, Satish Kumar *et al* demonstrated that the weight of renal stones produced by inserting zinc disk into the urinary bladder of rats receiving 1% EG was reduced by aqueous extract of *Asparagus racemosus*. This suggests that AR may be effective in prevention or curing urolithiasis.

In our study the Ethanolic extract of *Asparagus Racemosus* with doses of 800mg/kg and 1600mg/kg per orally to rats showed significant reduction in serum urea, creatinine, calcium and phosphorus levels in urolithiatic rats when compared to the positive control rats (Group II). These results were found to be statistically significant ( $p < 0.05$ ). In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood [12]. The possible mode of action of *Asparagus racemosus* may include excessive excretion or decrease in the concentration of urinary salts that prevent the supersaturation of the crystallizing salts. Earlier studies have demonstrated the diuretic property of *Asparagus racemosus*. This property also favors antiurolithiasis, either by hastening the process of dissolving or flushing of the preformed stones or by preventing the new stone formation in urinary system on prophylactic treatment. Other possible mode of action of *Asparagus racemosus* may be due to its antioxidant effect. There is in vivo evidence that hyperoxaluria induced peroxidative damage to the renal tubular membrane surface provides a favorable environment for individual calcium oxalate crystal attachment and subsequent development of the kidney stones. In a study by Thamilselvan S *et al* demonstrated that vitamin E administration completely prevented calcium oxalate crystal deposition in the kidney, by preventing hyperoxaluria-induced lipid peroxidation and tissue antioxidant imbalance [13]. There are many reports about antioxidant activity of *Asparagus racemosus*. Thus, it is possible that the *Asparagus racemosus* extract prevents stone formation via antioxidant effects. It has been reported that CaOx calculi such as struvite calculi may have a bacterial origin such as nanobacteria. [14]

Earlier studies have demonstrated the antibacterial activity of *Asparagus racemosus*. This antibacterial action of AR may prevent CaOx calculus formation. The exact mechanism underlying the antiurolithiatic effect is still unknown, but is apparently related to diuresis and lowering of serum concentrations of stone forming constituents. The protective effect against hyperoxaluria - induced lipid peroxidation may be contributory to the recovery of renal damage and the antibacterial activity may also be one of the possible modes of action. These effects could conclude the Antiurolithiatic Property of ethanolic extract of *Asparagus racemosus*. The phytochemical studies have demonstrated that flavanoids, saponins, polyphenols etc are the active principles in AR extracts. From the earlier studies it has been reported that flavanoids and saponins have diuretic activity [15, 16]. Flavonoids and polyphenols also have antioxidant effect. The active principle 9, 10-Dihydrophenanthrene has antibacterial effect [16]. All these chemical constituents may be responsible for the antiurolithiatic property of AR.

## 5. Conclusion

To conclude, Ethanol Extract of *Asparagus racemosus* possesses significant antiurolithiatic activity. The possible mechanism may be due to its diuretic, antioxidant, antibacterial and serum lowering property of stone forming constituents. These properties may be mediated by the presence of chemical components such as flavanoids, saponins, polyphenols and 9, 10-Dihydrophenanthrene in the ethanolic extract of *Asparagus Racemosus*.

**References**

- [1] Khan SR. Interactions between stone-forming calcific crystals and macromolecules. *Urol Int* 1997; 59:59-71.
- [2] Thomas B, Hall J. Urolithiasis. *Surgery* (Oxford). 2005; 23:129-33.
- [3] Grases F, Costa-Bauzá A, Ramis M, Montesinos V, Conte A: Simple classification of renal calculi closely related to their micromorphology and etiology. *Clin Chim Acta* 2002, 322:29-36.
- [4] Low RK, Stoller ML, Schreiber CK: Metabolic and urinary risk factors associated with Randall's papillary plaques. *J Endourol* 2000, 14:507-510.
- [5] Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB, Munch LC, Coe FL: Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney Int* 2003, 64:2150-2154.
- [6] Kim SC, Coe FL, Tinmouth WW, Kuo RL, Paterson RF, Parks JH, Munch LC, Evan AP, Lingeman JE: Stone formation is proportional to papillary surface coverage by Randall's plaque. *J Urol* 2005, 173:117-119.
- [7] de Water R, Noordermeer C, Houstmuller AB, Nigg AL, Stijnen T, Schroder FH, Kok DJ: Role of macrophages in nephrolithiasis in rats: an analysis of the renal interstitium. *Am J Kidney Dis* 2000, 36:615-625.
- [8] Muthukumar A, Selvam R: Renal injury mediated calcium oxalate nephrolithiasis: role of lipid peroxidation. *Ren Fail* 1997, 19:401-408.
- [9] Vaillie C, Debray C, Martin E, Souchard M, Roze C: On experimental ethylene glycol renal lithiasis in young rats before weaning. *Ann Pharm Fr* 1963, 21:201-206.
- [10] Debray C, Vaillie C, Fiehrer A, Martin E, Souchard M, Roze C: Experimental disease due to paired stresses. Humoral and visceral disturbances induced by tween 80 in rats with experimental oxalate nephritis caused by ethylene glycol. *J Physiol (Paris)* 1964, 56:707-726.
- [11] Thamilselvan S, Hackett RL, Khan SR: Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J Urol* 1997, 157:1059-1063.
- [12] Ghodkar PB. Textbook of medical laboratory technology. 1st ed. Mumbai: Bhalani Publishing House; 1994.
- [13] Thamilselvan S, Menon M. Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. *BJU Int*. 2005; 96:117-26.
- [14] Kramer G, Klingler HC, Steiner GE. Role of bacteria in the development of kidney stones. *Curr Opin Urol*. 2000; 10:35-8.
- [15] Chodera A, Dabrowska K, Sloderbach A, Skrzypczak L and Budzianowski J, Effect of flavonoid fractions of *Solidago virgaurea* on diuresis and levels of Electrolytes, *Acta pol pharm*, 1991;48:35-37.
- [16] Sood AR., Bajpai A. And Digits M, Pharmacological and biological studies on Saponins, *Indian. J. Pharmacol.* 1985; 17 (3): 178-179.