INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ANTIUROLITHIATIC ACTIVITY OF *SESAMUM INDICUM* LEAVES AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

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Article Received on: 29/05/19 Approved for publication: 02/08/19

DOI: 10.7897/2230-8407.1010297

ABSTRACT

The present study was undertaken to investigate the effects of *Sesamum indicum* (leaves) on experimentally induced kidney stones. Oxalate urolithiasis in male rats was induced experimentally by administration of 0.75% v/v ethylene glycol in drinking water for 28 days. The aqueous and alcoholic extracts of *Sesamum indicum* were administered to urolithiasis induced test group rats at three doses i.e. 100, 200 and 400 mg/kg respectively for 28 days. After 28 days, highly significant deposition of calcium oxalate in the kidneys was noticed along with increase in the urine volume, urinary oxalate, calcium levels and magnesium levels in urolithiasis induced group rats as compared to normal group rats. The serum analysis showed significant increase in the serum uric acid, serum creatinine, and blood oxalate in urolithiasis control group rats. Daily oral treatment with extracts not significantly reduced the quantity of calcium oxalate deposited in the kidneys but also reverted all the biochemical changes induced by ethylene glycol urolithiasis thus supporting its traditional claim.

Keywords: Calcium oxalate, ethylene glycol, urolithiasis, uric acid, creatinine.

INTRODUCTION

Urolithiasis is the third most common urinary tract disorder responsible for serious human suffering and economic cost to society. The worldwide incidence of Urolithiasis is quite high and in the north India more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate.[1,2] Hyperoxaluria is the main initiating factor of human idiopathic calcium oxalate (CaOx) stone disease. Oxalate is a powerful crystallization- driving factor present in the urine, retention of which enhances cell injury and causes early stages of lithogenesis.[3]

Urolithiasis can be promoted by calcium, oxalate, uric acid, inorganic phosphate, ammonium oxalate and bacterial products whereas can be inhibited by Urolithiasis inhibitors like citrate, pyrophosphate, magnesium, nephrocalcin, GAGs and acid polypeptides. Hence, the crucial predisposing factors that make an imbalance between levels of promoters and inhibitors of stone formation are low urine volumes, diet, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia, hypomagnesuria, lowurinary pH, cystinuria, and distal renal tubular acidosis.[4,5]

Currently, the available drug therapy for urinary stone includes, antibiotics (for struvite stones), allopurinol (for uric acid stone), opiates and NSAID'S (for relieving pain), and diuretics (for renal stone removal). Preventive management involves education of patients to decrease their risk of stone disease by modifying diet and hydration. Most kidney stones finally pass through the urinary tract on their own within 48 hours, with ample fluid intake. However, when dietary modification is ineffective, above mentioned pharmacological treatment should be contemplated. Additionally, Ketorolac injection and narcotics may be used for pain control when OTC pain-control medications are not effective and some medications like calcium channel blockers (nifedipine) and alpha blockers (tamsulosin) to increase the passage rates of kidney stones.[6]

The leaves of *Sesamum indicum* contain glycosides, flavonoids, alkaloids, carbohydrates saponins and phytosterols. Roots and leaves are emollient and a decoction of them forms a good hair-wash which will promote hair growth and blacken them. The leaves are useful in dysentery, cholera, nephropathy, uropathy and dermatopathy.

Various published journals and books have revealed that plant-based drugs are showing promising anti urolithiatic activity. Some of the plants reported for their anti urolithiatic activity are *Acorus calamus*[7], *Aerva lanata,*[8] *Asparagus racemosus*[9], *Melia azedarach* Linn,[10] *Moringa oleifera*[11] etc.

From the literature it was found that *Sesamum indicum* has also been traditionally indicated for treatment of kidney stones. Hence leaves extracts of this plant was select for the study of anti urolithiatic activity in ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS

Plant material

Sesamum indicum leaves collected from local area of Kanpur were authenticated by NISCAIR, New Delhi and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals

Ethylene glycol and Cystone were purchased from Sigma-Aldrich, Bangalore, India and Himalaya healthcare- Bangalore respectively. The following biochemical kits creatinine, uric acid, oxalate, sodium and potassium were purchased from Erba Diagnostics Mannheim GmbH, Germany.

Animals

Male Albino rats (Wistar strain) weighing between 150-200 g was acclimatized for 7 days under standard husbandry condition i.e.

- Room temperature - 26 ± 2^0 C
- Relative humidity - 45-55%
- Light/ dark cycle - 12:12 h

The animals were fed with a standard diet from Amrut Laboratories and Pranav Agro Industries Ltd. Sangli. Water was allowed under strict hygienic conditions. All animal studies were conducted in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) and all the procedures were followed as per rules and regulations.

Preparation of extracts

Preparation of alcoholic extract

The leaves powder was placed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colorless solvent in the siphon tube was taken as the completion of extraction. The extract was then shifted into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated[12].

Preparation of aqueous extract

About 100 g of powdered plant material was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with random shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50ºC[12].

These two extracts were stored in airtight containers in a refrigerator below 10ºC. The two extracts were analyzed for their color and consistency. The percentage yield was calculated with reference to air-dried powder material used for the extraction.

Toxicity studies

The acute toxicity of leaves of *Sesamum indicum* was determined by using albino rat of either sex (150-200 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts of leaves of *Seasamum indicum* and noted for the mortality up to 48 h study period (Short term toxicity). Based on the short-term toxicity study, the next dose of the individual extracts was calculated as per OECD guidelines No. 425. From the LD_{50} doses 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively[13].

Ethylene glycol induced kidney stone in rats

Male Albino rats weighing between 150-200 g each group containing 6 animals were divided into 9 groups.

- Group A - Normal Control (vehicle treated p.o, 10 ml/100gm)
- Group B- Toxicant (0.75% ethylene glycol in place of drinking water)
- Group C- Standard (EG (0.75%) + cystone (750 mg/kg, p.o)
- Group D- Alcoholic extract (low dose 100 mg/kg, p.o)
- Group E- Alcoholic extract (medium dose 200 mg/kg, p.o)
- Group F- Alcoholic extract (high dose 400 mg/kg, p.o)
- Group G- Aqueous extract (low dose 100 mg/kg, p.o)
- Group H- Aqueous extract (medium dose 200 mg/kg, p.o)
- Group I- Aqueous extract (high dose 400 mg/kg, p.o)

Experimental procedure

Albino rats weighting between (150-200 g) were divided into 9 groups of six rats in each. Group A served as normal control which was given with vehicle only; Group B with (0.75% ethylene glycol in drinking water); Group C with cystone (750 mg/kg, p.o) which served as standard. Animal in groups D, E and F were treated with three different doses (low, medium and high) of alcoholic and group G, H and I with aqueous extract. Group B, C, D, E, F, G, H and I were intoxicated with 0.75% ethylene glycol in drinking water. On the 28th day, 24 hours after the treatment urine sample were collected from all the animals and serum samples from each rat were collected, in all groups. P^H of urine was checked by using narrow ranges P^H (BDH) paper and urine volumes were noted. A drop of concentrated hydrochloric acid was added to the urine before being stored 4ºC; later sacrificed by overdose of ether. Right kidneys were removed and washed with saline, weighed and stored in 10% formaldehyde for histological studies.

Statistical analysis

All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). $P < 0.05$*, 0.01** and 0.001*** were considered as statistically significant.

RESULTS

In the present study the effect of the alcoholic extracts of leaves of *Sesamum indicum* (AELSI) and aqueous extract of leaves of *Sesamum indicum* (AQELSI) on normal kidney functions, was found to be non-toxic in nature. Ethylene glycol intoxication in normal rats elevated the serum levels of creatinine, uric acid and oxalate significantly. The rats treated with AELSI and AQELSI showed a significant reduction in the biochemical parameters elevated by ethylene glycol (Table 1)

Histopathological analysis revealed no calcium oxalate deposits or other abnormalities in the nephron segments of vehicle and standard treated group (Figure 1 and 3). On the other hand, many calcium oxalate deposits inside the renal tubules and dilation of the proximal tubules along with interstitial inflammation were observed in the renal tissue of urolithiatic rats (Figure 2). The number of calcium oxalate deposits in the renal tubules of Groups IV to IX rats was significantly less than the Group II (Figure 4 to 9).

Table 1: Effect of AELSI and AQELSI on serological constituents against EG induced urolithiasis

Groups	Treatment	Serum Parameters						
		Creatinine (mg/dl)	Uric acid (mg/dl)	Oxalates (mg/dl)	Calcium (mg/dl)	Magnesium (mEq/L)	Sodium (mEq/L)	Potassium (mEq/L)
Group I Normal	Vehicle 10 ml/kg p.o	0.50 ± 0.02	0.98 ± 0.02	2.20 ± 0.03	3.52 ± 0.04	0.66 ± 0.02	114.53 ± 1.70	5.01 ± 0.06
Group II Toxicant	Ethylene glycol 0.75% v/v	1.25 ± 0.02	1.96 ± 0.03	3.17 ± 0.03	0.73 ± 0.02	1.65 ± 0.03	167.28 ± 1.60	6.57 ± 0.04
Group III Standard	Cystone 750 mg/kg+ EG	0.57 ± 0.06**	1.13 ± 0.02**	2.37 ± 0.04**	3.35 ± 0.03**	0.87 ± 0.02**	126.96 ± 2.07**	5.41 ± 0.08**
Group IV Low dose	AELSI 100 mg/kg	1.18 ± 0.05^{ns}	1.91 ± 0.03^{ns}	3.03 ± 0.03*	0.87 ± 0.02^{ns}	1.60 ± 0.03^{ns}	159.43 ± 2.20*	6.38 ± 0.06^{ns}
Group V Med. dose	AELSI 200 mg/kg	0.86 ± 0.06**	1.46 ± 0.02**	2.68 ± 0.04**	2.24 ± 0.04**	1.32 ± 0.02**	145.01 ± 1.19**	5.87 ± 0.07**
Group VI High dose	AELSI 400 mg/kg	0.68 ± 0.03**	1.22 ± 0.02**	2.59 ± 0.03**	3.11 ± 0.05**	1.03 ± 0.03**	134.25 ± 2.03**	5.78 ± 0.04**
Group VII Low dose	AQELSI 100 mg/kg	1.15 ± 0.02*	1.88 ± 0.03^{ns}	3.01 ± 0.03*	0.88 ± 0.02^{ns}	1.55 ± 0.02*	159.30 ± 1.42*	6.30 ± 0.03*
Group VIII Med. dose	AQELSI 200 mg/kg	0.78 ± 0.02**	1.40 ± 0.02**	2.56 ± 0.02**	2.60 ± 0.06**	1.23 ± 0.02**	139.71 ± 2.05**	5.58 ± 0.03**
Group IX High dose	AQELSI 400 mg/kg	0.66 ± 0.01**	1.17 ± 0.01**	2.44 ± 0.03**	3.26 ± 0.03**	0.93 ± 0.02**	129.58 ± 1.45**	5.06 ± 0.04**

Values are mean ± S.E.M (n = 6), Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant
AELSI- Alcoholic extract of leaves of *Sesamum indicum*
AQELSI- Aqueous extract of leaves of *Sesamum indicum*

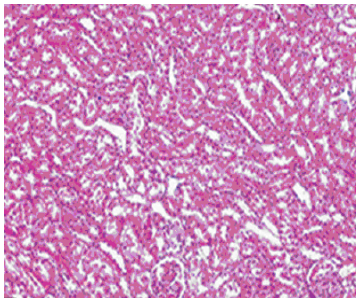

Figure 1: Histopathology of kidney of normal control

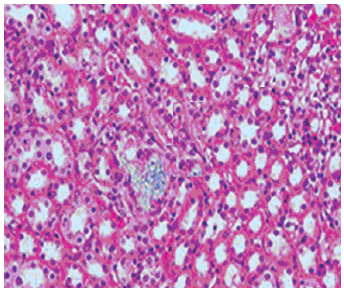

Figure 2: Histopathology of kidney of disease control

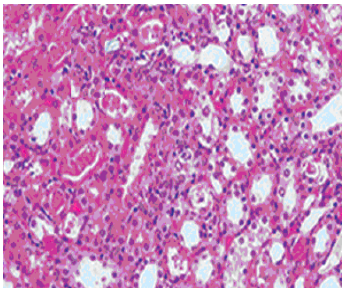

Figure 3: Histopathology of kidney of standard group

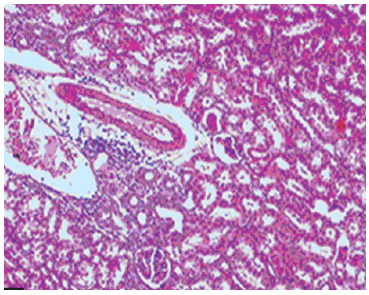

Figure 4: Effect of AELSI (Low) dose on EG induced urolithiasis in rat

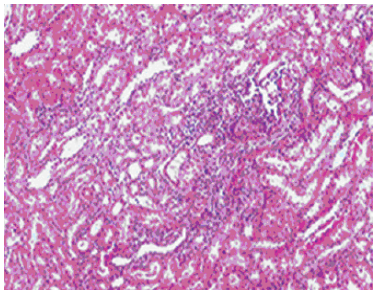

Figure 5: Effect of AELSI (Med) dose on EG induced urolithiasis in rat

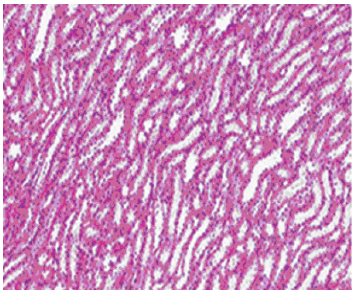

Figure 6: Effect of AELSI (High) dose on EG induced urolithiasis in rat

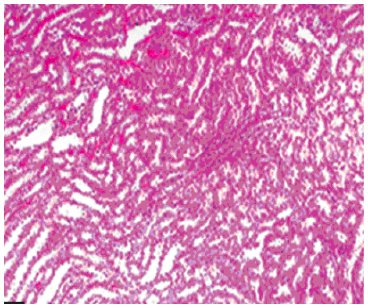

Figure 7: Effect of AQELSI (Low) dose on EG induced urolithiasis in rat

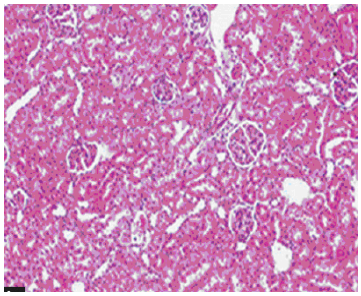

Figure 8: Effect of AQELSI (Med) dose on EG induced urolithiasis in rat

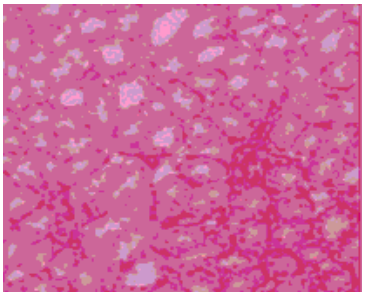

Figure 9: Effect of AQELSI (High) dose on EG induced urolithiasis in rat

DISCUSSION

As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of protective effect of the leaves of *Sesamum indicum* against ethylene glycol induced urolithiasis in rats.

In the present study, chronic administration of 0.75% v/v ethylene glycol aqueous solution to male Wistar rats produced hyperoxaluria.

Normal urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats. The magnesium levels return to normal on treatment with aqueous and alcoholic extracts of *Sesamum indicum*.

The increase in urinary uric acid excretion was observed in urolithiatic rats. Increased excretion of uric acid has been appeared in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of gycosaminoglycans. The prevalence of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggest its primary role in stone formation[15]. Treatment of *Sesamum indicum* leaf extracts lowered the excretion of uric acid and reduces the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) of kidney decreases due to the obstruction to the outflow of urine by stones in urinary system. Because of this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated in blood.[16]

In the present study, the positive control calculi-induced rats were found to have marked renal damage, consistent with the elevated serum levels of creatinine and uric acid. However, the treatment with AELSI and AQELSI inhibited these changes.

The mechanism of anti lithiatic activity of aqueous extract and alcoholic extract may involve the inhibition of oxalate induced toxic manifestations and free radical production along with enhancement of the body defense system. Drug treated group showing cyto protection due to its effect on prevention of deposition or aggregation of calcium oxalate in tubules, so the mechanical disruption of epithelium is less or protection against free radicals re-arrangements.

Microscopic examination of kidney sections derived from nephrolithiatic rats showed intra tubular and interstitial crystal deposits, consistent with the findings of others. In the present investigation histopathological evaluation showed the maximum prevention of crystal deposition which may be due to the active compound which is present in aqueous extract and alcoholic extracts. *Sesamum indicum* leaves have a high antioxidant capacity may be due to the presence of important phyto-constituents like glycosides, steroids and flavonoids may be prevents the calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria- induced per-oxidative damage to the renal tubular membrane surface which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

CONCLUSION

Various preliminary phytochemical analyses of the AELSI and AQELSI revealed the presence of carbohydrates, glycosides, proteins, steroids, flavonoids, alkaloids and saponins.

Treatment with medium dose (200 mg/kg) and high dose (400 mg/kg) of AELSI and AQELSI significantly prevented decrease in urine output as compared to lithiatic induced group. Test drugs significantly prevented decrease in urolithiatic promoters (calcium, oxalate and uric acid) and decrease in urolithiatic inhibitors (magnesium) as observed in various biological samples. Histopathology results were also significantly prevented by treatment groups.

It is found that AQELSI is more potent than AELSI that is confirmed by the physical and biochemical parameters followed by comparison of histological changes in kidneys.

Such activity may be apparently related to its diuretic action, decrease in promoters and increase in inhibitors level, antioxidant potential and partly due to saponification of crystals by saponins.

The presented data indicate that administration of aqueous and alcoholic extract of *Sesamum indicum* to the rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones, supporting folk information regarding anti urolithiatic activity of the plants. Exact mechanism underlying this effect is no clear, bur apparently related to antioxidant effect and lowering the stone forming constituents. Hence further research was suggested to explore the exact pharmacology of the drugs.

ACKNOWLEDGEMENTS

The authors thank the authorities of Maharishi Arvind Institute of Pharmacy, Jaipur and Kota College of Pharmacy, Kota, Rajasthan, India for providing necessary facilities.

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Cite this article as:

Nishant Singh Katiyar and Rajesh Asija. Anti urolithiatic activity of *Sesamum indicum* leaves against ethylene glycol induced urolithiasis in rats. Int. Res. J. Pharm. 2019;10(10):47-51 http://dx.doi.org/10.7897/2230-8407.1010297

Source of support: Nil, Conflict of interest: None Declared