

Antilithiatic activity of *Hibiscus sabdariffa* Linn. on ethylene glycol-induced lithiasis in rats

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Abstract

The ethanolic extract of leaves of *Hibiscus sabdariffa* Linn. (EEHS) was evaluated for its antilithiatic activity in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75 %) in adult male albino Wistar rats for 28 days. The ionic chemistry of urine was altered by ethylene glycol (EG), which elevated the urinary concentration of crucial ions, viz. calcium, phosphate, uric acid and oxalate thereby contributing to renal stone formation. The EEHS, however, significantly ($P < 0.05$) reduced the elevated level of these ions in urine. Also, it elevated concentration of urinary magnesium, which is considered as one of the inhibitors of crystallization. All these observations revealed that EEHS has curative effect on stone formation induced by ethylene glycol.

Keywords: Antilithiatic, *Hibiscus sabdariffa*, Roselle, Nephrolithiasis, Urolithiasis, Renal calculi, Ethylene glycol.

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Introduction

An important reason for acute and chronic renal failure, lithiasis (stone formation), includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in men, while phosphate stones happen more in women. The pathogenesis of lithiasis seems to be multifactorial and complicated¹. Medical management of lithiasis, today, includes lithotripsy and surgical procedures. Disappointingly, the underlying risk factors are not corrected by these techniques; hence there is a need to continue the medical supervision and

therapy to prevent stone recurrence². The herbal sources have become an vital area in the search for new antilithiatic drugs, because their parts as such, extracts and compounds isolated from them have demonstrated a variety of biological activity. In present study the antilithiatic activity of *Hibiscus sabdariffa* Linn. (Malvaceae) has been investigated scientifically to validate its folkloric use for lithiasis in South India.

H. sabdariffa is an annual erect shrub, distributed throughout the tropical Africa and Asia. It is commonly known as Roselle and it is cultivated in hotter regions of India as a monsoon (April-November) crop. The plant is reported to have antioxidant^{3,4},

hypolipidemic⁴, chemopreventive⁵ and diuretic properties^{6,7}.

Materials and Methods

Animals

Adult male albino Wistar rats were used for this study with the weight range of 150-200 g. They were maintained under 12h dark/light cycle in well ventilated polypropylene metabolic cages (3-4/cage) at $25 \pm 2^\circ\text{C}$. They were fed with standard pellet diet (AMRUT Pranav Agro Industries Limited) and had free access

to water. The animals were maintained in the above said conditions for a week before the experiment. This study has the approval from Institutional Animal Ethics Committee (IAEC).

Preparation of the extract

The leaves of *H. sabdariffa* were collected from the farmlands of Guntur district, Andhra Pradesh, India. Care was taken to collect only the healthy leaves. The collected leaves were authenticated at the Department of Botany, The American College, Madurai and a voucher specimen (PC.05129) has been deposited in Division of Pharmacology, KM College of Pharmacy, Madurai, India. The leaves were then shade dried, coarsely powdered in such a way that it passed through sieve No.20 and was retained on sieve No.40. About 500 g of the dry powder was extracted continuously in Soxhlet apparatus with 99% ethanol for 72 hours. After 72h, the solvent was evaporated to obtain the crude extract (5.85%) (EEHS). The extract was then dried under vacuum and suspended in water before use. The phytochemical screening gave positive results for proteins, carbohydrates, glycosides, flavonoids, tannins and saponins.

Experimental design

Lithiasis was induced in rats by using the method of Selvam *et al*⁸. The animals were selected from acclimatized crowd and randomly divided into 4 groups of 6 each. Group I served as normal control and were maintained on commercial pellet feed, ordinary drinking water throughout the study period (28 days). Group II animals (lithiatic control) were fed with 0.75% ethylene glycolated water

ad libitum for 28 days. Group III animals were fed with 0.75 % ethylene glycolated water *ad libitum* along with EEHS (50 mg/kg body weight) by gastric intubation for 28 days. Group IV animals were fed with ordinary drinking water and EEHS (50 mg/kg body weight) by gastric intubation for 28 days. Urine was collected on days 7, 14, 21 and 28 for 24h by keeping the animals in polypropylene metabolic cages. The collected urine was analyzed for calcium (using calcium liquid kit: Raichem method)⁹, magnesium (Erba Magnesium Aresnazo Method, Endpoint-TRANSASIA)⁹, oxalates (Hodgkinson and Williams procedure)¹⁰, inorganic phosphates (using Phosphorous reagent kit: Raichem method)¹¹ and protein (at 650 nm)¹² using standard methods. The volume of urine collected from all groups was recorded.

Ethylene glycol, being reported to be renotoxic, renal function was assessed at the end of the study by estimating the serum and urine creatinine levels. Finally, the prevalence of lithiasis was confirmed by histopathological studies of the kidneys isolated from the sacrificed animals.

Histopathological studies

The isolated kidneys were weighed and transferred to 10% neutralized formalin (pH 7.4). Pathological changes were observed in the sections of kidney fixed in paraffin that were stained with hematoxylin and eosin.

Statistical analysis

The results are expressed as mean $\pm$ SEM. Data was evaluated using ANOVA-Single Factor test followed by

Newman-Keuls test. Probability values less than 0.05 were considered significant.

Results

Ionic parameters of urine

The urinary output of all the four groups of rats (control and experimental) on 28th day is as follows: The urinary volume of the normal control rats was 6.10 ± 0.30 ml/day/rat, while in the EG treated rats of Group II, it was reduced to 3.90 ± 0.40 ml/day/rat. However, in the drug-treated group (Group III) the urinary volume increased significantly ($P < 0.05$) to 5.20 ± 0.30 ml/day/rat.

The concentrations of the various ions in the collected urine were investigated and found to fluctuate drastically after the treatment with EG. On 28th day, the concentration of oxalate (14.53 ± 0.3 mg/24h) was increased significantly in Group II animals. Group III animals that were treated with EEHS showed a significant reduction in the oxalate excretion (2.93 ± 0.54 mg/24h). Similarly, the excretion of calcium (5.3 ± 0.2 mg/24h) and phosphate (14.2 ± 0.1 mg/24h) in the urine of Group II animals was increased on the 28th day following the treatment with ethylene glycolated water. But these elevated levels were decreased in Group III animals to 1.2 ± 0.15 mg/24h and 8.77 ± 0.04 mg/24h for calcium and phosphate, respectively which were treated with the extract.

Contradictorily the excretion of magnesium (0.30 ± 0.52 mg/24h) decreased gradually in Group II animals on 28th day following EG treatment. But in Group III animals, it showed an enhanced excretion of magnesium (0.86 ± 0.01 mg/24h). The amount of protein excreted by Group II animals was

increased to $9.90 \pm 0.91 \text{ mg/24h}$ following EG treatment, but in normal control rats it was $3.87 \pm 0.08 \text{ mg/24h}$, whereas in the extract treated animals (Group III) it was $3.15 \pm 0.06 \text{ mg/24h}$. There were no significant changes in the ionic parameters and volume of urine in Group IV animals, treated with EEHS and ordinary drinking water. All the values discussed above are presented in Table 1 & 2.

Table 1: Effect of *H. sabdariffa* leaves extract on urinary excretion of various ionic parameters (Oxalate, Calcium & Magnesium)

	Treatment group	Before EG treatment	After EG treatment (Days)			
			7	14	21	28
Oxalate	Group I	0.36 ± 0.54	0.37 ± 0.1	0.36 ± 0.1	0.37 ± 0.1	0.37 ± 0.1
	Group II	0.43 ± 0.26	7.45 ± 0.17^a	10.23 ± 0.1^a	13.17 ± 0.2^a	14.53 ± 0.3
	Group III	0.39 ± 0.26	1.45 ± 0.1	2.4 ± 0.12	2.75 ± 0.2	2.93 ± 0.11
	Group IV	0.41 ± 0.1	7.4 ± 0.11^b	5.46 ± 0.2^b	4.06 ± 0.23^b	2.27 ± 0.54^b
Calcium	Group I	0.72 ± 0.01	0.71 ± 0.01	0.72 ± 0.01	0.82 ± 0.01	0.80 ± 0.01
	Group II	0.76 ± 0.6	2.6 ± 0.6^a	6.5 ± 0.23^a	5.87 ± 0.14^a	5.3 ± 0.2^a
	Group III	0.77 ± 0.12	1.05 ± 0.1	0.9 ± 0.17	0.99 ± 0.12	1.2 ± 0.15
	Group IV	0.78 ± 0.24	4.7 ± 0.2^b	3.53 ± 0.2^b	2.84 ± 0.06^b	2.7 ± 0.06^b
Magnesium	Group I	1.17 ± 0.1	0.92 ± 0.01	1.2 ± 0.11	1.35 ± 0.6	1.22 ± 0.08
	Group II	1.3 ± 0.21	1.2 ± 0.17^a	0.7 ± 0.77^a	0.4 ± 1.56^a	0.3 ± 0.52^a
	Group III	1.08 ± 0.09	0.73 ± 0.008	0.80 ± 0.01	0.54 ± 0.01	0.86 ± 0.01
	Group IV	1.32 ± 0.14	1.2 ± 0.11^b	1.1 ± 0.09^b	1.1 ± 0.23^b	1.4 ± 0.14^b

Values are expressed as md/24 h urine sample. Values were provided as mean $\pm$ SEM for six animals of each group.

^a Significantly different from normal control; ^b Significantly different from lithiatic control; $P < 0.05$ was considered significant.

Table 2 : Effect of *H. sabdariffa* extract on urinary excretion of various ionic parameters (Protein & Phosphate)

	Treatment group	Before EG treatment	After EG treatment (Days)			
			7	14	21	28
Protein	Group I	3.54 ± 0.27	3.25 ± 0.08	3.4 ± 0.01	3.67 ± 0.06	3.87 ± 0.08
	Group II	3.41 ± 0.43	6.9 ± 0.72^a	8.7 ± 0.62^a	9.5 ± 0.72^a	9.9 ± 0.91^a
	Group III	3.26 ± 0.1	3.57 ± 0.14	3.55 ± 0.17	3.32 ± 0.1	3.15 ± 0.06
	Group IV	3.35 ± 0.11	7.47 ± 0.12^b	6.83 ± 0.2^b	6.46 ± 0.12^b	5.9 ± 0.68^b
Phosphate	Group I	7.07 ± 0.1	7.7 ± 0.08	7.12 ± 0.08	7.1 ± 0.07	7.22 ± 0.1
	Group II	7.91 ± 0.1	12.4 ± 0.1^a	13.43 ± 0.2^a	14.1 ± 0.08^a	14.2 ± 0.1^a
	Group III	7.83 ± 1.23	9.45 ± 0.1	8.2 ± 0.1	9.1 ± 0.07	8.77 ± 0.04
	Group IV	7.01 ± 0.32	10.0 ± 0.3^b	11.1 ± 0.3^b	10.73 ± 2.8^b	11.1 ± 0.4^b

Values are expressed as md/24 h urine sample. Values were provided as mean $\pm$ SEM for six animals of each group.

^a Significantly different from normal control; ^b Significantly different from lithiatic control; $P < 0.05$ was considered significant.

Serological parameters

Serum analysis was carried out and the values are given in Table 3. A significant increase in creatinine levels was observed in Group II when it was compared to Group I. Animals in Group III, after treating with the plant extract, showed restoration in creatinine to normal limits. Moreover in Group III animals, the creatinine clearance was also improved by the extract.

Histopathological data

It was observed that microcrystals were deposited in the sections of kidneys of rats treated with ethylene glycol. It was also observed that tubules were dilated, damaged and inflammatory cells were infiltrated into the interstitial space. But, improvement in the above said symptoms were observed in the kidney sections of rats treated with the extract. The results are shown in Figs. 1, 2, & 3.

Discussion

Different chemicals used to induce lithiasis in experimental animals include ethylene glycol (EG), glycolic acid and ammonium oxalate¹³. Kidney, being the principal target for EG toxicity and its administration to the experimental animals for more than 4 weeks, resulted in substantial excretion of oxalate and deposition of microcrystals in kidneys¹⁴. Therefore, in this study EG was preferred to induce lithiasis. Alteration in urine volume and composition were observed after the induction of lithiasis. As the volume of urine excreted by group III animals were more when compared to that of the volume excreted by the Group II animals, this reinforces the plant's diuretic property^{6,7}. Such an effect may be

Table 3 : Effect of *H. sabdariffa* on serological parameters on 28th day of lithiasis

Treatment groups	Serum creatinine (mg/dl)	Creatinine clearance (ml/min)
Group I	0.52 ± 0.17	0.68 ± 0.01
Group II	1.70 ± 0.12 ^a	0.05 ± 0.02 ^a
Group III	0.60 ± 0.01 ^b	0.52 ± 0.04 ^b
Group IV	0.52 ± 0.03 ^b	0.63 ± 0.05 ^b

Values are expressed as mean ± SEM for six animals of each group.

^a values are significantly different from normal control; ^b values are significantly different from lithiatic control; $P < 0.05$ was considered significant.

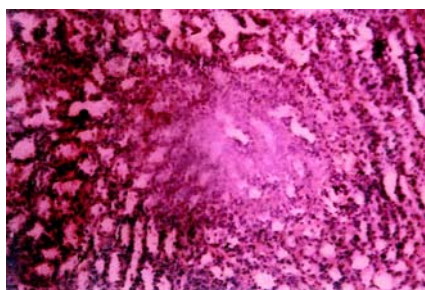


Fig. 1: Section of rats kidney showing normal epithelial lining and tubules (Group I)

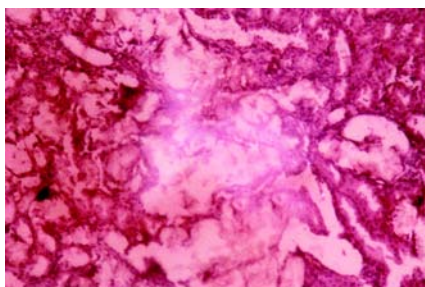


Fig. 2: Section of rats kidney showing epithelial lining (degenerated) and dilated tubules (Group II)

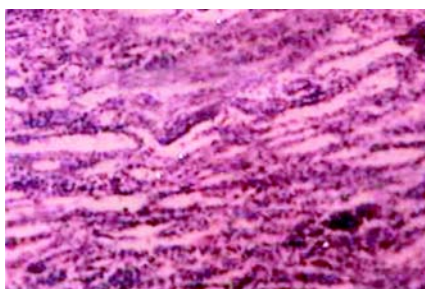


Fig. 3: Section of rats kidney showing tubules and epithelial lining comparatively similar to normal (Group III)

advantageous in the lithiatic condition, as an increased urine output is recommended to reduce the possibility of stone formation.

It has been suggested that, ethylene glycol being oxidized into oxalic acid by non-specific dehydrogenase lead to hyperoxaluria¹⁵ which is considered to be the key factor in the induction of lithiasis¹⁶. It was observed that the amount of calcium and oxalate excreted following the administration of ethylene glycol in Group II animals was increased, which suggest that calcium oxalate stones might have been formed. Previous study report states that more than 80% of the renal stones are made up of calcium oxalate and calcium phosphate¹⁷. But on administration of the extract to the animals, the amount of calcium and oxalate levels in urine were reduced in Group III, thus reducing the intensity of sympathetic circumstances for crystallization of calcium oxalate.

Moreover, the amount of phosphate excreted in urine was increased in Group II animals following EG administration, which means that beside calcium oxalate, calcium phosphate has also been formed. Since, there is a decrease in the phosphate level of the

animals in Group III, it is apparent that the extract has inhibition over the precipitation of both calcium phosphate and calcium oxalate.

In Group II, the magnesium level in the urine was decreased, which is a common feature in the stone formers, whereas in Group III the same was increased, thus reducing the intensity of crystallization.

In stone formers and animal models with hyperoxaluric conditions, increased excretion of proteins are reported which has an influence in the formation and growth of crystals^{18,19}. A similar condition was observed in the ethylene glycol treated rats, which reduces the situations favourable for growth of the crystals.

Animals in Group II showed increase in serum creatinine levels than the animal in control group, which reveals that the chemical used is renotoxic. Low serum creatinine level and improved creatinine clearance was seen in Group III, which states that the kidney function is improved.

Conclusion

This study confirms the effective use of *Hibiscus sabdariffa* in kidney malfunctioning in general and especially stone formation. The study also supports Roselle leaves applications in folklore medicine for same purposes.

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