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ORIGINAL PAPER

Inhibition of calcium oxalate crystal deposition on kidneys of urolithiatic rats by *Hibiscus sabdariffa* L. extract

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Abstract The present study aims at systematic evaluation of the calyces of *Hibiscus sabdariffa* to establish its scientific validity for anti-urolithiatic property using ethylene glycol-induced hyperoxaluria model in male albino rats. Administration of a mixture of 0.75% ethylene glycol and 2% ammonium chloride resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. The decrease in the serum calcium concentration indicates an increased calcium oxalate formation. Supplementation of aqueous extract of *H. sabdariffa* at different doses (250, 500 and 750 mg/kg body weight) significantly lowered the deposition of stone-forming constituents in the kidneys and serum of urolithiatic rats. These findings have been confirmed through histological investigations. Results of in vivo genotoxicity testing showed no significant chromosomal aberrations in the bone marrow cells of ethylene glycol-induced rats. The plant extracts at the doses investigated induced neither toxic nor lethal effects and are safe. It can be concluded that the calyces of *H. sabdariffa* are endowed with anti-urolithiatic activity and do not have genotoxic effects. Thus, it can be introduced in clinical practices and medicine in the form of orally administered syrup after further investigations and clinical trials.

Keywords Urolithiasis · *Hibiscus sabdariffa* L. · Ethylene glycol · Crystals · Genotoxicity

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Introduction

Urolithiasis is an extremely painful disease that afflicts the human population since ancient times [14]. It is a urinary tract disorder characterized by the presence of solid deposits such as urinary calculi (also known as stones) or excessive amounts of crystals in the urinary tract. These solid deposits/calculi can form anywhere in the urinary collecting system, but most often calculi arise in the kidney (nephrolithiasis) [19]. It occurs more frequently in men than women but rare in children [33], affecting approximately 12% of the men and 5% of the women by the age of 70. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, growth and aggregation, and ending with retention within the urinary tract. The etiology of stone formation is a multifactorial process which may relate to diet, urinary tract infection, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque, microliths, etc. [12]. The principal causative factor of the formation of stones is attributed to the supersaturation of precipitating salts. One of the most important phenomena that characterize urolithiasis is its high recurrence. Thus, appropriate management is required to treat urolithiasis and therefore, it is worth looking for an alternative to those conventional methods, such as the use of medicinal plants.

Medicinal plants are of great economic importance in the Indian subcontinent. Plants remain the basis for a large proportion of the medications used today for the treatment of variety of diseases. A number of researchers have documented the use of traditional medicinal plants in India [15]. A variety of plants including those used by traditional medical practitioners grow luxuriantly in Manipur (23°50′–25°42′N; 92°58′–94°45′E), a region in the north-eastern part of India which happens to be within the Indo-Burmese

mega-biodiversity hot-spot [24]. The Indian indigenous system of medicine provides abundant data on plants available for the treatment of urolithiasis. Among the vast number of medicinal plants that are claimed to be anti-urolithiatic, *Hibiscus sabdariffa* L. (family: Malvaceae) is the one for which systematic pharmacological studies have not been carried out on its calyces to support the claim made. Hence, the present study aims at systematically evaluating the calyces of this plant to establish its scientific validity for anti-urolithiatic property using ethylene glycol-induced hyperoxaluria model in rats and to assess the genotoxicity of this plant. Thus, scientific investigations on the indigenous medicine prepared from plant products used by the Tribals and Meiteis of Manipur may prove to be of great pharmacological importance leading to the advent of novel drugs, which could be at par with the modern allopathic medicines in terms of efficacy, minimal side-effects and cost affordability.

Hibiscus sabdariffa commonly known as Roselle or Jamaican Sorrel is an annual erect shrub that grows to 6 feet or more and is mostly branched. Stems are robust and glabrous. Leaves are alternate, long-petiolate, palmately divided into 3–7 lobes with serrate margins and bear short-peduncled light-yellow flowers with a reddish centre at the base of the staminal column. The flowers are in axillary or in terminal racemes. The calyx becomes fleshy when enlarged at maturity creating a bright fleshy red, acid fruit of about $1^1/_4$ inches. *H. sabdariffa* is cultivated in the hotter regions of India as a monsoon (April–November) crop. Besides the pleasant aroma and beauty of the plant itself, it possesses medicinal attributes. As a medicine, it is used as a therapeutic, laxative, chemopreventive [21], anti-hypertensive, cholesterol-lowering medicine [10] and an antioxidant agent [10, 37]. It also lowers hepatotoxicity and reduces fever. Almost all the parts are considered diuretic and antiscorbutic [23, 41] in their action. The fibrous part of *Hibiscus* is used in the production of twins and cord known as "Roselle hemp".

Materials and methods

Plant sample

The fresh calyces of *H. sabdariffa* were collected from various areas of Imphal-West (24°37′N and 93°30′E) district, India. A voucher number (Deb 1377) was assigned to it after depositing in the Herbarium of Manipur University, Imphal.

Preparation of plant extract

The plant samples were washed in fresh running tap water and air-dried for about 10 min. For the preparation of aqueous extract, 10 g of the plant sample was extracted in 100 ml of deionized water with the help of a mortar and pestle and homogenized for uniformity. Then, the homogenate was centrifuged at $3{,}000 \times g$ for 10 min to get a clear supernatant. Finally, the clear supernatant was decanted and filtered through Whatman No. 1 filter paper and stored at 4°C for future use.

Pharmacological screening for anti-urolithiatic activity

Animal selection

Male albino rats (*Rattus norvegicus* albus.) weighing about 150–200 g were selected for the study. The animals were maintained under 12 h dark/light cycle in well-ventilated polypropylene metabolic cages at (25 ± 2°C) and fed with standard pellet diet and had free access to drinking water. The animal care and the experimental protocols were performed in accordance with the guidelines of the Institutional Animal Ethical Committee (IAEC). Experiments on albino rats were conducted with the approval (No. MU/8-199/06/UGC dated 03-09-2008) of IAEC.

Acute toxicity studies

Maximum tolerated dose (MTD) was determined by treatment of the test system (albino rat, *Rattus norvegicus* albus.) with various concentrations of plant extracts as described by Carrol [3]. For this purpose, male albino rats weighing about 150–200 g of 5 animals each were taken for each dose. The different doses of aqueous extract of *H. sabdariffa* (Table 1) were administered orally with a plastic disposable syringe, fitted with a feeding needle. All the groups of rats were kept under observation for 24 h.

Ethylene glycol-induced urolithiasis model

The effect of oral administration of aqueous extract of *H. sabdariffa* on calcium oxalate urolithiasis induced by ethylene glycol was studied using male albino rats. Animals were divided into nine groups of 5 each. Group I, normal/control rats given normal regular diet and water ad libitum; Group II, urolithiatic rats given drinking water containing a mixture of 0.75% ethylene glycol [v/v] (EG) and 2% ammonium chloride [w/v] (AC); Group III, urolithiatic rats given standard anti-urolithiatic drug, cystone (750 mg/kg body weight); Groups (IV–VI), urolithiatic rats given aqueous extract of *H. sabdariffa* at the doses of 250, 500 and 750 mg/kg body weight; Groups (VII–IX), normal rats given aqueous extract of *H. sabdariffa* at the doses of 250, 500 and 750 mg/kg body weight. All the groups of rats were given drinking water ad libitum and the treatment duration was for 28 days.

Table 1 Exploratory trials for determination of MTD (maximum tolerated dose) of aqueous extract of *Hibiscus sabdariffa* L.

Dose (mg/kg body weight)	Mortality (%)
0	0
250	0
500	0
750	0
1,000	0
1,250	0
1,500	0
1,750	0
2,000	0
2,010	0
2,020	20
2,030	40
2,040	80
2,050	100

Assessment of anti-urolithiatic activity

Serum analysis

After the experimental period, blood was collected from the retro-orbital region of the rat under anesthetic conditions. Serum was separated by centrifugation at 10,000×*g* for 10 min [16] after which the levels of calcium [22], phosphorus [11], urea and creatinine [25] were assessed.

Kidney analysis

The anesthetized rats (with chloroform) were sacrificed by cervical dislocation. The abdomen was dissected and both the kidneys were removed. The isolated kidneys were cleaned off extraneous tissues. The left kidney was oven-dried at 80°C for 24 h, after which the kidneys were weighed. About 1 g of the dried kidney sample was boiled in 100 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate obtained was again centrifuged at 2,000×*g* for 10 min and the supernatant was separated [4] after which the levels of calcium [22] and phosphorus [11] in the kidney were assessed.

Histological analysis

The tissue pieces taken from the kidney of the rats were fixed in neutral buffered formalin (10%) and subsequently embedded in paraffin. The sections (about 5-µm thick) were stained using hematoxylin and eosin stains [6] to study the histopathological changes and to check calcium oxalate crystal deposition. Tissues slices were photographed using a CCD camera fitted to trinocular phase contrast microscope-UNILAB (GE-52TRH) with 400× magnification.

Genotoxicity testing

The anesthetized rats were sacrificed by cervical dislocation and the bone marrow was taken out from either of the femur to perform cytogenetic analysis. The metaphase chromosomal plates were prepared according to the conventional air-drying method [38] with minor modifications. In brief, the bone marrow taken out from one of the femurs was aspirated, flushed with normal saline (0.9% NaCl), treated with hypotonic solution (0.54% KCl) for about 20–30 min, centrifuged at 800×*g* for 5 min and the pellet was fixed in freshly prepared chilled Carnoy's fluid (3 methanol:1 glacial acetic acid) after 2–3 changes with this fixative along with centrifugation. The slides were prepared by standard air drop method. They were then dried and stained with 10% Giemsa (Sigma) prepared in Sorrenson's buffer, pH 5.6. The stained slides were rinsed with distilled water. Chromosomal aberrations per 200 cells were scored under 400× magnifications. Chromosomal aberrations such as chromatid breaks, centromeric gaps, fragments and deletions were scored. Aberrations were identified according to the criteria given by Savage [30].

Micronucleus assay was performed according to the method of Schmid [31] with minor modifications. Briefly, bone marrow taken out from the femur was aspirated out into 1 ml RPMI-1640 (without L-glutamine and sodium bicarbonate), centrifuged at 200×*g* for 5 min and the pellet was resuspended in 0.5 ml of fetal calf serum (heat inactivated at 56°C for 30 min). The smears of cells were prepared on pre-cleaned, pre-coded dried slides and fixed in absolute methanol. The slides containing the cells were stained with 10% Giemsa (Sigma) prepared in Sorrenson's buffer, pH 5.6 according to the method of Vives Corrons et al. [40] with minor modifications and examined under a microscope. 1,000 cells were counted blind for each animal. Photographs were taken using a CCD camera fitted to trinocular phase contrast microscope-UNILAB (GE-52TRH) using oil immersion at 1,000-fold magnification.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values are presented as means ± standard error (SE). Values of $p < 0.05$ were considered significant.

Results

Based on the results obtained from acute toxicity testing, MTD for the *H. sabdariffa* was found to be 2,010 mg/kg

Table 2 Effect of aqueous extract of the calyces of *Hibiscus sabdariffa* L. on kidney and serum parameters in control and experimental animals

Groups	Kidney (mg/g)			Serum (mg/dl)		
	Calcium	Phosphorus	Calcium	Creatinine	Phosphorus	Urea
Group I	3.41 ± 0.03^{a}	2.59 ± 0.09^{a}	4.51 ± 0.17^{b}	0.49 ± 0.03^{a}	3.05 ± 0.12^{a}	52.74 ± 0.03
Group II	9.09 ± 0.04^{d}	4.06 ± 0.35^{b}	3.00 ± 0.16^{a}	1.87 ± 0.05^{b}	8.01 ± 0.27^{b}	65.71 ± 0.05
Group III	$3.61 \pm 012^{a,b}$	2.59 ± 0.07^{a}	4.50 ± 0.17^{b}	0.51 ± 0.03^{a}	3.06 ± 0.12^{a}	53.85 ± 0.04
Group IV	4.14 ± 0.24^{c}	2.71 ± 0.70^{a}	4.40 ± 0.08^{b}	0.56 ± 0.05^{a}	3.13 ± 0.09^{a}	61.05 ± 0.15
Group V	4.13 ± 0.05^{c}	2.71 ± 0.64^{a}	4.42 ± 0.09^{b}	0.55 ± 0.07^{a}	3.14 ± 0.08^{a}	58.95 ± 0.06
Group VI	4.13 ± 0.05^{c}	2.65 ± 0.30^{a}	4.48 ± 0.06^{b}	0.54 ± 0.07^{a}	3.10 ± 0.06^{a}	54.74 ± 0.05^{c}
Group VII	4.11 ± 0.03^{c}	2.66 ± 0.30^{a}	4.47 ± 0.07^{b}	0.54 ± 0.06^{a}	3.10 ± 0.06^{a}	54.75 ± 0.07^{c}
Group VIII	$3.97 \pm 0.07^{b,c}$	2.66 ± 0.30^{a}	4.48 ± 0.09^{b}	0.54 ± 0.06^{a}	3.09 ± 0.06^{a}	54.75 ± 0.08^{c}
Group IX	$3.95 \pm 0.08^{b,c}$	2.61 ± 0.33^{a}	4.49 ± 0.06^{b}	0.52 ± 0.06^{a}	3.08 ± 0.06^{a}	54.73 ± 0.07^{c}

Values are expressed as means ± standard error ($n = 5$). For each column, values followed by the same letters (a–d) are not statistically different at $p < 0.05$ as measured by Tukey HSD test

body weight. The therapeutic doses of the plant extract were taken as 250, 500 and 750 mg/kg body weight.

In the present study, chronic administration of aqueous solution of a mixture of 0.75% ethylene glycol and 2% ammonium chloride in drinking water to male albino rats significantly increased the deposition of calcium oxalate stones. Serum and kidney analyses revealed that the levels of renal stone components were increased grossly in urolithiatic rats (Table 2, Group II) when compared with the control group (Table 2, Group I). This shows strong indication of renal and hepatic impairment. The decrease in the serum calcium concentration indicates utilization of calcium for calcium oxalate formation ultimately depositing in the kidney site, thereby subsequently elevating its level in kidneys of urolithiatic rats. However, supplementation of aqueous extract of *H. sabdariffa* at different doses (250, 500 and 750 mg/kg body weight) significantly lowered the deposition of stone-forming constituents in the kidneys and serum in both the treated sets when compared to the cystone-treated rats (Table 2, Group III). The treatment of aqueous extract of *H. sabdariffa* at different doses significantly reduced the deposition of the crystalline components in both the sets (Table 2, Groups IV–VI and Groups VII–IX). The serum urea and phosphorus levels were remarkably increased in urolithiatic rats (Table 2, Group II), while the level of creatinine in the serum was only slightly elevated in Group II indicating a marked renal damage. However, treatment of *H. sabdariffa* extract in both the sets (Table 2, Groups IV–VI and Groups VII–IX) significantly lowered the elevated serum levels of urea, phosphorus and creatinine.

Histological investigations revealed that rats treated with ethylene glycol and ammonium chloride had large deposits of calcium oxalate crystals in all parts of the kidney. Tubular dilation and degeneration of epithelial lining also occurred (Fig. 1b, Group II). Such deposits were not present in normal rats (Fig. 1a, Group I), cystone-treated rats (Fig. 1c, Group III), normal rats treated with the extract at all doses (Groups VII–IX) and urolithiatic rats treated with extract at the dose of 750 mg/kg body weight (Fig. 1f, Group VI). Slight traces of crystals were observed in urolithiatic rats treated with extract at the doses 250 and 500 mg/kg body weight (Fig. 1d, e Groups IV and V). There is less degeneration of epithelial lining and tubules thereby indicating a marked improvement in these groups too. The observed structural abnormalities in bone marrow cells in various groups of treated rats were illustrated in Tables 3, and 4. Results of in vivo genotoxicity testing showed no significant chromosomal aberrations (centromeric gaps, chromatid breaks, deletions, and fragments) in bone marrow cells of ethylene glycol-induced rats although, there is a slight increase in the micronuclei formation. The plant extracts at the doses investigated induced neither toxic nor lethal effects and is safe when compared with the control groups. There is no significant micronuclei formation in these groups and hence, it can be concluded that oral administration of *H. sabdariffa* did not have any genotoxic effect.

Discussion

Urolithiasis is a stone-disorder due to an imbalance between inhibitors and promoters in the kidneys and human kidney stones are mainly composed of calcium oxalate type of stones [7]. Many in vivo models have been developed for investigating the mechanisms underlying in stone formation and ascertaining the effect of various therapeutic agents on the development and progression of urolithiasis [1, 2, 9, 17, 18, 20].

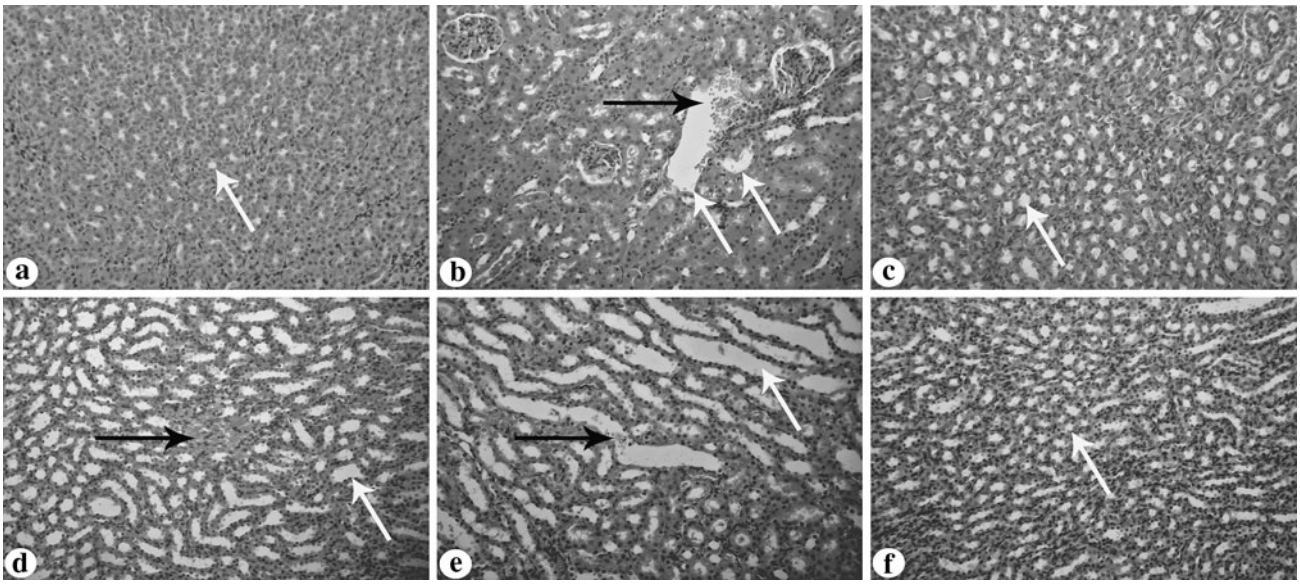

Fig. 1 Kidney section of control rat with normal epithelial lining and tubules (**a**), kidney section of urolithiatic rat showing dilated tubules, degenerated epithelial lining and crystal deposit (**b**), kidney section of urolithiatic rat treated with cystone (750 mg/kg body weight) showing epithelial lining and tubules comparatively similar to that of normal rats (**c**), kidney section of urolithiatic rat treated with aqueous extract of *H. sabdariffa* (250 mg/kg body weight) showing less degeneration of epithelial lining and tubules with lower rate of crystal deposition (**d**), kidney section of urolithiatic rat treated with aqueous extract of *H. sabdariffa* (500 mg/kg body weight) showing less degeneration of epithelial lining and tubules with very low rate of crystal deposition (**e**), kidney section of urolithiatic rat treated with aqueous extract of *H. sabdariffa* (750 mg/kg body weight) showing epithelial lining and tubules comparatively similar to that of normal rats (**f**); *black arrows* denote crystal deposition and *white arrows* denote tubules

Table 3 Effect of aqueous extract of *Hibiscus sabdariffa* L. on rat bone marrow

Groups	Normal metaphases/ 200 cells	Centromeric gaps	Chromatid breaks	Deletions	Fragments	Total aberrations
Group I	196.00 ± 0.70	1.20 ± 0.49	0.80 ± 0.49	1.40 ± 0.40	0.60 ± 0.24	4.00 ± 0.71
Group II	192.60 ± 0.67	2.00 ± 0.32	1.60 ± 0.25	2.20 ± 0.37	1.60 ± 0.24	7.40 ± 0.67
Group III	194.60 ± 0.51	1.40 ± 0.40	1.20 ± 0.49	1.80 ± 0.58	1.00 ± 0.45	5.40 ± 0.51
Group IV	193.40 ± 0.68	1.80 ± 0.49	1.40 ± 0.40	2.00 ± 0.32	1.40 ± 0.50	6.60 ± 0.68
Group V	193.80 ± 0.80	1.60 ± 0.25	1.40 ± 0.24	1.80 ± 0.49	1.40 ± 0.40	6.20 ± 0.80
Group VI	194.40 ± 0.40	1.60 ± 0.24	1.20 ± 0.20	1.60 ± 0.24	1.20 ± 0.37	5.60 ± 0.40
Group VII	194.60 ± 0.40	1.40 ± 0.51	1.20 ± 0.58	1.60 ± 0.24	1.20 ± 0.37	5.40 ± 0.40
Group VIII	194.20 ± 0.49	1.20 ± 0.49	1.40 ± 0.50	1.80 ± 0.58	1.40 ± 0.51	5.80 ± 0.49
Group IX	194.00 ± 0.32	1.60 ± 0.24	1.20 ± 0.58	1.80 ± 0.32	1.40 ± 0.25	6.00 ± 0.32

No significant changes were observed as determined by Tukey's post hoc test. Values are expressed as means ± standard error ($n = 5$)

Rats are the most frequently used animal models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans [1]. Rat models of calcium oxalate urolithiasis induced by either ethylene glycol alone or in combination with other drugs such as ammonium chloride were often used to study the pathogenesis of kidney crystal deposition [9]. In the present study, male albino rats were also treated with solutions containing a mixture of 0.75% ethylene glycol and 2% ammonium chloride for 28 days. All positive control rats (Group II) developed calcium oxalate depositions during that time. The present results also showed that the administration of ethylene glycol caused statistical increase in the levels of phosphorus, urea and creatinine and a decrease in the level of calcium in the calculi-induced or urolithiatic rats.

Selvam [32] reported that stone formation was also caused by hyperoxaluria which resulted in increased renal retention and excretion of calcium and phosphate. The decrease in serum calcium concentration indicates an increase of urinary calcium and calcium oxalate stone formation. This suggestion is in agreement with several studies like Rajagopal et al. [26] who reported that the level of serum calcium was decreased and urinary calcium

Table 4 Effect of aqueous extract of *Hibiscus sabdariffa* L. on rat bone marrow micronuclei

Groups	Polychromatic micronucleated erythrocytes	Normochromatic micronucleated erythrocytes	% Micronucleus
Group I	2.20 ± 0.20	0.80 ± 0.37	0.30 ± 0.04
Group II	3.80 ± 0.58	2.60 ± 0.50	0.64 ± 0.07
Group III	2.60 ± 0.24	1.20 ± 0.37	0.38 ± 0.03
Group IV	3.60 ± 0.75	2.20 ± 0.20	0.58 ± 0.09
Group V	3.60 ± 0.60	2.00 ± 0.32	0.56 ± 0.06
Group VI	3.40 ± 0.24	1.80 ± 0.37	0.52 ± 0.04
Group VII	3.00 ± 0.71	1.40 ± 0.60	0.44 ± 0.12
Group VIII	3.20 ± 0.58	1.60 ± 0.51	0.48 ± 0.10
Group IX	3.20 ± 0.49	2.20 ± 0.37	0.54 ± 0.07

No significant changes were observed as determined by Tukey's post hoc test. Values are expressed as means ± standard error ($n = 5$)

increased in rats treated with ethylene glycol. Soundararajan et al. [34] showed that calcium oxalate excretion was significantly increased in the urine of ethylene glycol-induced urolithiatic rats. They also stated that ethylene glycol disturbed oxalate metabolism by increasing the substrate availability resulting in an increase in the activity of oxalate synthesizing enzymes in these rats. Moreover, several investigations demonstrated that ethylene glycol treatment increased urinary calcium excretion significantly in urolithiatic rats [5, 16, 39].

It has been reported that administration of ethylene glycol to rats increased excretion of phosphorus in stone formers [8] and hyperoxaluric rats [27, 35]. It seems that increased urinary phosphorus excretion along with oxalate stress provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [28]. However, the phosphorus level was brought down to normal after treatment with aqueous extract of *H. sabdariffa* thereby, reducing the risk of stone formation.

It has also been observed that there is a marked decrease in the glomerular filtration rate (GFR) due to the obstruction of urine outflow by stones in urinary system during urolithiasis, resulting in the accumulation of waste products, particularly the nitrogenous substances such as urea and creatinine in the blood [13]. It has also been reported that there is an increased lipid peroxidation and decreased antioxidant potential in the kidneys of rats supplemented with a calculi-producing diet [29, 36]. Elevated serum levels of urea and creatinine indicate that there is a marked renal damage. However, the curative and prophylactic treatment with aqueous extract of *H. sabdariffa* causes diuresis and hastens the process of dissolving the preformed stones and prevention of new-stone formation in the urinary system. The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR and the anti-lipid peroxidative property of *H. sabdariffa*.

Histopathological investigations revealed the presence of polymorphic irregular calcium oxalate crystals in the lumina of tubules accompanied by edema and cast formation which caused dilation of proximal tubules along with interstitial inflammation in both the ethylene glycol-induced urolithiatic groups. This might attribute to oxalate formation and also caused extensive intertubular hemorrhages and congestion of blood vessels. Atmani et al. have reported that crystal deposits were intensely birefringent, polycrystalline, and arranged in rosette characteristic of calcium oxalate crystals which proved adhesion and retention of pentides within renal tubules. These histological observations support the presence and growth of renal calculi in renal medulla region as observed in human urolithiasis. Supplementation of aqueous extract of *H. sabdariffa* at the doses of 250 and 500 mg/kg body weight revealed the presence of moderate to few crystals along with mild appearance of edema and dilation in tubules, and crystals were present focally indicating the ability of aqueous extract of *H. sabdariffa* to dissolve the pre-formed stones to some extent. Similarly, when anti-urolithiatic drug, cystone or *H. abdariffa* extract was administered at the dose of 750 mg/kg body weight, no crystals were observed in both the cases indicating the ability of extract of *H. sabdariffa* to dissolve pre-formed stones to a greater extent as that of cystone. These histological studies support the calcium oxalate deposition data in kidneys by ethylene glycol induction and its treatment by supplementation with aqueous extract of *H. sabdariffa*.

The results of cytogenetic investigations demonstrated that aqueous extract of *H. sabdariffa* did not produce any type of toxic effects in bone marrow cells in the doses administered. No significant aberrations were observed in the chromosomes of experimental rats. *H. sabdariffa* contains several beneficial compounds which contribute to the overall biological activity of the extract. The best response occurred after administration at a dose of 750 mg/kg body weight.

Conclusions

The present study concludes that administration of aqueous extract of *H. sabdariffa* effectively prevented the development of urolithiasis in male albino rats. These findings support the use of *H. sabdariffa* as an alternative medicine to prevent urolithiasis. However, the mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone-forming constituents. This shows that there is still a wide scope for using plant drugs in the treatment of

urolithiasis, as modern drugs induce side-effects after long-term use and unable to prevent recurrence of kidney stones. Further, rigorous chemical and pharmacological studies on the isolated active components of *H. sabdariffa* may prove rewarding. Thus, these results indicate that the calyces of *H. sabdariffa* are endowed with anti-urolithiatic activity and do not have genotoxic effects. Hence, it can be introduced in clinical practices and medicine in the form of orally administered syrup after further investigations and clinical trials.

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