Short Communication

Antiurolithiatic effect of lithocare against ethylene glycol-induced urolithiasis in Wistar rats

Sumaiya I. Lulat, Yogesh Chand Yadav, R. Balaraman, Rajesh Maheshwari

Department of Pharmacy, Sumandeep Vidyapeeth University, Piparia, Vadodara, Gujarat, India

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Correspondence to:
Dr. R. Balaraman,
E-mail: rbalaraman2000@gmail.com

ABSTRACT

Aim: This study is aimed to investigate the protective effect of Lithocare (LC) (a polyherbal formulation) against ethylene glycol (EG) induced urolithiasis in Wistar rats.
Materials and Methods: The protective effect of LC (400 and 800 mg/kg) was evaluated using EG-induced urolithiasis in rats.
Results: Administration of EG in drinking water resulted in hyperoxaluria, hypocalcemia as well as an increased renal excretion of phosphate. Supplementation with LC significantly reduced the urinary calcium, oxalate, and phosphate excretion dose-dependently. There was a significant reduction in the levels of calcium, oxalate as well as a number of calcium oxalate crystals deposits in the kidney tissue of rats administered with LC in EG-treated rats. There was a significant reduction in creatinine, urea, uric acid, and blood urea nitrogen when LC was administered in EG-treated rats.
Conclusions: From this study, it was concluded that the supplementation of LC protected EG-induced urolithiasis as it reduced the growth of urinary stones. The mechanism underlying this effect might be due to its antioxidant, diuretic, and reduction in stone-forming constituents.

KEY WORDS: Ethylene glycol, lithocare, polyherbal formulation, urolithiasis, Wistar rats

Introduction

Urinary calculi are the third most prevalent disorder of the urinary system. Approximately, 80% of these calculi composed of calcium oxalate.[1] Urine is normally a super-saturated solution, and only some individuals are prone to this disease. One reason for this is the presence of inhibitors of lithogenesis in urine, including macromolecules, citrate, and magnesium.[2] Thus, an imbalance between the promoters such as low urine volume, calcium, oxalate, uric acid, phosphate, and inhibitors may represent a potential factor in lithogenesis. Various therapies including thiazide diuretics and alkali-citrate are being used in an attempt to prevent recurrence, but scientific evidence for their efficacy is less convincing. In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with little side effects.[3]

At present, there are various polyherbal formulations available in the market for the treatment of urolithiasis. Lithocare (LC) is one of the polyherbal formulations (Bacfo Pharmaceuticals India Limited, Noida) that consist of *Crataeva nurvala* (200 mg), *Boerhaavia diffusa* (200 mg), and *Asteracantha longifolia* (100 mg). The dried bark of *C. nurvala* is used the raw drug in traditional systems of medicine in India such as Ayurveda and siddha. The decoction of bark has been internally administered to cure diseases such as renal calculi, dysuria, helminthiasis, inflammations, and abscesses. The decoction exhibits actions such as carminative, laxative, thermogenic, diuretic, lithontriptic, expectorant, and demulcent.[4] *B. diffusa* has diuretic properties[5] and is

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used by diabetics to lower blood sugar,[6] *B. diffusa* has shown antibacterial activity, mainly against Gram-negative bacteria[7] Extracts of *B. diffusa* leaves have shown antioxidant and hepatoprotective properties in pharmacological models.[8] *A. longifolia* has aphrodisiac properties and is a stimulant to the male genital system and beneficial for the treatment of sexual debility, premature ejaculation, and erectile failure. It is also used in the diseases such as urinary stones, rheumatoid arthritis, edema, thirst, and gout. The seeds bestow excellent results in urinary ailments such as dysuria, urinary calculi, and cystitis.[9]

However, so far no scientific study has been reported regarding the antiurolithiatic property of LC (*C. nurvala*, *B. diffusa*, *A. longifolia*). Therefore, this study is aimed to investigate the protective effect of LC against ethylene glycol (EG) induced urolithiasis and its possible underlying mechanisms using male Wistar albino rats.

Materials and Methods

Animals

Adult male Wistar rats weighing between 200 and 250 g were used in this study. The animals had free access to standard rat pellet with water supplied *ad libitum* under strict hygienic conditions. They were housed in standard conditions of temperature (22°C ± 2°C, relative humidity 55% ± 5% and 12 h light/dark cycles) were used. All experimental protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with Committee for the Purpose of Control and Supervision of Experiment on Animal guidelines, Ministry of Social Justice and Empowerment, Government of India. The experiment was conducted in accordance with accepted standard guidelines for the care and use of animals in scientific research.

Acute Toxicity Study

Acute oral toxicity study of LC (a polyherbal formulation) was carried out according to OECD guidelines 425. The acute toxicity study of LC was performed on male Wistar rats. Increasing doses of LC (50, 200, 400, 1000, and 2000 mg/kg) were orally administered to groups of 3 animals for each dose after a 12 h fast. The signs and symptoms associated with LC were observed after 0, 30, 60, 120, 180, and 240 min and then once in a day for the next 14 days. The animals were observed continuously for any mortality, behavior such as general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, and sedation up to 14 days and at the end of the period, the number of survivors were recorded.

Ethylene Glycol-induced Urolithiasis in Rats

EG-induced urolithiasis model was used to assess the antiurolithiatic activity in male albino Wistar rats. Urolithiasis was induced by administration of EG (0.75% w/v, p.o.) in drinking water for 28 days *ad libitum*.[10]

Experimental Design

Six groups of rats ($n = 6$) used, in which Group I (control) was administered carboxyl methyl cellulose (5 ml/kg of 0.5% w/v) for 28 days. Group II (LC treated) was administered LC (800 mg/kg, p.o.) for 28 days. Group III (EG-treated) was administered EG alone (0.75% v/v) in drinking water for 28 days. Group IV (Standard drug treated) EG-treated animals received standard drug cystone (750 mg/kg, p.o.) for 28 days. Group V (LC treated) EG-treated animals received LC (400 mg/kg, p.o.) for 28 days. Group VI (LC treated) EG-treated animals received LC (800 mg/kg, p.o.) for 28 days.

After 28 days, animals were anesthetized under light diethyl ether and withdrawn blood sample from retro-orbital sinus of rats by using glass capillaries for biochemical estimation. The kidneys were retrieved, dissected, washed with saline for estimation of antioxidant enzymes and preserved in a formalin solution (10%) for further histological analysis.

Assessment of Antiurolithiatic Activity

Collection of urine

Urine samples at early morning were collected on 21 and 28 days. One drop of urine was placed on a glass slide for observation of CaOx crystals using a light microscope.

Urine samples (24 h) were collected on the 28th day by keeping the animals in an individual metabolic cage. The animal had free access to drinking water during urine collection period. A drop of concentrated hydrochloric acid was added to the collected urine before being stored at 4°C. The parameters such as urinary output, pH, calcium, uric acid, phosphate, and magnesium were estimated from urine.

Collection of serum

Blood was collected from the retro-orbital plexus of rat under light ether anesthesia, using glass capillaries. Blood was collected in 2 ml eppendorf tubes. After allowing it to clot in open for 15 min, it was centrifuged at 5000 rpm for 20 min for separation of serum. Serum obtained was stored at −20°C until further biochemical parameters estimation such as calcium, creatinine, uric acid, urea, and blood urea nitrogen (BUN).

Kidney Homogenate Analysis

At the end of the experiment, the rats were sacrificed by cervical decapitation and kidneys were excised, isolated. Further, they were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. Half portion of the isolated kidney was kept in crushed ice. They were cross chopped with a surgical scalpel into fine slices and were chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. 10% (w/v) homogenate of the tissues was prepared in 0.1 M Tris hydrochloride buffer (pH 7.4) in a homogenizer at a speed of 2500 rpm. The homogenate was centrifuged at 5000 rpm for 20 min (−4°C) using a cooling centrifuge. The supernatant obtained after centrifuged was used for the estimation of various marker enzymes. Clear supernatant was separated and used to estimate catalase,[11] glutathione (GSH),[12] and malondialdeide (MDA).[13]

Histopathology

After sacrifice, kidney of each group was rapidly dissected out and washed immediately with saline and fixed in 10% phosphate-buffered formalin. Paraffin-embedded specimens were cut into 5 μm-thick sections and stained with hematoxylin and eosin. The sections were examined under the light microscope (Olympus B × 20, Tokyo, Japan) for the presence of histopathological changes and photomicrographs (Olympus DP12 camera, Japan) were taken.

Statistical Analysis

The results were presented as a mean ± standard error of the mean. The difference among data was statistically analyzed using one-way ANOVA followed by the Tukey test to determine the level of significance using (Prism, GraphPad version 5, GraphPad Software, Inc). Differences between the data were considered significant at $P < 0.05$.

Results

The oral administration of LC in rats up to the dose 2000 mg/kg did not show any sign of toxicity, and there was no mortality for 14 days.

The microscopic (×45 and × 100) examination of urine of normal control group did not show any crystal while EG alone treated group exhibited abundant and larger calcium oxalate crystals. Treatment with LC (400 and 800 mg/kg, p.o.) showed less number as well as the size of crystals as compared to EG-treated group [Figure 1].

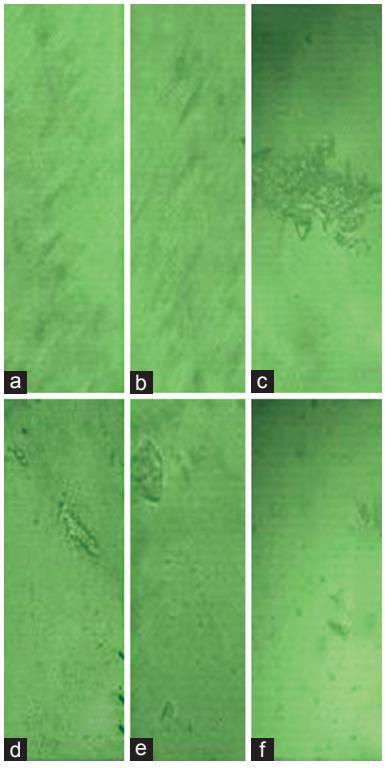
EG-treated group showed a significant ($P < 0.001$) increase in urine output, urine pH, uric acid, calcium, phosphate, urine oxalate, and a decrease in magnesium as compared to control animals. However, treatment with LC (400 and 800 mg/kg, p.o.) showed a significant decrease in urine pH ($P < 0.01$; $P < 0.001$), uric acid ($P < 0.01$; $P < 0.001$), calcium ($P < 0.001$), phosphate ($P < 0.01$; $P < 0.001$), urine oxalate ($P < 0.01$; $P < 0.001$) and an increase in magnesium ($P < 0.001$), urine output ($P < 0.05$; $P < 0.001$), respectively [Table 1 and Figure 2].

EG-treated group showed significant ($P < 0.001$) increase calcium, creatinine, uric acid urea, and BUN levels in serum as compared to normal control group. The treatment with LC (400 and 800 mg/kg, p.o.) exhibited a significant decrease calcium ($P < 0.01$; $P < 0.001$), creatinine ($P < 0.001$), uric acid ($P < 0.05$; $P < 0.001$), urea ($P < 0.001$), and BUN ($P < 0.001$) levels in serum as compared to EG-treated group, while 400 mg/kg treated animals did not show any significant changes in creatinine, urea and BUN as compared to EG-treated rats [Table 2].

In an EG-treated group, there was significant ($P < 0.001$) increase in kidney weight, MDA and a decrease GSH, catalase levels as compared to control group. LC (800 mg/kg, p.o.) treatment showed a significant ($P < 0.001$) reduction in kidney weight, MDA levels and an increase GSH, catalase levels when compared to control group. However, 400 mg/kg treated rats did not show any significant effect on kidney weight, MDA, GSH and catalase in renal tissue [Table 3].

In histopathology study, ethyl glycol treated group showed a mark tubules dilation and crystal deposition. However, treatment with LC (400 and 800 mg/kg, p.o.) significantly reduced tubules dilation and crystal deposition [Figure 3].

Figure 1: Microscopic study of calcium oxalate crystals in rat urine. CaOx crystals, viewed under light microscope (×100), in 24 h urine from (a) control animals shows absence of crystals, (b) only Lithocare treated group (800 mg/kg) shows absence of crystals, (c) the untreated group i.e. ethylene glycol treated shows numerous large crystals in urine, (d) ethylene glycol + cystone and the groups receiving lithocare formulation, (e) ethylene glycol + 400 mg/kg, and (f) ethylene glycol + 800 mg/kg for 28 days shows fewer small CaOx crystals in urine compared to ethylene glycol treated animals



Discussion

In this study, male rats were used for EG-induced urolithiasis. Since, earlier studies showed lesser stone deposition in female rats as compared to male rats.[14,15] EG (0.75%) administration in male Wistar resulted in hyperoxaluria. It was significantly decreased by LC (800 mg/kg) treatment.

LC treated group showed a significant increase in urine output as compared to EG-treated group rats. EG-treated group showed depressed the glomerular filtration rate because of the obstruction to the flow of urine by stones in the urinary system. Due to this, waste products such as urea, creatinine, BUN and uric acid get accumulated in blood indicating a marked renal damage.

EG-treated group showed a significant increase MDA levels and decrease levels of antioxidant potential in renal tissue.

Figure 2: Effect of Lithocare on various urinary parameters in ethylene glycol-induced urolithiasis. Values are expressed as mean ± standard error of mean, (n = 6), one-way ANOVA followed by the Tukey test. Ethylene glycol: Ethylene glycol. ###$P < 0.001$ as compared to control group **$P < 0.01$, ***$P < 0.001$ as compared to ethylene glycol treated group

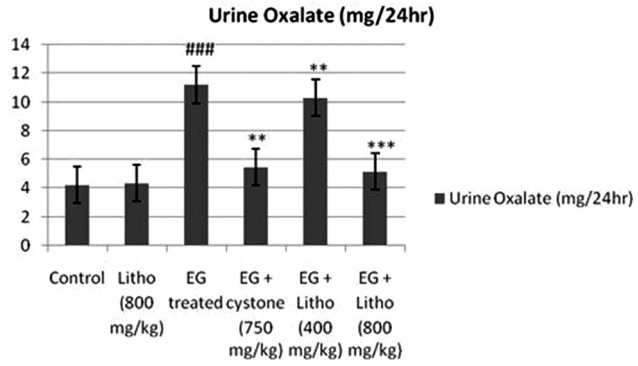


Table 1:

Effect of lithocare on various urinary parameters in ethylene glycol-induced urolithiasis

Groups	Urine output (ml/24 h)	pH	Calcium (mg/24 h)	Uric acid (mg/24 h)	Phosphate (mg/24 h)	Magnesium (mg/24 h)
Control	25.00±0.83	6.47±0.07	3.86±0.48	2.61±0.16	5.68±0.036	3.18±0.02
LC (800 mg/kg)	34.40±1.96	7.14±0.24	4.30±0.18	3.01±0.14	5.23±0.11	4.00±0.28
EG-treated	35.00±0.63###	9.48±0.27###	6.81±0.29###	6.57±0.35###	9.39±0.20###	1.47±0.20###
EG + cystone (750 mg/kg, p.o.)	49.00±1.87***	6.97±0.16***	4.18±0.01***	2.96±0.08***	5.92±0.19***	3.24±0.12***
EG + LC (400 mg/kg, p.o.)	41.40±0.50*	7.21±0.17**	6.73±0.28	5.23±0.28**	7.74±0.48**	2.20±0.20
EG + LC (800 mg/kg, p.o.)	46.80±1.20***	6.76±0.24***	4.23±0.09***	3.21±0.13***	6.05±0.34***	3.35±0.24***

Values are expressed as mean±SEM, (*n*=6), one-way ANOVA followed by the Tukey test. ###*P*<0.001 as compared to control group, **P*<0.05, ***P*<0.01, ****P*<0.001 as compared to ethylene glycol treated group. EG=Ethylene glycol, SEM=Standard error of mean, LC=Lithocare

Table 2:

Effect of lithocare on various serum parameters in ethylene glycol-induced urolithiasis

Groups	Calcium (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	BUN (mg/dL)
Control	6.98±0.86	0.77±0.047	3.78±0.46	28.20±2.88	13.16±1.349
LC (800 mg/kg)	6.31±0.067	1.78±1.055	3.12±0.29	29.48±3.39	13.76±1.58
EG-treated	10.96±0.24###	3.82±0.26###	8.26±1.08###	73.62±8.97###	34.37±4.19###
EG + cystone (750 mg/kg, p.o.)	7.13±0.045***	0.92±0.02***	4.65±0.173***	28.70±0.44***	13.40±0.20***
EG + LC (400 mg/kg, p.o.)	8.52±0.46**	1.48±0.12	5.640±0.30*	69.19±9.01	32.31±4.21
EG + LC (800 mg/kg, p.o.)	7.24±0.099***	0.84±0.02***	4.16±0.23***	37.80±3.73***	17.65±1.74***

Values are expressed as mean±SEM, (*n*=6), one-way ANOVA followed by the Tukey test. ###*P*<0.001 as compared to control group, **P*<0.05, ***P*<0.01, ****P*<0.001 as compared to ethylene glycol treated group. EG=Ethylene glycol, SEM=Standard error of mean, LC=Lithocare, BUN=Blood urea nitrogen

Table 3:

Effect of lithocare on various kidney parameters in ethylene glycol-induced urolithiasis

Groups	Kidney weight (g)	MDA (nmol/ mg of protein)	GSH (nmol/ mg of protein)	Catalase (µmol/ H_2O_2/min)
Control	0.93±0.02	0.59±0.13	7.72±0.24	37.9±0.50
LC (800 mg/kg)	1.05±0.02	0.73±0.10	6.97±0.28	37.8±0.24
EG-treated	2.56±0.35###	4.61±4.61###	4.24±0.10###	16.9±1.81###
EG + cystone (750 mg/kg, p.o.)	1.14±0.05***	1.30±1.30***	7.01±0.04***	29.1±0.47***
EG + LC (400 mg/kg, p.o.)	2.38±0.37	4.06±0.29	4.04±0.12	17.2±0.80
EG + LC (800 mg/kg, p.o.)	0.74±0.22***	2.04±0.18***	6.88±0.04***	26.0±0.69***

Values are expressed as mean±SEM, (*n*=6), one-way ANOVA followed by the Tukey test. ###*P*<0.001 as compared to control group, ****P*<0.001 as compared to ethylene glycol treated group. EG=Ethylene glycol, SEM=Standard error of mean, LC=Lithocare, MDA=Malondialdeide, GSH=Glutathione

Elevated oxalate concentration in urine has been reported to induce lipid peroxidation and cause renal damage by reacting with polyunsaturated fatty acids in the cell membrane.[16,17] In EG-treated rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid that are markers of glomerular and tubular damage. Treatment of LC prevented the elevation of serum levels of these markers and inhibited MDA levels.

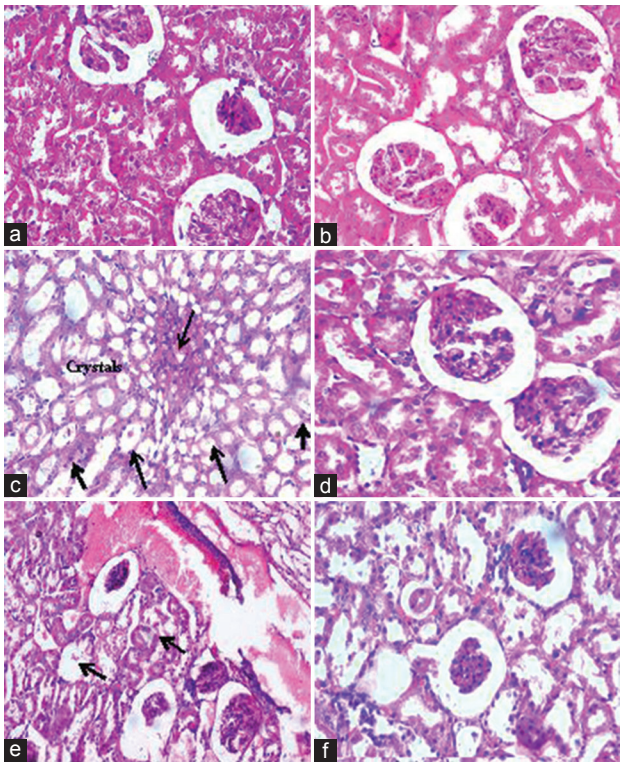
Treatment with LC showed a significant reduction in kidney weight, MDA levels and an increase in GSH, catalase levels indicating a curative effect of LC against EG-induced oxidative stress. This activity could be contributed by LC due to the presence of lupeol[18] which seems to have an antiurolithiatic activity against EG-treated rats. LC treatment groups were shown to decrease intracellular calcium. This might be due to the increased bioavailability of nitric oxide that in turns activate 3, 5' cyclic guanosine monophosphate that controlled the increase in intracellular calcium levels.

Microscopic examination of kidney sections of EG-induced urolithiatic rats showed polymorphic irregular crystal deposits inside the tubules that caused dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Treatment with LC decreased the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

Conclusion

These results indicate that administration of LC reduced and prevented the growth of urinary stones. Therefore, LC was helpful to prevent the early stages of stone development. The mechanism underlying this

Figure 3: Ethyl glycol treated group (c) shown, mark tubules dilation and crystal deposition. Histopathology of treated groups with ethylene glycol + extract (400 mg/kg) and ethylene glycol + extract (800 mg/kg) reduced significantly ethylene glycol-induced urolithiasis. (a) Histopathology sections of kidney plate normal liver, (b) extract treated (800 mg/kg), (c) ethylene glycol treated (0.75% v/v), (d) ethylene glycol + cystone treated (750 mg/kg), (e) ethylene glycol + Lithocare treated (400 mg/kg), and (f) ethylene glycol + Lithocare treated (800 mg/kg). Magnification × 20 for each section



effect was mediated possibly through a good antioxidant, nephroprotective property, and lowering the concentration of urinary stone-forming constituents due synergistic effect of their LC components.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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