

Antiuro lithiatic activity of ethanol leaf extract of *Ipomoea eriocarpa* against ethylene glycol-induced urolithiasis in male Wistar rats

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ABSTRACT

Objective: The objective of this study was to investigate the prophylactic and curative effect of the ethanol leaf extract of *Ipomoea eriocarpa* (*Convolvulaceae*) (IEE) in ethylene glycol-induced urolithiasis in rats.

Materials and Methods: Thirty male Wistar rats were divided into five groups ($n = 6$). All the groups received stone-inducing treatment till 28th day, comprising 1% ethylene glycol (v/v) with 1% ammonium chloride (w/v) for 4 days, followed by 1% ethylene glycol alone in water, except Group I (Control). Group II received only stone-inducing treatment till 28th day. Group III (Standard) received cystone (500 mg/kg) from 15th day till 28th day. Group IV (Prophylactic) received IEE (200 mg/kg) from 1st day till 28th day and Group V (Curative) received IEE (200 mg/kg) from 15th day till 28th day. Various biochemical parameters such as phosphorus, calcium, magnesium, urea, and creatinine levels were evaluated using urine, serum, and kidney homogenate. The kidneys were also sectioned and examined histopathologically under light microscope to study the kidney architecture and calcium oxalate deposits.

Results: The IEE treatment (prophylactic and curative) significantly ($P < 0.001$) restored the parameters in urine, serum, and kidney homogenate to near-normal level. The histopathological examinations revealed that calcium oxalate crystal deposits in the renal tubules and congestion and dilation of the parenchymal blood vessels were significantly reverted after IEE treatment.

Conclusions: The leaf extract of *I. eriocarpa* reduces and inhibits the growth of urinary stones showing its effect as an antiuro lithiatic agent.

KEY WORDS: Calcium oxalate, ethylene glycol, *Ipomoea eriocarpa*, urolithiasis

Introduction

Nephrolithiasis or urolithiasis, commonly known as kidney or renal stone, is a highly prevalent clinical problem that affects about 20% of the human population. A majority of urinary stones are composed of phosphates, oxalates, cystine, and uric acid. Almost 80% of these calculi are composed of calcium oxalate (CaOx).^[1] Kidney stone formation is a complex process which is the outcome of several physio-chemical events such

as supersaturation, nucleation, crystal growth, aggregation, and retention.^[2] Medical treatment and extracorporeal shock wave lithotripsy are extensively used for the removal of calculi. However, continuous exposure to shock waves may cause acute kidney injury, infection and decrease in kidney function, and an increase in stone relapse.^[3] Drug treatment has shown some feasibility, but not without side effects.^[4]

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Several pharmacological and clinical studies on traditional medicinal plants used in the treatment of kidney stones have publicized their therapeutic potential in various *in vitro* as well as *in vivo* models. Various herbal plants such as *Flos carthami*,^[5] *Costus spiralis*,^[6] *Costus igneus*,^[7] *Herniaria hirsuta*,^[8] *Tribulus terrestris*,^[9] and *Scoparia dulcis*^[10] have successfully proved as prophylactic and curative medicine for urolithiasis.

Ipomoea eriocarpa is an annual semi-aquatic plant which belongs to the *Convolvulaceae* family. It is distributed throughout the tropical Asia, South American and African tropical regions, and Northern Australia. The leaves are consumed as a vegetable in the North Eastern and Eastern region of India. In addition, the plant has many unspecified medicinal uses in India. The extract of the plant is used for the treatment of headache, fevers, ulcers, leprosy, rheumatism, and epilepsy.^[11] Scientific investigation of *I. eriocarpa* recently demonstrated antipyretic,^[12] antioxidant,^[13] toxicity, cerebroprotective,^[14] antinociceptive,^[15] antisecretory,^[16] antimicrobial, anthelmintic, insecticidal,^[17] anti-arthritis, and anti-diabetic activities.^[18]

The *in vitro* antiurolithiatic activity of the ethanol leaf extracts of *I. eriocarpa* (IEE) has shown good results^[13] and hence, the present study has been designed to study the *in vivo* antiurolithiatic property of the leaf extract of *I. eriocarpa* on ethylene glycol-induced urolithiasis in male Wistar rats.

Materials and Methods

Plant

The leaves of *I. eriocarpa* were collected from Kamrup and Udalguri district of Assam, during the month of June, 2013. The plant was identified as *I. eriocarpa* at the Botany Department, Gauhati University, Assam, by Dr. P. P. Baruah (Accession number: 18081). The voucher specimen of the plant was deposited at the college for further reference.

Preparation of Extracts

Leaves of *I. eriocarpa* were shade dried and powdered to get coarse granules which were stored in an air tight container in the dark. The 25 g of the powder was subjected to continuous extraction in Soxhlet apparatus using absolute ethanol for 7 h (IEE). The extract (IEE) was filtered and evaporated under reduced pressure to give a viscous mass. The concentrated crude extracts were stored at 4°C in a refrigerator and used for further study.

Animals and Treatment

Thirty male Wistar rats (180–220 g) were used in the present experimental study.^[19] All the experimental procedures and protocols used in this study were approved from the Institutional Animal Ethics Committee (VIT/IAEC10th/March 14th/No. 36). The animals were kept in polypropylene cages and maintained under standardized conditions (temperature 27°C ± 1°C, humidity 60% ± 4%, and natural lighting), fed with a standard diet and water. The rats were divided into five groups, a minimum of six animals were used in each group. Group I served as the control regimen and received regular food and drinking water. Group II–V received stone inducing treatment till 28th day, comprising 1% ethylene glycol (w/v) with 1% ammonium chloride (w/v) for 4 days, followed by 1% ethylene glycol alone in water. Group II received only

stone-inducing treatment till 28th day. Group III served as the standard regimen and received antiurolithiatic drug, cystone (500 mg/kg), from 15th day till 28th day. Group IV and V served as prophylactic and curative regimens, respectively. Group IV received IEE (200 mg/kg) from 1st day till 28th day and Group V received IEE (200 mg/kg) from 15th day till 28th day.

Measurement of Body Weight and Water Intake

Body weight (%) and water intake (24 h) were determined at the end of the 28th day for each group.

Biochemical Analysis

Collection and analysis of urine

Urine samples (24 h) were collected by keeping the rats in metabolic cages. During the urine collection period, animals had free access to drinking water. Urine samples were analyzed for the phosphorus,^[20] calcium,^[21] and magnesium.^[22]

Serum analysis

Blood was collected from the rats in each group after sacrificing the animals, and serum was separated by centrifugation at 6000 rpm for 15 min and analyzed for phosphorus,^[20] calcium,^[21] urea, and creatinine.^[23]

Kidney homogenate analysis

The animals were sacrificed by cervical decapitation and the abdomen was cut open to remove the kidneys from each animal and perfused using phosphate buffer saline. The isolated kidneys were cleaned off and the left kidney was finely minced and 20% homogenate was prepared in Tris–HCl buffer (0.02 mol/L) of pH 7.4. Total kidney homogenate was used for assaying tissue phosphorus,^[20] calcium,^[21] and magnesium.^[22]

Kidney histopathology

The isolated right kidney was preserved in 10% formalin solution. Sections were cut with 5 µm thickness and using 4 Leica RM-2126 microtome, they were mounted on slides after staining with hematoxylin and eosin. The slides were observed under light microscope to study the kidney architecture and CaOx deposits.

Statistical Analysis

Results were expressed in terms of mean ± standard error mean. Differences among data were determined using one-way ANOVA test followed by Dunnett's multiple comparison test (GraphPad Software, Inc., version 5, CA, USA.) and $P < 0.05$ was considered statistically significant.

Results

Measurement of Body Weight and Water Intake

The baseline parameters such as body weight and water intake, recorded before the start of treatment, were almost same for all the groups. The parameters recorded after the treatment are shown in Table 1. The urolithiatic treatment had shown loss in body weight of the animals in the stone-induced group ($P < 0.001$ vs. Group I), whereas the other group animals showed significant gain in their body weights after the experiment. Water intake was not significantly different among the groups except the stone-induced group which was significantly high as compared to the control group ($P < 0.001$ vs. Group I).

Table 1:**Effect of *Ipomoea eriocarpa* on parameters in urolithiatic male Wistar rats**

Parameter (unit)	Group I (control)	Group II (stone-induced)	Group III (standard)	Group IV (prophylactic)	Group V (curative)
Change in body weight (%)	14.92±3.15	-2.76±4.08 ^{*a}	6.79±2.32 ^{*a,b}	8.54±4.14 ^{*a,b}	4.27±2.98 ^{*a,b}
Water intake (mL/24 h)	6.48±1.32	12.97±2.32 ^{*a}	8.76±1.98 ^b	7.87±3.21 ^b	10.45±1.10 ^a
Urine (mg/dl)					
Phosphorus	6.12±0.98	13.12±1.30 ^{*a}	10.21±2.10 ^{*a,‡b}	9.89±1.23 ^{*a,‡b}	10.54±0.23 ^{*a,‡b}
Calcium	8.02±1.22	13.97±0.76 ^{*a}	11.09±1.46 ^{‡a,‡b}	10.98±1.89 ^{‡a,‡b}	11.40±1.40 ^{‡a,‡b}
Magnesium	5.21±1.45	1.24±1.10 ^{*a}	2.34±1.57 ^{‡a}	2.56±0.87 ^{‡a}	1.97±0.87 ^{‡a}
Serum (mg/dl)					
Phosphorus	8.10±2.01	14.02±0.89 ^{*a}	10.78±0.87 ^{‡a,‡b}	10.79±1.67 ^{‡a,‡b}	11.02±0.77 ^{‡a,‡b}
Calcium	8.32±0.83	14.78±0.57 ^{*a}	11.24±1.90 ^{‡a,*b}	11.09±3.21 ^{‡a,*b}	12.09±1.21 ^{*a,‡b}
Urea	13.78±3.02	26.32±2.78 ^{*a}	16.78±1.23 ^{‡a,*b}	17.76±1.35 ^{*a,*b}	18.56±2.00 ^{*a,*b}
Creatinine	0.35±1.40	1.32±1.23	0.87±2.10	0.79±1.78	0.92±2.08
Kidney homogenate (mg/g)					
Phosphorus	2.67±0.78	7.81±0.56 ^{*a}	3.13±1.20 ^b	3.20±2.10 ^b	3.42±1.23 ^b
Calcium	9.79±1.32	16.79±0.87 ^{*a}	12.45±0.89 ^{‡a,*b}	12.34±1.45 ^{‡a,*b}	12.78±0.98 ^{‡a,*b}
Magnesium	3.85±1.50	0.81±1.3 ^{‡a}	2.41±0.76	2.46±0.87	1.91±0.56

Values for urine parameters are measured in 24 h urine sample. All values are stated as mean±SEM (n=6). ^aComparisons are made with Group I, ^bComparisons are made with Group II, ^{*}P<0.001, [‡]P<0.01, [‡]P<0.05. SEM=Standard error of mean

Biochemical Analysis

Urine analysis

The concentration of urine phosphorus, calcium, and magnesium present in Group I–V is shown in Table 1. In the present experiment, administration of 1% ethylene glycol in drinking water to male Wistar rats caused significant ($P < 0.001$ vs. Group I) increase of phosphorus and calcium concentration and decrease in the magnesium concentration in urine of the stone-induced group (Group II). However, treatment with IEE at 200 mg/kg significantly ($P < 0.01$ vs. Group II) reduced the phosphorus and calcium excretion and increased the magnesium excretion in urine in both the prophylactic and curative groups (Group IV and V, respectively) and were comparable to the standard group (Group III, cystone-treated).

Serum analysis

Renal function was evaluated by measuring serum phosphorus, calcium, urea, and creatinine in Group I–V [Table 1]. The concentration of phosphorus, calcium, urea, and creatinine in the serum was significantly ($P < 0.001$ vs. Group I) increased in the stone-induced group indicating renal damage. However, treatment with IEE significantly ($P < 0.001$ vs. Group II) reduced the concentrations of phosphorus, calcium, urea, and creatinine in the serum in both the prophylactic and curative groups to a near-normal level and were comparable to the standard group.

Kidney homogenate analysis

The deposition of crystalline components in the renal tissue, namely phosphorus and calcium was significantly ($P < 0.001$ vs. Group I) increased in the stone-induced group [Table 1], whereas magnesium level was decreased. Treatment with IEE significantly ($P < 0.001$ vs. Group II) reduced the concentrations of phosphorus and calcium and increased the magnesium in both the prophylactic and curative groups to a near-normal level as compared to the standard group.

Kidney histopathology

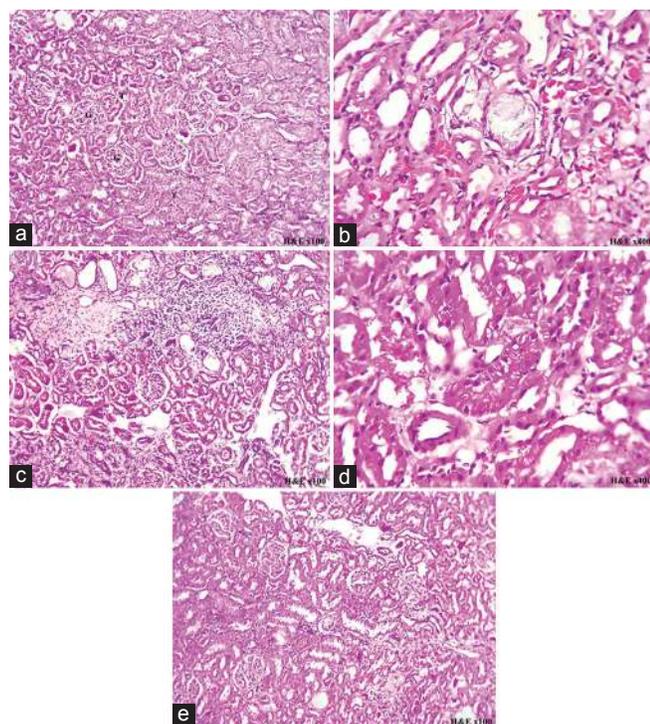
Kidney histopathological analysis revealed no CaOx crystal deposit or other abnormalities in the kidney of the control group (Group I) as shown in Figure 1a. On the other hand, many CaOx crystal deposits in the renal tubules and congestion and dilation of the parenchymal blood vessels were seen in the renal tissue of the stone-induced group (Group II) as shown in Figure 1b. In the standard group (Group III), the kidney showed normal architecture with dilation of tubules in the cortico-medullary junction, minima interstitial inflammation, and occasional renal tubules showed CaOx crystal deposits [Figure 1c]. In the prophylactic group (Group IV), the kidney showed normal architecture and few renal tubules that revealed vacuolar degeneration with no CaOx crystal deposits [Figure 1d]. However, in the curative group (Group V), the kidney showed normal architecture and occasional renal tubules that revealed CaOx crystal deposit [Figure 1e].

Discussion

In the present experiment, male Wistar rats were chosen to induce urolithiasis with 1% ethylene glycol (w/v) with 1% ammonium chloride (w/v). The urinary system of male rats resembles that of humans, and previous studies have proved that stone formation in female rats was considerably less as compared to the male rats.^[19]

It have been reported earlier that ethylene glycol causes hyperphosphaturia, hypercalciuria, and hypomagnesemia leading to urolithiasis.^[19] We also found elevated concentration of phosphorus and calcium and lowered concentration of magnesium in the urine of the stone-induced group [Table 1]. The increased calcium occurs due to excessive tubular damage in the kidney, leading to excretion of intracellular calcium via urine. Hypercalciuria leads to elevated phosphorus leakage. Elevated urinary phosphorus excretion along with oxalate stress appears

Figure 1: (a) Control group (H and E, ×100); (b) stone-induced group (H and E, ×400); (c) standard group (H and E, ×100); (d) prophylactic group (H and E, ×400); (e) curative group (H and E, ×100)



to provide an appropriate environment for stone formation by forming calcium phosphate crystals, which epitaxially causes CaOx deposition.^[19] In the stone-induced group, there was a considerable increase in the levels of phosphorus as compared to the control group. However, during the administration of IEE, the calcium and phosphorus level decreased to a near-normal level in Group IV and V, which proves that IEE is effective in inhibiting hypercalciuria and hyperphosphaturia. Magnesium is considered as a potent inhibitor of CaOx crystals because it decreases supersaturation.^[19,24] Magnesium level was significantly decreased in the stone-induced group as compared to control group, due to super saturation and metabolic acidosis. Administration of IEE restored the magnesium excretion in Group IV and V compared to the stone-induced group. These results were in agreement with other previous reports.^[19,24]

In urolithiasis, the stones in the urinary tract obstruct urine outflow resulting in the decreased glomerular filtration rate. This leads to the deposition of waste products in the blood, mainly nitrogenous substances such as urea and creatinine.^[19] Therefore, in the present experiment, serum concentrations of phosphorus, calcium, urea, and creatinine were determined. As shown in Table 1, the phosphorus, calcium, urea, and creatinine levels in the stone-induced group were higher than the control group. However, the IEE treatments significantly reduced the phosphorus, calcium, urea, and creatinine levels. Our results are compatible with the previous findings.^[24,25] In the stone-induced group, the high level of these parameters was because of the CaOx stone formation in the urinary tract, which led to the deposition of waste products in the blood. However, treatments with IEE inhibited the stone formation and lowered these parameters.

Increase in calcium levels in the renal tissues of the stone-induced group was observed due to accumulation of crystalline material as CaOx. The IEE treatment significantly suppresses this rise in intracellular calcium. Increased phosphorus level and decreased magnesium level were also observed in the renal tissues of the stone-induced group. However, IEE treatment restored these levels in the prophylactic and curative groups. These results were compatible with the previous findings.^[19,25]

The biochemical analyses were also aided by the histopathological examinations of the kidney. Microscopic examinations of the kidney sections of the stone-induced groups showed CaOx crystal deposits in the renal tubules and congestion and dilation of the parenchymal blood vessels. The prophylactic group showed few renal tubules with vacuolar degeneration and no CaOx crystal deposits. Whereas, the curative group showed occasional renal tubules with CaOx crystal deposit.

Conclusions

The results indicate that administration of leaf extract of *I. eriocarpa* reduces and inhibits the growth of urinary stones. It is also seen that the prophylactic effect is more efficient than the curative effect. Therefore, the leaf extract of *I. eriocarpa* is useful to prevent the recurrence of urolithiasis as it proved its effect on the early stages of stone development. The mechanism causing this effect is still unspecified, but is possibly related to increased diuresis and lowering of urinary concentrations of stone-forming components.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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