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Parveen Kumar Goyal
Ph.D., Research Scholar, IKG PTU, Kapurthala-144603, Punjab, India
Department of Pharmacology, Hindu College of Pharmacy, Sonepat-131001, Haryana, India

Santosh Kumar Verma
Department of Pharmaceutical Sciences, CT Institute of Pharmaceutical Sciences, Jalandhar-144020, Punjab, India
Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttrakhand-247661, India

Prof. (Dr.) Anil Kumar Sharma
Former Director, Department of Pharmaceutical Sciences, CT Institute of Pharmaceutical Sciences, Jalandhar, Punjab-144020, India

Correspondence:
Prof. (Dr.) Anil Kumar Sharma
Former Director, Department of Pharmaceutical Sciences, CT Institute of Pharmaceutical Sciences, Jalandhar, Punjab-144020, India
Email: aksharma91@gmail.com

Antilithiatic potential of *Vernonia cinerea* against calcium oxalate calculi in experimental rats

Parveen Kumar Goyal, Santosh Kumar Verma, Anil Kumar Sharma*

ABSTRACT

Objectives: The present manuscript was focused on evaluating the antilithiatic potential of *Vernonia cinerea* extract (VCE) against calcium oxalate calculi using experimental model. **Methods:** The drinking water containing ethylene glycol (0.75% v/v) and ammonium chloride (1% w/v) was used to induce hyperoxaluria in Wistar rats. Thirty-six rats, divided into following six groups (each containing six animals), were treated with vehicle (normal control), ethylene glycol and ammonium chloride (urolithic), Neeri (standard), 100, 300 and 500 mg/kg, VCE (tests). The experimental protocol involved the estimation of different biochemical parameters in urine, serum, kidney homogenates, and histopathological examinations of the kidney. **Results:** The urolithic rats showed the presence of oxalate crystals in renal tubules and significant changes in biochemical parameters like decreased creatinine clearance, increased urinary levels of oxalates, urea, calcium, phosphorus, uric acid, proteins, decreased urinary magnesium levels; increased serum levels of urea nitrogen, uric acid, calcium, phosphorus, lactate dehydrogenase; increased calcium, phosphorus, and oxalate contents in kidney homogenates. It altered the renal architecture and impaired the functions. The extract significantly ($p< 0.05$) reversed the biochemical changes in urine, serum and kidney homogenates in a dose-dependent manner. It improved the renal functions as indicated by improved creatinine clearance, reduced lactate dehydrogenase activity and restoration of renal architecture towards normal. **Conclusions:** *Vernonia cinerea* showed significant antilithiatic potential against oxalate calculi in glycolated rats.

Keywords: *Vernonia cinerea*, Urolithiasis, Hyperoxaluria, Ethylene glycol, Calcium oxalate calculi.

INTRODUCTION

Urolithiasis, the presence of one or more calculi in the urinary tract, is a serious, debilitating problem throughout the world, affecting approximately 12% of the population. It is a recurrent renal disease affecting about 4-8% population in the UK, 15% in the US, 20% in Gulf countries, and 11% in India. It is more prevalent between the ages of 20 to 40 in both sexes [1, 2]. The risk of developing urolithiasis in adults appears to be 5-9% in Europe, 12% in Canada, 13-15% in the USA and 1-5% in the Eastern hemisphere. The highest risks have been reported in Asian countries with lifetime recurrence rates of up to 50% [3, 4]. More than 75% renal stones are composed of calcium oxalate (CaOx) and occur in two forms i.e. CaOx monohydrate (Whewellite) and dihydrate (Weddellite) [4, 5].

Most of the kidney stones, that remained tiny enough, travels through the urinary tract and pass out on its own even without being noticed by the patient. The larger stones that fail to pass through urinary tract require medical help. In the modern system of medicines, renal stones are usually treated by surgical and interventional procedures like percutaneous nephrolithotomy, ureteroscopy, extracorporeal shock wave lithotripsy etc. These surgical procedures are prohibitively costly for the common man, need careful follow-up for a long time and moreover, the recurrence is quite common. The modern system of medicines lacking clinically satisfactory drugs that can be used to dissolve the renal stones or to prevent the stone formation and recurrence; therefore the physicians have to be dependent on alternative medicines. In alternative systems of medicines, many remedies have been employed during the ages to treat urinary stone; and most of these have originated from plants. These herbal remedies have proved to be quite useful, though rationale behind their use is not well established through systematic pharmacological studies except for some composite formulations and few individual plants. In order to obtain the standardized herbal medicines, there is a constant need of exploring the new herbal drugs for their antilithiatic potentials using modern systematic pharmacological techniques.

Vernonia cinerea (L.) Less. (Family: Asteraceae), commonly known as Sahadevi and Purple Fleabane, is an important medicinal plant which chemically contains luteolin, vernolides, amyrins, sterols, terpenes, mucilages, alkaloids, flavonoids, amino acids, etc [6-9]. It has been used as the vital ingredient in many herbal composite formulations like Neeri (Aimil Pharmaceuticals India Ltd.), Cystone (Himalaya Drug Company, India) etc recommended for renal calculi. It has traditionally been used as anodyne, anti-inflammatory, diaphoretic, diuretic, lithotriptic, stomachic, anti-periodic, etc.

It has also been recommended for leucoderma, psoriasis, chronic skin diseases, colic, dysuria and renal calculi [10, 11]. It has been scientifically substantiated for anti-inflammatory [12], antibacterial [13], antifungal [14], antidiarrhoeal [15], antidiabetic [16], antipyretic [17], antioxidant [18], hepatoprotective [19], nephroprotective potentials [20], etc but not for its potential against renal calculi. The present manuscript is aimed at evaluating the antilithiatic potential of *V. cinerea* against oxalate stones in experimental rats.

MATERIALS AND METHODS

Plant material

The dried whole plant of *Vernonia cinerea* (L.) Less., authenticated by the botanist Dr. H. B. Singh, (Ref. No. AIMIL/PD/2015) was obtained as a gift sample from Aimil Pharmaceutical India Limited, New Delhi.

Chemicals and reagents

Ethylene glycol was purchased from Loba Chemie, Mumbai, India. Ammonium chloride was purchased from Thermo Fisher Scientific India Pvt Ltd. Mumbai, India. All diagnostic kits used for estimating the biochemical parameters were obtained from ERBA Diagnostic Mannheim GmbH, Germany. Neeri, the polyherbal antiurolithiatic formulation, was obtained as a gift sample from Aimil Pharmaceutical India Limited, New Delhi. All other chemicals and reagents used were of the at least analytical grade.

Experimental animals

Healthy adult Wistar rats of either sex, weighing between 150-250 g and equivalent age (4-5 months) group, were procured from Panacea Biotec Limited, Lalru (India). They were housed in polypropylene cages (62 cm x 30 cm x 12 cm) and kept in standard laboratory conditions at 25±2 ºC with alternate light and dark cycle of 12 hours each. All the experimental animals were allowed free access to pellet diet and water. Prior to start the experimental protocol, the rats were acclimatized to experimental laboratory conditions for at least one week. The study protocol was duly approved by the Institutional Animal Ethical Committee (IAEC) of CT Institute of Pharmaceutical Sciences, Jalandhar (Punjab), India [Registration No. 1001/PO/a/06/CPCSEA]. The experimental handling and care of animals were taken as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests, Government of India, New Delhi.

Preparation of extract

In order to prepare the hydroethanolic extract of *V. cinerea*, the coarsely powdered drug (100 g) was mixed with 60% ethanol (2500 ml) in a stoppered glass container and kept at 45°C for five days with occasional shaking. Thereafter, the contents were filtered through Whatman No. 1 filter paper. The filtrate so obtained was concentrated under vacuum using a rotary evaporator and the concentrated contents were then dried to constant weight at 45°C in a hot air oven. The dried extract was kept in a sterile glass container and labeled as VCE i.e. *V. cinerea* extract.

Animal model for urolithiasis

Ethylene glycol (EG) and ammonium chloride (AC) induced hyperoxaluria animal model was used to assess the antilithiatic potential. The rats were treated with 0.75% v/v EG and 1.0% w/v AC in drinking water *ad libitum* for first three days to accelerate the lithiasis followed by only 0.75% v/v EG in drinking water for remaining period of six weeks study [21-23].

Experimental design

Thirty-six Wistar rats were randomly divided into six groups (each containing six animals) and designated as:

Group I (Normal control): The rats were maintained on pellet diet and water *ad libitum* for six weeks. The vehicle (10% v/v Tween 80) was orally administered once daily.

Group II (Urolithic control): The rats were treated with drinking water containing 0.75% v/v EG and 1.0% w/v AC for first three days and followed by the treatment with only 0.75% v/v EG containing drinking water *ad libitum* for the remaining period of study. The animals were allowed free access to pellet diet through the whole study tenure. After two weeks of treatment, the vehicle was orally administered once daily for remaining four weeks period.

Group III (Standard groups): The rats were treated similarly as in the urolithic control group II for first two weeks. After two weeks of treatment, the standard drug Neeri (500 mg/kg; p.o.) was administered to the animals for remaining four weeks.

Group IV to VI (Test groups): For first two weeks, the rats were treated similarly as in group II. After two weeks of treatment, the rats of different groups were administered with VCE (100, 300 and 500 mg/kg; p.o. respectively) for next four weeks.

All the extracts were emulsified in 10% v/v Tween 80. After two weeks, all the animals of group II-VI were individually kept in metabolic cages for 24 hours and urine samples were collected. The urine samples were microscopically analyzed for the presence of CaOx crystals. The animals showing the crystals of CaOx in urine were selected for further study.

Biochemical analysis

Collection and analysis of urine

After the completion of six weeks experimental period, the animals were individually kept in metabolic cages for 24 hours and urine samples were collected. The volume and pH of collected urine samples were measured. The urine volumes were divided into two parts. One part was acidified with 1-2 drops of 1N hydrochloric acid. Both, the acidified and non-acidified samples of urine were centrifuged at 1500 rpm for 10 minutes to remove the debris. The calcium, magnesium and phosphorus contents were estimated in acidified urine using standard reagents kits (Erba Diagnostics Mannheim, GmbH, Germany). The non-acidified urine sample was subjected to estimate the oxalate contents by Hodgkinson's method [24]. The urinary creatinine, urea, uric acid, and total proteins contents in the non-acidified sample were also estimated using commercially available standard reagent kits (Erba Diagnostics Mannheim, GmbH, Germany).

Serum analysis

After collecting the urine, the blood samples were collected from retro-orbital sinus of each animal and serum was separated by centrifugation at 2000 rpm for 10 minutes. The serum was then quantitatively analyzed for creatinine, urea nitrogen, uric acid, calcium, magnesium, phosphorus, and lactate dehydrogenase (LDH) using commercially available standard reagent kits (Erba Diagnostics Mannheim, GmbH, Germany).

Kidney tissue homogenate analysis

After collecting the blood samples, the animals were sacrificed by cervical dislocation. The abdomen was cut opened and both the kidneys were carefully isolated. The isolated kidneys were cleaned of the extraneous tissues in ice-cold saline and weighed. One kidney was preserved in 10% buffered neutral formalin for the purpose of histological studies. The other kidney was sliced into two equal halves. One-half was dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1.0 N hydrochloric acid for about 30 minutes and homogenized. The homogenate was then centrifuged at 2000g for 10 minutes and the supernatant was separated [25-27]. The supernatant was used for estimating the calcium and phosphorus contents with commercially available kits (Erba Diagnostics Mannheim, GmbH, Germany). The oxalate contents were measured by Hodgkinson's method [24]. The other half of kidney was minced and a 10% homogenate was prepared in tris-HCl buffer pH 7.4 [28, 29]. The homogenate was then used for estimating the LDH level with commercially available reagent kits (Erba Diagnostics Mannheim, GmbH, Germany).

Histopathological studies

The kidneys, fixed in 10% buffered formalin (pH 7.0) solution, were dehydrated with ascending grades of ethanol and embedded in paraffin. The 4-6 µm thick sections of paraffin kidney were cut, mounted on slides, deparaffinised and rehydrated with descending grades of ethanol. After staining with hematoxylin and eosin, the kidney sections were examined for the presence of calcium oxalate crystals and various pathological changes like tubular necrosis, glomerular and tubular architecture etc [30, 31].

Data Analysis

All the values were expressed as mean ± SEM (standard error mean). The data were analyzed by employing One-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism 5.0 software. The values of $p<0.05$ were considered as statistically significant.

RESULTS

Urine analysis

The effects of VCE on different urinary parameters were represented in Table 1. The EG and AC administration in drinking water induced urolithiasis and showed marked elevation in urinary levels of oxalates, calcium, phosphorus, urea, uric acid and proteins in lithic rats (group II) when compared with normal control (group I). It acidified the urine, lowered the urine volume and magnesium (stone inhibiting factor) level. The VCE (100, 300, 500 mg/kg, p.o.) significantly reversed these urinary changes in a dose-dependent manner and restored towards normal ranges. The results were also found to be comparable with a standard herbal formulation Neeri as depicted in Table 1.

Serum analysis

The rats in urolithic group showed increased serum levels of calcium, phosphorus, urea, urea nitrogen, uric acid and marker enzyme of renal injury i.e. LDH. The creatinine clearance was also found to be prominently altered which in turn elevated the serum creatinine level. The serum magnesium level was found to be lower when compared with that of the control group. The VCE, in a dose-dependent manner, significantly reversed the above mentioned biochemical changes towards normal ranges and depicted in Table 2. The effects of VCE were found to be comparable with the standard drug. It increased creatinine clearance and improved the renal functions. The reduced LDH level in VCE treated groups also indicated that it might restore the normal architecture of renal tubules by treating the renal injury.

Kidney tissue homogenate analysis

The analysis of kidney tissues homogenate showed the marked increase in the level of oxalates, calcium, and phosphorus in urolithiasis induced animals. The VCE significantly decreased the oxalate level in a dose-dependent manner. It restored the altered levels of calcium and phosphorus towards normal ranges. It significantly ($p< 0.05$) reduced the LDH activity in kidney. The effects of VCE were found to be comparable with that of standard and shown in Table 3.

Table 1: Effects of VCE on various urinary biochemical parameters in lithiatic rats

Parameters	Group I	Group II$^{\$}$	Group III$^{\#}$	Group IV$^{\#}$	Group V$^{\#}$	Group VI$^{\#}$
Ur. Vol. (ml/24hrs)	19.70±0.71	10.62±0.42***	21.22±0.68***	13.75±0.65	16.73±1.02***	17.68±0.95***
pH	7.77±0.27	6.25±0.12***	8.25±0.14***	7.35±0.07***	7.33±0.02***	7.83±0.24***
Oxalate (mg/dl)	0.34±0.05	4.82±0.42***	0.87±0.13***	3.08±0.19***	2.18±0.25***	0.94±0.06***
Ca (mg/dl)	1.19±0.17	4.86±0.42***	0.33±0.08***	2.86±0.44**	2.47±0.36***	1.44±0.31***
Mg (mg/dl)	4.70±0.10	3.62±0.15***	4.94±0.17***	4.71±0.04***	5.06±0.06***	5.01±0.13***
P (mg/dl)	1.37±0.10	3.92±0.20***	0.90±0.06***	3.01±0.15*	2.46±0.20***	1.90±0.27***
Urea (mg/dl)	476.87±16.17	925.53±22.26***	314.88±32.94***	775.47±21.10**	634.28±12.37***	486.05±28.26***
UAC (mg/dl)	2.19±0.14	9.97±0.58***	3.24±0.15***	6.40±0.39***	4.91±0.11***	3.62±0.22***
Proteins (g/dl)	0.13±0.02	0.79±0.04***	0.14±0.02***	0.48±0.07***	0.33±0.03***	0.28±0.03***

$^{\$}$ compared with normal control (Group I).

$^{\#}$ compared with lithiatic control (Group II).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Ur. Vol. - Urine volume, Ox - Oxalate, Ca - Calcium, Mg - Magnesium, P - Phosphorus, UAC - Uric acid

Table 2: Effects of VCE on various biochemical parameters in serum of lithiatic rats

Parameters	Group I	Group II$	Group III#	Group IV#	Group V#	Group VI#
Ca (mg/dl)	8.78±0.38	14.09±0.88***	7.22±0.39***	9.87±0.43***	9.24±0.24***	8.04±0.33***
Mg (mg/dl)	2.11±0.23	1.38±0.06	3.24±0.20***	1.76±0.10	2.47±0.22**	2.47±0.29**
P (mg/dl)	4.71±0.27	7.44±0.38***	4.04±0.28***	6.14±0.17*	5.10±0.17***	4.70±0.22***
UAC (mg/dl)	0.39±0.03	2.04±0.27***	0.46±0.07***	0.54±0.10***	0.58±0.03***	0.42±0.04***
BUN (mg/dl)	19.66±0.87	48.86±3.77***	16.56±0.74***	31.31±1.59***	25.65±0.52***	21.61±1.01***
Cre (mg/dl)	0.46±0.08	1.56±0.11***	0.52±0.04***	0.99±0.05***	0.87±0.06***	0.68±0.04***
Cre Clr (mg/min.)	0.59±0.04	0.42±0.03***	0.68±0.05***	0.59±0.04***	0.60±0.05***	0.67±0.06***
LDH (U/l)	848.92±108.2	2396.34±217.5***	1014.26±98.7***	1938.64±186.6***	1576.20±144.6***	1225.74±106.2***

$ compared with normal control (Group I).
# compared with lithiatic control (Group II).
* p<0.05, ** p<0.01, *** p<0.001
Ca - Calcium, Mg - Magnesium, P - Phosphorus, UAC - Uric acid, BUN - Blood urea nitrogen, Cre - Creatinine, Cre clr - Creatinine clearance LDH - Lactate dehydrogenase

Table 3: Effects of VCE on biochemical parameters in kidney homogenates of lithiatic rats

Parameters	Group I	Group II$	Group III#	Group IV#	Group V#	Group VI#
Oxalate (mg/g)	2.8±0.19	7.34±0.57***	3.3±0.26***	5.13±0.47***	4.37±0.41***	3.83±0.25***
Ca (mg/g)	4.34±0.37	11.7±0.89***	4.92±0.51***	8.15±0.76***	6.84±0.51***	5.43±0.49***
P (mg/g)	1.49±0.18	4.63±0.52***	1.77±0.24***	4.11±0.52	3.46±0.51***	2.12±0.32***
LDH (U/g)	1.98±0.26	5.04±0.44***	2.88±0.32***	4.76±0.38	3.72±0.28***	3.15±0.26***

$ compared with normal control (Group I).
# compared with urolithic control (Group II).
*** p<0.001
Ca - Calcium, P - Phosphorus, LDH - Lactate dehydrogenase

Histopathological studies

The examinations of images of renal histopathological slides, taken at 400 x under light microscope equipped with a digital camera, shown in figure 1 were found to support the results obtained in the biochemical analysis of urine, serum, and kidney homogenates. The rats of the control group (Fig.1 A) showed normal renal architecture without any crystal deposition. In the urolithic rats, large sized crystals of CaOx were observed along with glomerular atrophy, damaged tubular cells and deranged renal organizations (Fig. 1 B). The VCE treated groups (IV, V, and VI shown in Fig. 1 D, E, and F respectively) significantly restored the normal renal architecture in a dose-dependent manner when compared with lithiatic group. No CaOx crystals were observed, the cellular organizations of proximal and distilled convoluted tubules were restored. The normal glomerular sizes and renal architecture were recovered. The effects of VCE were found to be comparable with that of standard Neeri as shown in figure 1 C.

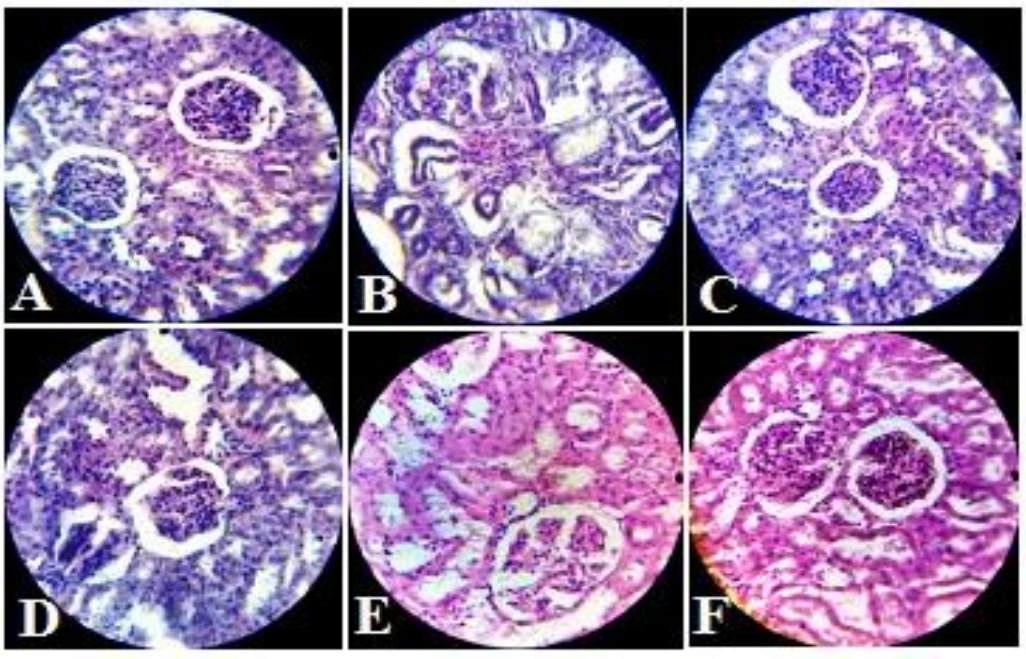

Figure 1: Histological images (400 x) of rat kidney sections of different groups. **(A)** Normal control treated with vehicle only. **(B)** Lithiatic control treated with EG and AC. **(C)** Standard group treated with Neeri. **(D-E)** Test groups treated with 100, 300, and 500 mg/kg VCE respectively.

DISCUSSION

For pre-clinical antilithiatic evaluation, EG and AC-induced urolithiasis is the highly reliable and most commonly used animal model. Rats are the most commonly employed experimental animals for studying CaOx renal stones as the oxalate metabolism in rats is considered almost similar to that of human beings [22]. EG, when administered to rats in drinking water, is readily absorbed, and increase the substrate availability for oxalate synthesizing enzymes like glycolic acid oxidase (GAO) and LDH in rats. By substrate mediated induction of GAO in liver and LDH in liver and kidney, the hyperoxaluria occurs which is one of the major risk factors for CaOx urolithiasis [29, 32]. The oxalates, as poorly soluble, readily precipitate as CaOx and damage the epithelial lining of renal tubules that leads to adhesion and retention of crystals [33, 34]. Furthermore, the administration of AC along with EG causes the acidification of urine and supposed to support urolithiasis [35]. In light of the above context, we employed EG and AC induced urolithiasis animal model for evaluating the antilithiatic potential of VCE.

The supersaturation of urine, the primary requirement for urinary crystallization and stone formation, mainly depends on urinary pH, solute concentration, and complexation [31, 36]. In the present study, administration of EG and AC in drinking water to rats significantly decreased the urine volume and increased oxalate concentration that favored the crystallization of oxalates by supersaturating the urine. The hyperoxaluria, increased concentration of urinary oxalates, is considered as the major risk factor of CaOx urolithiasis. It is usually associated with renal cell injury that facilitates the adherence and retention of oxalate crystals. It also alters the cell membrane integrity, increases the production of free radical and decreases the antioxidant levels that enhance the cell death [37, 38]. Furthermore, it also acidified the urine and considered to facilitate the stone formation [39]. The VCE, when compared with urolithic rats (group II), significantly prevented the supersaturation of urine by increasing urine volume, decreasing oxalate concentrations and increasing the urinary pH. The increased urine volume also facilitated the mechanical expulsion of tiny stones. Furthermore, when compared with lithiatic group, the VCE significantly reduced the oxalate levels in kidney tissue homogenates which also supported its inhibitory potential against hyperoxaluria.

The urinary crystallization of CaOx and stone formation is considered to be affected by various stone promoting and inhibiting factors. The calcium and phosphates are considered as stone promoting inorganic factors while the magnesium is stone inhibiting [40]. Hypercalciuria favors the nucleation and precipitation of CaOx in urine that leads to crystal growth [41]. Hypercalcemia was also reported in urolithiasis patients [42]. Increased urinary phosphorus level has also been reported in hyperoxaluric rats [43]. In the present study, elevated levels of calcium and phosphorus in both urine and serum were observed in urolithic rats when compared with that of normal control. The VCE significantly decreased the calcium and phosphorus in urine as well as serum. It also increased the urine and serum magnesium levels which were also reduced in lithic animals as shown in Table 1 and 2. Multiple cations, in hyperoxaluric condition, tend to complex with oxalates to form urinary salts of oxalate. The magnesium salts are soluble while the calcium salts are insoluble and facilitate the precipitation of calculi of CaOx [44]. The VCE also significantly decreased the calcium and phosphorus levels in kidney tissue homogenates as expressed in Table 3 which support the antilithiatic potentials.

Once the crystals of CaOx produced, they agglomerate, tend to retain in renal tubules and develop into stones. The stones then tend to damage the renal tissues, obstruct the urine outflow, deteriorate the renal functions and decrease the glomerular filtration rate that facilitates the accumulation of various waste products especially non-protein nitrogen (NPN) substances in blood [31, 45, 46]. In the present study, lithiatic rats showed increased serum levels of uric acid, blood urea nitrogen (BUN), creatinine and decreased the creatinine clearance as depicted in Table 2. The increased BUN, serum creatinine, and reduced creatinine clearance are the markers of tubular and glomerular damage in the kidney. These changes evident the significant impairment of renal functions [45]. Uric acid is also reported to promote the growth of CaOx crystals [47]. The VCE significantly decreased the BUN and serum uric acid level in a dose-dependent manner. It also lowered the elevated uric acid and urea levels in urine towards normal. It improved the creatinine clearance and reduced the serum creatinine levels. The VCE, when compared with lithiatic rats (group II), appreciably restored the renal functions towards normal.

The EG increased the activity of LDH which is an oxalate synthesizing enzyme present in the liver and kidney. It is also considered as the marker enzyme of cellular injury. It is released when any injury to a cell or cell membrane, which leads to cell death, occurs [48]. The increased LDH activity indicated the increased oxalate synthesis as well as the enhanced injury of renal epithelial cells. The raised serum LDH level was observed in hyperoxaluric rats (group II). The VCE significantly reduced the LDH levels in serum and kidney tissue homogenates, inhibited the oxalate synthesis and injury to epithelial cells.

In the present study, proteinuria was observed in EG and AC treated rats (group II) which indicated the dysfunctions of proximal tubules [49]. The VCE, when compared with the lithiatic group, significantly lowered the urinary level of proteins in a dose-dependent manner and improved the tubular functions.

The examination of histological slides also supported the results of biochemical changes in the urine and serum. The lithiatic group showed the deposition of oxalate crystals in renal tubules, shrinkage of glomeruli, and altered renal architecture. The oxalate crystals are usually associated with injury of renal epithelial cells that facilitate the adhesion and tubular retention of crystals, as the injury to epithelial cells exposes the cell surface to a variety of crystal adhesion molecules [50]. This also alters the integrity of cell membrane, increases the free radical production and decreases the antioxidant levels that enhance the cell death. The interactions between injured tubular epithelial cells and oxalate crystals are considered to have a significant role in urolithiasis [37, 38]. The VCE significantly restored the normal cellular organization, renal architecture, and renal functions. No crystal deposition was observed, and cellular integrity was recovered.

CONCLUSION

The present study concluded that the VCE exhibited significant antilithiatic potentials against CaOx calculi in EG intoxicated rats. It significantly restored the EG mediated biochemical changes in different parameters of urine, serum, and kidney tissue homogenates towards normal ranges. It reinstated the normal renal architecture and improved the renal functions.

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Conflict of Interest

The authors declared no conflict of interests.

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