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Antinephrolithiatic activity of *Ananas comosus* extract against experimentally induced renal calculi in rats

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Abstract: The present study evaluates the prophylactic role of *Ananas comosus* ethanolic extract (ACEE) against sodium oxalate (NaOx) - induced nephrolithiasis. Forty two rats were allocated into the following set of groups (6 rats/set group). Normal rats divided to two groups, one of them received distilled water (Control group) and the other received ACEE (1000 mg/kg body weight, p.o) for 7 consecutive days. Urolithiatic rat groups which divided into five subgroups injected with sodium oxalate (70 mg NaOx /kg body weight, i.p) for 7 days; and concurrently received oral administration of distilled water (Urolithiatic group, Vehicle), ACEE and Cystone. Interestingly, ACEE showed a beneficial effect in preventing stone formation. Significant reductions were obtained in the urinary and serum calcium and phosphate excretion along with an increase in magnesium excretion in urolithiatic rats treated with ACEE. Urolithiatic rats treated with ACEE and cystone significantly increased the urinary volume. Administration of ACEE caused significant amelioration in renal function which suggests antilithiatic activity of ACEE. Moreover, urolithiatic rats treated with ACEE significantly attenuated oxidative damage induced by NaOx. In conclusion, ACEE has antilithiatic efficacy may be due to its diuretic activity, antioxidant activity, beside its bioactive constituents which affecting calcium oxalate crystallization.

Keywords: *Ananas comosus* ethanolic extract, nephrolithiasis, sodium oxalate, urinary stone, oxidative stress.

INTRODUCTION

Urinary stone (calculi) formation is one of the most abundant urologic illnesses that occur in approximately 12% of the global population (Gilhotra & Christina, 2011; Sathya & Kokilavani, 2012). First urinary stones formation back to 4800 B.C. in an Egyptian mummy (E1 Amrah Egypt). Urinary calculi are hardened mineral deposits formed in kidney, bladder and ureters and termed as urolithiasis (Vidhya *et al*., 2013). But, the stones that formed in the kidney known as a renal calculus and termed as nephrolithiasis. Kidney stone arises from an imbalance between the kidney's requirement to reservation fluid and the need to extrude waste products of low solubility. Thereby, the major causative factor for stone formation is the supersaturation of precipitating salts in the urine (Janapareddi *et al*., 2013). Urine is typically supersaturated with most stone making salt compounds, but it holds chemicals that prevent crystal formation in the urinary tract (Vennila & Mariyal, 2015). Calcium nephrolithiasis is the most common form of renal stone syndrome as calcium oxalate (CaOx) is the chief crystalline component of renal stones where it represents 75% of all renal stones (Lee *et al*., 2012).

Interaction between renal epithelial cells and CaOx crystals stimulate the reactive oxygen species (ROS) generation (Umekawa *et al*., 2009). Tsujihata *et al*. (2006) clarified that CaOx crystals prompt renal epithelial cell damage through lipid peroxidation. In fact, oxalate disturbs the electron transport chain in the mitochondria and so persuades the leakage of free radicals (Jonassen *et al*., 2004). Normally, cells restricts the over production of free radical through the development of antioxidant defense system. But, in nephrolithiasis case oxidative stress (OxS) increased as a result of antioxidant system dysfunction (Jonassen *et al*., 2003).

Open surgery used for the lithiatic treatment has steadily vanished in the last 30 years and substituted by minimal invasive techniques such as extracorporeal shock wave lithotripsy (ESWL) or ureteroscopy (URS). The ESWL and URS tools considered as revolutionized in the nephrolithiasis treatment (Vennila & Mariyal, 2015). However, these techniques are not the satisfactory method for complete cure of nephrolithiasis yet. Since, they are expensive, have several adverse effects including renal injury and/or renal dysfunction as well as increase in stone recurrence (Devkar *et al*., 2016).

Despite enormous progresses drug remedy, there is no sincerely acceptable medication regardless to those medications about renal calculi. The recent resurgence of phytotherapy comes from several factors such as effectiveness of plant medicines, lesser side effects and even supplementation compared to modern medicines. Indeed, the World Health Organization has also paid importance to the use of herbal drugs (Rathod *et al*., 2012). Previous studies explored some natural products that can act as a promising candidate for the prevention and treatment of lithiasis (Das&Malipeddi, 2016 and Dinnimath *et al*., 2017). This may be due to their phyto-

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constituents that act by multiple mechanisms like diuretic activity, crystallization inhibition activity, lithotriptic activity, antioxidant activity, antimicrobial activity, analgesic and anti-inflammatory activity (Vennila & Mariyal, 2015). *Ananas comosus* is the third most important fruit crop in the tropical and subtropical regions of the world after banana and citrus (Lu *et al*., 2014). Its antioxidant activity was reported (Lu *et al*., 2014) and it has the major mineral constituents such as potassium, magnesium, and citrate that help in the renal stone inhibition (Chutipongtanate *et al*., 2012 and Lu *et al*., 2014).

Thereby, the present study aimed to evaluate a prophylactic role of the *Ananas comosus* ethanolic extract (ACEE) as a prophylactic agent against sodium oxalate (NaOx)-induced nephrolithiasis in rats and compared its efficacy with cystone, which is a standard antilithiatic drug.

MATERIAL AND METHODS

Drug and chemicals

Cystone was purchased from local pharmacy, sodium oxalate (NaOx) was purchased from El-Gomhoria chemical industry (Egypt). Kits for all biochemical parameters were procured via Biodiagnostic (El Moror St., Dokki, Egypt) and Spectrum companies.

Preparation of Ananas comosus ethanolic extract (ACEE)

Ripe mature *Ananas comosus* fruit was purchased from local vegetable markets of Egypt, chopped into small pieces, shade dried and coarsely powdered. About 300g of *A. comosus* powder was taken in a beaker and 1L of ethanol was added and kept in the shaker for 24h then filtered. The extract obtained was evaporated to dryness in rotary evaporator and used for various evaluations.

Experimental design and protocol

Forty two rats were separated into the following groups (6 rats/group). (I) Normal rats received (i) distilled water (control group) or (ii) *Ananas comosus* ethanolic extract (1000 mg/kg body weight) (ACEE group) orally for 7 days. (II)Urolithiatic groups; rats injected with sodium oxalate, NaOx (70 mg/kg body weight) i.p. for 7 days and concurrently received oral administration of (i) distilled water (urolithiatic group, vehicle), (ii) 500 mg ACEE, (iii) 750 mg ACEE, (iv) 1000 mg ACEE/kg body weight, and (v) standard antilithiatic drug, cystone (750 mg/kg body weight) group. All doses of ACEE and standard drug were suspended in distilled water and given by oral route.

In vitro antioxidant test

To assess the antioxidant capacity of ACEE; the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition efficacy was done according to Brand *et al*. (1995). The antioxidant capacity of ACEE was compared with ascorbic acid as a reference standard and different concentrations (10-80mg/ml) were prepared for each one.

Animals

Male Wistar albino rats (*Rattus norvegicus*) ranged between 130-150g were purchased from the National Research Center (Egypt). Only male rats were designated to induce urolithiasis as the urinary system of male rats mimic that of the human being system, additionally, the stone deposition in female rats can be neglected (Mitra *et al*., 1998). Animals were housed for 7 days in standard environmental conditions (22±1ºC, humidity 60±5%, and natural day/night cycle) and free access to chow and tap water. Experiment was performed according to the guidelines of the Care and Use of Laboratory Animals (8^{th}edition) and the study was approved by the Institutional Animal Care and Use Committee (IACUC) (CUFS/S/PHY/25/14) of the Faculty of Science, Cairo University, Egypt.

Induction of experimental urolithiasis

To induce urolithiasis, 70 mg sodium oxalate (NaOx) / kg body weight was injected intraperitoneally for 7 days to the rats (İlhan *et al*., 2014).

Animal handling and specimen collection

At the termination of the experimental period, rats were weighed and each one kept individually in metabolic cages for 24h and a urine sample was collected. Urine was freed from fecal contamination. Animals had free access to drinking water during the urine collection period. After urine collection, the animals were euthanized and blood was collected by heart puncture. Blood was centrifuged for 10 minute at 3000 rpm for serum preparation. Two kidneys were excised from each animal, weighed relative to its rat body weight. The right kidney immediately blotted by filter paper to remove traces of blood and stored at -80^{o}C for biochemical analysis. The left kidney fixed in 10% formalin and used for histopathological examination.

Urine analysis

The urine volume was measured by using the measuring cylinder. Furthermore, pH of urine was measured using Mission Urinalysis Reagent Strips. The collected urine samples were centrifuged for 10 minutes and the urine supernatant was used for analysis of calcium, phosphorus, magnesium, creatinine, urea, and uric acid using Biodiagnostic kits (Dokki, Giza, Egypt).

Serum analysis

The collected serum was used for kidney function markers such as creatinine (Bartles *et al*., 1972), urea, uric acid, and blood urea nitrogen (BUN) or urinary urea nitrogen (UUN) (Tietz *et al*., 1990). As well as calcium,

phosphorus, and magnesium were analyzed in serum using Biodiagnostic kits according to the methods adopted by Gindler and King (1972); El-Mezabani *et al*. (1977); Grindler *et al*. (1971), respectively.

Kidney homogenate preparation

A 10% kidney tissue homogenate was prepared using Tris-HCl buffer (0.1M, pH 7.4). The homogenate was centrifuged at 860×g for 15min. at 4^{o}C and the obtained supernatant was used to determine the oxidant/antioxidant markers.

Determination of oxidative/antioxidative parameters

MDA concentration is a key indicator for lipid peroxidation and it was estimated by Ohkawa *et al*. (1979), glutathione reduced (GSH) (Beutler *et al*., 1963), superoxide dismutase (SOD) (Nishikimi *et al*., 1972), catalase (Aebi, 1984), and glutathione- S- Transferase (GST) (Habig *et al*., 1974) were determined in kidney supernatant according to manufactures instructions using Bio-diagnostic kits (Giza, Egypt).

Kidney histopathological examination

For microscopic evaluation, the 10% formalin fixed left kidneys of all groups, dehydrated in a gradient of ethanol, embedded in paraffin, and then cut into 5µ serial sections. Then the slides were deparaffinized, stained with hematoxylin and eosin, and then examined under a light microscope to detect the aggregations of CaOx crystal deposits in the renal tubules.

STATISTICAL ANALYSIS

Results are presented as mean values±SEM of six animals per group. Statistical differences between groups were statistically analyzed using one-way ANOVA followed by Duncan's test. A p value < 0.05 considered statistically significant. SPSS software (version 20) was used for statistical computations.

RESULTS

Anti-free radical activity of Ananas comosus extract

ACEE seems to have anti free-radical activity as it can scavenge DPPH free radical equivalent to standard scavenger (Ascorbic acid). ACEE has the highest DPPH scavenging activity at 10 mg/ml as at this concentration it can inhibit 99.28% of DPPH free radical whereas the % inhibition of ascorbic acid was 95.6% (fig. 1).

Urinary output and pH

Table 1 shows data of some physical parameters of urine obtained at the completion of the experiment/group. The present study revealed that non-significant change in the urinary volume was observed between control and ACEE (1000 mg/kg body weight). But, the urinary output of NaOx (urolithiatic) group was significantly decreased (P<0.05), as compared with control rats. Whereas the urine volume of urolithiatic rats treated with ACEE (500, 750, and 1000 mg/kg body weight) have significantly increased (P<0.05), as compared with untreated urolithiatic one. Additionally, cystone increased urinary volume of urolithiatic rats significantly (P<0.05). Urine pH in control normal rats was found to be acidic. On the induction of urolithiasis, pH becomes slightly alkaline in untreated urolithiatic rats as compared with the control rats. After completion of the study, urolithiatic groups treated with ACEE at the different selected doses and cystone have restored urinary pH to acidic when compared to the respective untreated group.

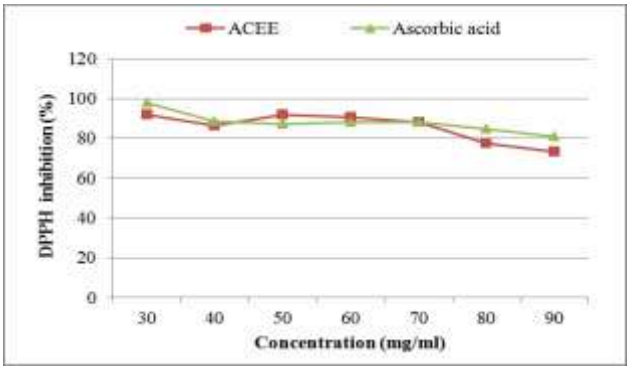

Fig. 1: DPPH radical scavenging activity of ACEE.

Another important finding obtained by light microscopy examination is the formation of urinary pyramidal-shape calcium oxalate dehydrate crystals (arrows) in the urolithiatic group (fig. 2 C). It is appeared that these crystals were prevented to form or dissoluted by ACEE administration (fig. 2 D-F). Similarly, cystone prevent the formation of crystals (fig. 2 G). This finding gives an indication about the ability of ACEE to prohibit crystal formation as standard antilithiatic drug, cystone.

Effect of ACEE on urinary markers

Significant decrease (P<0.05) in urinary calcium, and creatinine levels was observed in the ACEE group as compared to control one (table 2). Excretion of calcium and phosphate was grossly increased (P<0.05) in urolithiatic rats, as compared with control rats. Conversely, the level of magnesium in urine reduced significantly (P<0.05) in urolithiatic rats relative to control level. Supplementation with ACEE to urolithiatic rats modulated the altered levels of calcium, phosphate and magnesium significantly (P<0.05) when compared with untreated urolithiatic rats. However, significant rise (P<0.05) in urinary creatinine, urea, uric acid levels was revealed in the urolithiatic group in comparison to the control group. Urinary creatinine, urea, uric acid excretion were decreased significantly (P<0.05) after administration of urolithiatic rats with ACEE (500, 750, 1000 mg/kg body weight) when compared with untreated group. The ameliorative results of ACEE were consistent with cystone–treated rats.

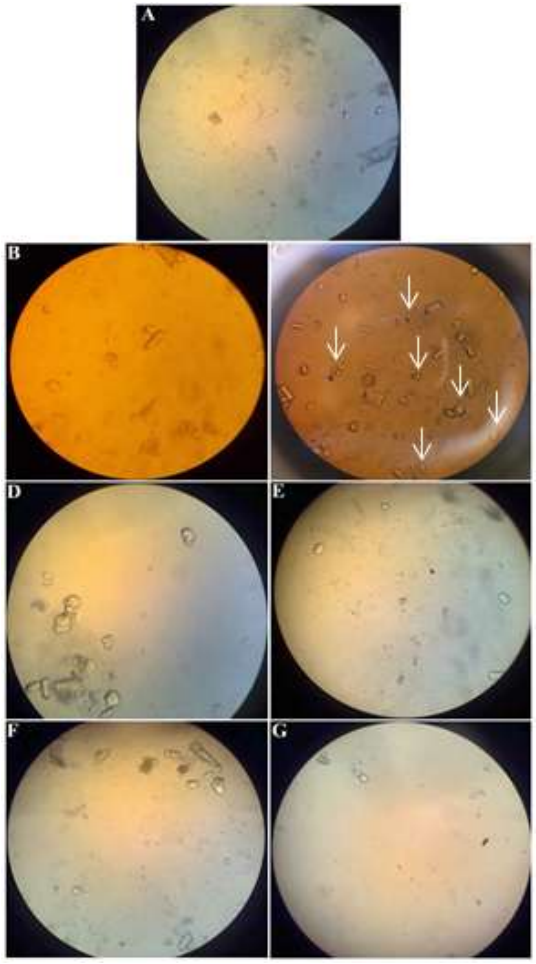

Fig. 2: Light microscopy of urinary calcium oxalate crystals of (A) Control group. (B) ACEE (1000 mg/kg). (C) Urolithiatic group showing pyramidal-shape calcium oxalate dehydrate crystals (arrows). (D) Urolithiatic group treated with ACEE (500 mg/kg). (E) Urolithiatic group treated with ACEE (750 mg/kg). (F) Urolithiatic group treated with ACEE (1000 mg/kg). (G) Urolithiatic group treated with cystone.

Effect of ACEE on serum kidney function markers

There was no significant difference between the serum kidney functions of ACEE treated and control rats except for phosphate and uric acid levels (table 3). However, NaOx injection for 7 days resulted in significant elevation ($P<0.05$) of serum calcium, phosphate, creatinine, urea, uric acid, and BUN concentrations comparable with control levels. On the other hand, serum magnesium level of urolithiatic rats decreased significantly ($P<0.05$) as compared to control rats. These findings indicate impaired renal functions in the NaOx urolithiatic rats. Treatment of urolithiatic rats with ACEE (500, 750 and 1000 mg/kg) significantly reversed ($P<0.05$) the serum alterations and back all the values to near normal value, when compared to urolithiatic rats. Treatment with cystone significantly decreased ($P<0.05$) the levels of these parameters, as compared with urolithiatic rats. These results give a supportive evidence of the similarity of ACEE with that of the standard drug, cystone.

Effect of ACEE on kidney weight

Statistically, there is a non-significant change in the relative kidney weight of ACEE and that of control group (fig. 3). Conversely, the relative kidney weight was significantly raised ($P<0.05$) in urolithiatic rats compared with control rats. Urolithiatic groups treated with 500, 750, or 1000 mg ACEE/kg body weight appears as a cystone effect, as they diminished the relative kidney weight significantly ($P<0.05$) in comparison with untreated ones.

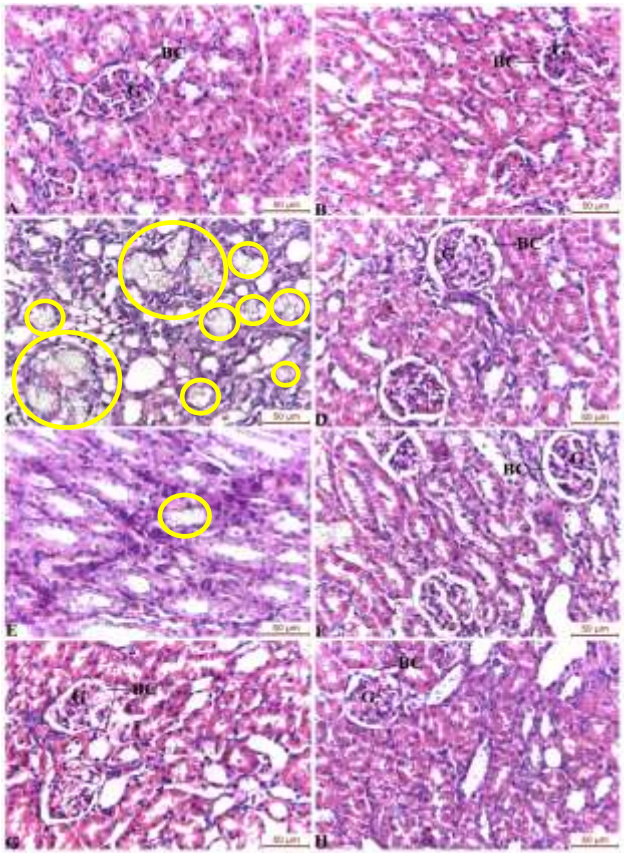

Fig. 3: Effect of ACEE on relative kidney weight of urolithiatic group.

Effect of ACEE on kidney oxidative/antioxidative markers

There is no marked change between renal oxidative and antioxidative markers of ACEE and control rats except for catalase activity. Stone induction enhanced the renal peroxidation by increasing MDA content significantly ($P<0.05$) and inhibited the antioxidant molecules levels including GSH, GST, SOD, and catalase significantly ($P<0.05$) as compared to control animals (Table 4). Interestingly, co-administration of ACEE (500, 750, 1000 mg/kg body weight) to urolithiatic rats reversed the oxidative/antioxidant changes significantly ($P<0.05$) relative to the untreated rats. Similarly, cystone caused

significant amelioration (P<0.05) in the oxidative markers of untreated urolithiatic rats.

Effect of ACEE on renal calculi deposition using histopathological evaluation

Normal group showed intact architecture of medullary and papillary tubules (fig. 4a). Histological analysis revealed no abnormalities in the nephron segment of the ACEE group, as compared with control rats (fig. 4b). On the other hand, intratubular calcium oxalate crystals deposits were observed in the renal tissues of urolithiatic rats and caused secondary tubular dilation (fig. 4c). ACEE (500 mg/kg) partially prevented calcium oxalate crystals in renal tissue, as the number of calcium oxalate deposits in the tubules were less than untreated urolithiatic group in their number and size. Whereas, there are no calcium oxalate deposits or other abnormalities in the nephron segment of the urolithiatic rats treated with ACEE (750, 1000 mg/kg). The kidney section was almost near normal in urolithiatic rats who received cystone. These findings suggest the efficacy of ACEE to be antinephrolithiatic agent.

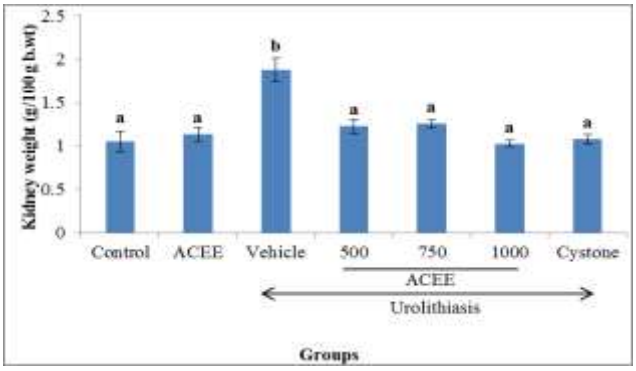

Fig. 4: Photomicrograph of kidney sections of rats stained by hematoxylin and eosin (HE, X 100). A) Section of control rats showing normal cytoarchitecture of renal tissue. B) Section of ACEE treated rats showing the normal glomeruli and tubular region as control rats. C) Section of urolithiatic rats showing deposition of multiple crystals (yellow circle) and tubular dilatation. D&E) sections of rat kidney treated with ACEE (500 mg/kg) showing marked decrease in size and number of crystal deposition. F&G) ACEE (750, 1000 mg/kg) treatment more or less reversed the pathological changes to normal. H) Section of cystone treated rats (750 mg/kg) showing regenerative changes in glomeruli and tubules.

DISCUSSION

The prevalence of nephrolithiasis increasing worldwide, perhaps due to the change in lifestyle, diet, and climate (Zuckerman and Assimos, 2009). The available antiurolithiatic treatments are cost, ineffective in many cases, have side effects rather than their major drawback of stones recurrency (Devkar *et al*., 2016). Therefore, the prophylactic treatment is highly recommended to control the urolithiasis. Phytotherapy can be a best source used in folk medicine to treat kidney stones (Yasir and Waqar, 2011). The present study investigates the effect of *Ananas comosus* ethanolic extract (ACEE) against nephrolithiasis induced by sodium oxalate (NaOx) in male rats.

Oxalate plays a key role in urolithiasis pathogenesis (Mandavia *et al*., 2013). The present study showed that intraperitoneal injection of NaOx caused a significant increase in relative kidney weight of rat, which may be due to increased crystal depositions in the kidney. This supported by histological study in which plenty of intratubular crystals deposited in renal tissue. The renal epithelial injury promotes crystal retention, as epithelial injury exposes a variety of crystal adhesion molecules on epithelial surfaces (Bijarnia *et al*., 2008). The abundance of crystals cause dilation of the tubules causing narrowed cytoplasm and this may be the reason of spreading the hematoxylin dye in urolithiatic tissue.

The study of the urinary chemistry is a good indication of the extent of stone formation, as the type of stones formed is pH-dependant and can be predicted from the pH of the fasting urine (Gupta *et al*., 2012). The current study revealed that NaOx trigger CaOx crystals as the pH of urine in the urolithiatic group is appropriate to form this type of crystal. Touhami *et al*. (2007) and Puotinen &Straus (2010) supported the present result as they clarified that CaOx formed in slightly alkaline medium. Association of crystals with renal tubular cells is a potential factor of urolithiasis (Touhami *et al*., 2007). The current study expected that nephrolithiasis and urolithiasis may be occurred due to the increase calcium excretion, and low urinary volume which are promoters of stone formation; beside the decreased urinary magnesium concentration which means decreasing in stone inhibitors. This reflects the super saturation of urine with stone-forming constituents which is the causative factor in

Table 1: Effect of ACEE on pH and urine volume of urolithiatic rats

Parameters	Control	ACEE (1000 mg/kg)	Urolithiatic				
			Vehicle	ACEE (500 mg/kg)	ACEE (750 mg/kg)	ACEE (1000 mg/kg)	Cystone
Urine volume (ml/24 h)	12.50± 1.41^{ac}	9.67± 0.75^{a}	5.75± 0.54^{b}	11.67± 0.95^{d}	13.42± 0.84^{ac}	12.83± 1.01^{acd}	11.42± 0.80^{cd}
pH of urine	Acidic	Acidic	Slightly alkaline	Acidic	Acidic	Acidic	Acidic

calculogenesis. This expectation is in line with Akila *et al*. (2011), who clarified that urolithiasis occurs as a result of supersaturation of urine which takes place due to imbalance between the stone promoters and inhibitors. Additionally, the higher urinary phosphate excretion, which achieved in the present study, is a good condition for CaOx stone formation (Soundararajan *et al*., 2006; Vidhya *et al*., 2013).

Interestingly, ACEE showed a beneficial effect in preventing stone formation. In consonance with the report of Akila *et al*. (2011), administration of cystone or ACEE to urolithiatic rats decreased the relative kidney weight and significantly raised the levels of stone inhibitors (magnesium and urinary volume) as compared to the untreated urolithiatic group. Diuretic action is necessary to increase the amount of fluid going through the kidneys and flush out the deposits. The increased urine output of cystone or ACEE treated rats dilutes the saturation of urinary electrolytes which evidenced by significant decrease of urinary calcium and phosphates and this confirm the diuretic action of ACEE. Thereby, there is a lesser chance of precipitation and disability for stone formation. This interprets the inhibitory action of ACEE against stone formation as there is a report attributing the antilithiatic activity of the plants to their diuretic property (Ghelani *et al*., 2016).

Elevations of biochemical parameters such as serum urea, uric acid and creatinine are considered reliable for

Table 2: Effect of different doses of ACEE on urinary excretion parameters of urolithiatic group

Parameters (mg/dl)	Control	ACEE (1000 mg/kg)	Urolithiatic				
			Vehicle	ACEE (500 mg/kg)	ACEE (750 mg/kg)	ACEE (1000 mg/kg)	Cystone
Calcium	33.53 ± 2.40^{a}	28.62± 1.40^{b}	50.57± 1.17^{c}	18.47± 0.41^{d}	19.95± 0.41^{d}	18.18± 0.42^{d}	20.00± 0.47^{d}
Phosphate	2.23± 0.16^{a}	2.76± 0.19^{ac}	4.35± 0.20^{b}	2.70± 0.20^{ac}	2.78± 0.11^{c}	2.63± 0.18^{ac}	2.57± 0.14^{ac}
Magnesium	6.38± 0.23^{a}	6.10± 0.19^{a}	3.47± 0.15^{c}	5.08± 0.21^{bd}	5.13± 0.15^{bd}	5.60± 0.22^{d}	5.10± 0.09^{bd}
Creatinine	24.93± 0.32^{a}	23.38± 0.21^{b}	30.13± 0.61^{c}	25.72± 0.28^{a}	22.33± 0.33^{d}	20.68± 0.25^{e}	20.55± 0.19^{e}
Urea	1.06± 0.08^{a}	1.13± 0.05^{a}	1.91± 0.10^{b}	1.05± 0.09^{a}	1.15± 0.10^{a}	1.06± 0.07^{a}	1.06± 0.09^{a}
Uric acid	8.03± 0.12^{a}	8.22± 0.18^{a}	12.85± 0.20^{b}	9.03± 0.19^{c}	9.37± 0.30^{c}	8.87± 0.16^{c}	7.93± 0.12^{a}
UUN	4.97± 0.37^{a}	5.33± 0.22^{a}	8.87± 0.43^{b}	5.37± 0.33^{a}	5.68± 0.31^{a}	5.2± 0.184^{a}	5.37± 0.24^{a}

Table 3: Effect of different doses of ACEE on serum kidney function markers of urolithiatic group

Parameters (mg/dl)	Control	ACEE (1000 mg/kg)	Urolithiatic				
			Vehicle	ACEE (500 mg/kg)	ACEE (750 mg/kg)	ACEE (1000 mg/kg)	Cystone
Calcium	8.07± 0.25^{ac}	8.30± 0.28^{a}	12.70± 0.71^{b}	6.92± 0.22^{d}	8.25± 0.26^{ac}	7.55± 1.13^{acd}	7.18± 0.28^{cd}
Phosphate	8.83± 0.22^{a}	7.93± 0.3^{b}	11.13± 0.43^{c}	8.32± 0.27^{ab}	6.53± 0.24^{d}	6.82± 0.09^{d}	7.08± 0.13$^{d.}$
Magnesium	7.9± 0.21^{a}	7.6± 0.23^{ad}	4.77± 0.17^{c}	6.15± 0.5^{b}	6.23± 0.2^{b}	6.93± 0.16^{bd}	7.27± 0.28^{ad}
Creatinine	0.85± 0.02^{a}	1.02± 0.06^{ae}	2.5± 0.1^{b}	1.33± 0.09^{cd}	1.49± 0.08^{d}	1.17± 0.06^{ce}	0.86± 0.02^{a}
Urea	21.07± 0.35^{ab}	22.1± 0.61^{b}	31.38± 0.49^{c}	19.32± 0.25^{d}	21.48± 0.62^{b}	19.82± 0.58^{ad}	21.63± 0.45^{b}
Uric acid	5.52± 0.15^{a}	6.17± 0.19^{b}	9.4± 0.17^{c}	6.8± 0.2^{d}	7.0± 0.16^{d}	6.8± 0.2^{d}	7.17± 0.26^{d}
BUN	9.7± 0.16^{ab}	10.28± 0.28^{b}	14.58± 0.21^{c}	10.00± 0.12^{ab}	10.00± 0.57^{ab}	9.38± 0.52^{a}	10.07± 0.54^{ab}

All values are means ± SEM (n= 6). Values with different superscript letters are significantly different ($P < 0.05$).

Table 4: Effect of different doses of ACEE on kidney lipid peroxidtion and antioxidant parameters of urolithiatic group.

Parameters	Control	ACEE (1000 mg/kg)	Urolithiatic				
			Vehicle	ACEE (500 mg/kg)	ACEE (750 mg/kg)	ACEE (1000 mg/kg)	Cystone
MDA (nM/g. tissue)	14.12± 0.65^{a}	16.12± 1.63^{ab}	24.53± 0.96^{c}	17.28± 1.24^{ab}	14.43± 1.87^{ab}	18.07± 0.33^{b}	16.38± 0.47^{ab}
GSH (mg/g. tissue)	7.00± 0.27^{abd}	7.28± 0.27^{bd}	2.97± 0.26^{c}	7.53± 0.18^{d}	7.72± 0.10^{d}	6.75± 0.21^{ab}	6.38± 0.27^{a}
GST (U/g. tissue)	1.53± 0.04^{ab}	1.65± 0.12^{b}	0.66± 0.04^{c}	1.41± 0.03^{ad}	1.25± 0.04^{d}	1.38± 0.03^{ad}	1.43± 0.04^{a}
SOD (U/g. tissue)	72.65± 1.53^{ab}	74.97± 1.14^{b}	39.98± 3.18^{c}	68.22± 1.17^{ad}	61.32± 1.01^{e}	67.17± 1.01^{d}	65.78± 2.02^{de}
Catalase (U/g. tissue)	2.50± 0.09^{a}	2.04± 0.12^{b}	1.38± 0.07^{c}	1.90± 0.04^{b}	2.19± 0.17^{b}	2.00± o.10^{b}	2.11± 0.15^{b}

All values are means ± SEM (n= 6). Values with different superscript letters are significantly different ($P < 0.05$).

probing drug-induced nephrotoxicity in animals and man (Gujjala *et al*., 2016). Stones formation leads to an obstruction to the flow of urine in urinary system. This causes renal dysfunction which resulted in accumulation of nitrogenous waste substances such as creatinine, urea, and uric acid in the blood (Aggarwal *et al*., 2012). This interprets the significant increase of these renal markers in urolithiatic group in the present study, as elevation of serum renal function markers indicates renal impairment due to hyperoxaluria. Administration of cystone or ACEE caused significant amelioration in renal function (urea, creatinine and uric acid) which suggests antilithiatic activity of ACEE that prevents renal impairment caused by hyperoxaluria.

Oxidative stress acts as an essential mediator in the pathophysiology of urolithiasis (Devkar *et al*., 2016). Calcium oxalate crystal deposition is accompanied with severe oxidative stress to renal tissue (Peng *et al*., 2015). The present study showed that NaOx injection caused extensive oxidative damage as reflected from increased levels of MDA and decreased level of GSH as well as enzymatic antioxidant enzymes (GST, SOD, and CAT). The present results are in consistent with the results of Gupta *et al*. (2012); Sailaja *et al*. (2012) and Pawar&Vyawahare (2017). They reported that NaOx significantly pronounce the release of malondialdehyde in kidney and reduced the activity of SOD and catalase. This alteration of oxidant/antioxidant system may be due to renal tissue damage induced by oxalate which reacting with polyunsaturated fatty acids in cell membranes causing lipid peroxidation as Karadi *et al*. (2006) clarified. Additionally, Thamilselvan *et al*. (2000) disclosed that low level of renal glutathione, which achieved in present study, favor lipid peroxidation and retain calcium and oxalate in the kidney.

Moreover, urolithiatic rats treated with cystone or ACEE significantly attenuated oxidative damage induced by NaOx. This appeared via the reduction of the renal MDA level and repletion of antioxidant molecules when compared with untreated urolithiatic rats. This may be due to the antiradical scavenging activity of ACEE which revealed by DPPH assay. The antioxidant activity of ACEE may help in its antilithiatic activity. As it was reported that the plant extracts that protect against the development of OxS may have antilithiatic activity (Hadjzadeh *et al*., 2007 and Grases *et al*., 2009). The effectiveness of ACEE as a prophylactic agent in urolithiasis was also confirmed histologically. As the renal tissue of urolithiatic rats treated with ACEE have no crystal deposition in comparison with untreated one.

The present study attributes the antilithiatic (either urolithiatic or nephrolithiatic) activity of ACEE to collective effects that may contribute in its mechanism such as (1) its potassium content (Lu *et al*., 2014). As it was reported that potassium depletion contributes to the renal stone formation (Sriboonlue *et al*., 1991). (2) Its magnesium content (Lu *et al*., 2014), magnesium can form complexes with oxalate in the gut and form a soluble complex (Vidhya *et al*., 2013). Therefore, it can decrease CaOx supersaturation through decreasing oxalate absorption and urinary excretion (Liebman and Costa, 2000). Consequently, decline the concentration available for CaOx precipitation. Therefore, magnesium can be considered as a potent inhibitor of CaOx stone formation. (3) Its citric acid content (Prabha and Rangaiah, 2014) which is the principal organic acid presents in *A. comosus*. Escribano *et al*. (2009) reported that citrate can be used as prophylactic agent to prevent kidney stone for a long time by inhibiting stone formation particularly effective against the CaOx stone. Chutipongtanate *et al*. (2012) added that citrate not only dissolves CaOx crystals, but also it can detach these crystals from renal tubular cells. The potency of citrate to inhibit CaOx crystals comes from its ability to coat the surface of growing calcium crystals or to its ability to complexed with calcium, and hence reduce the concentration of CaOx (Basavaraj *et al*., 2007). (4) Its diuretic activity

which is attributed to the presence of potassium as Alok *et al*. (2008) mentioned. (5) Its antioxidant activity which confirmed by DPPH assay.

CONCLUSION

ACEE has noteworthy anti-nephrolithiatic activity as ACEE not only increased the level of stone inhibitors but also decreased the level of stone promoters in urine consequently prevent renal calculi formation. Its antilithiatic ability may attribute to its diuretic and antioxidant activities rather than its bioactive constituents. There was no dose dependent increment in the effect which favors the use of ACEE in lowest does (500 mg/kg body weight).

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