

Anti-Urolithiatic Effect of *Pericampylus glaucus* against Ethylene Glycol Induced Urolithiasis in Male Sprague Dawley Rats

(Kesan Anti-Urolitiatik *Pericampylus glaucus* terhadap Urolitiasis Teraruh Etilena Glikol dalam Tikus Jantan Sprague Dawley)

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ABSTRACT

Pericampylus glaucus is a common plant used traditionally for the remedies of urinary and kidney stones. Hence, present research was aimed to investigate the antiurolithiatic effect of ethanol extract of *P. glaucus* against ethylene glycol induced urolithiasis in male Sprague Dawley rats. The nephrolithiasis was induced by ethylene glycol (0.75% v/v) in drinking water over a period of 30 days. The animals were divided into normal, lithiatic and extract treated group that received ethanolic extract of *P. glaucus* at dose rate of 200 and 400 mg/kg (b.wt) by mouth. The calcium, phosphate, urine pH, oxalate, magnesium in urine and histopathological examination of the kidney were observed. A significant ($p < 0.01$) increased in the levels of phosphate, calcium, oxalate, and an imbalance in kidney with decreased magnesium excretion in urine were observed in ethylene glycol induced urolithiasis as compared to normal control. Treatment with ethanolic extract of *P. glaucus* at dose of 200 and 400 mg/kg (b.wt) decreased significantly ($p < 0.01$) the hyperoxaluria, calcium, phosphate, oxalate with significant ($p < 0.05$) increased retention of urinary magnesium and improvement in the renal function as matched to lithiatic group. The histopathological examination showed the deposition of a large number of calcium oxalate crystal in lithiatic group while smaller depositions in 200 mg/kg (b.wt) extract treated group. The results confirmed the antiurolithiatic effect of *P. glaucus* in male Sprague Dawley rats and validate its traditional uses against urinary stone.

Keywords: Anti-urolithiatic; ethylene glycol induced; hyperoxaluria; *Pericampylus glaucus*

ABSTRAK

Pericampylus glaucus adalah tumbuhan yang biasa digunakan secara tradisi untuk pemulihan kencing dan batu karang. Oleh itu, penyelidikan ini bertujuan untuk mengkaji kesan antiurolitiatik ekstrak etanol *P. glaucus* terhadap urolitiasis teraruh etilena glikol dalam tikus jantan Sprague Dawley. Nephrolithiasis telah diaruh etilena glikol (0.75% v/v) dalam air minuman selama 30 hari. Tikus tersebut dibahagikan kepada kumpulan normal, litiatik dan kumpulan terawat ekstrak yang mendapat ekstrak etanolik *P. glaucus* pada kadar dos 200 dan 400 mg/kg (b.wt) melalui mulut. Kalsium, fosfat, pH air kencing, oksalat, magnesium dalam air kencing dan pemeriksaan histopatologi buah pinggang telah dicerapkan. Peningkatan signifikan ($p < 0.01$) tahap fosfat, kalsium, oksalat dan ketidakseimbangan pada buah pinggang dengan penurunan magnesium perkumuhan dalam air kencing dicerapkan dalam urolithiasis teraruh etilena glikol berbanding kawalan normal. Rawatan dengan ekstrak etanol *P. glaucus* pada 200 dan 400 mg/kg (b.wt) menurunkan dengan ketara ($p < 0.01$) hiperoksaluria, kalsium, fosfat, oksalat dengan peningkatan ketara ($p < 0.05$) pengekalan kencing magnesium dan peningkatan fungsi buah pinggang agar sama dengan kumpulan litiatik. Pemeriksaan histopatologi menunjukkan pemendapan sejumlah besar kristal oksalat kalsium dalam kumpulan litiatik manakala deposisi yang lebih kecil dalam ekstrak 200 mg/kg (b.wt) kumpulan terawat. Hasil mengesahkan kesan antiurolitiatik *P. glaucus* pada tikus jantan Sprague Dawley dan mengesahkan kegunaan secara tradisi terhadap batu karang.

Kata kunci: Anti urolitiatik; etilena glikol teraruh; hiperoksaluria; *Pericampylus glaucus*

INTRODUCTION

The development of stony concretions in body either in the urinary system or gall bladder are known as lithiasis. Nephrolithiasis and urolithiasis are the common types of lithiasis being claimed as of the main source of renal failure in human as reported in literature (Prasad & Bharathi 2007). These types of lithiasis are present in 12% of worldwide

population, affecting mostly male with recurrence rate are (70-80%) as compared to female (47-60%) (Awari et al. 2009; Choubey et al. 2010). Calcium oxalate contributes 75-90% of the total kidney stones followed by magnesium ammonium phosphate 10-15%, cystine 0.5-1% and uric acid 3-10% (Ashok & Vishwanathswamy 2010). There is no proper treatment and physicians just medicate the pain

until the stones get out their own as previously reported in research paper (Chitme et al. 2010). Lithotripsy and surgical procedures are also carried out but the risks factors associate with these therapy are not corrected until now (Betanabhatla et al. 2009). Furthermore, medical management of lithiasis involved surgical exclusion and extracorporeal shock lithotripsy techniques (Sathish et al. 2010). But, the side effects are linked with these therapeutic techniques. Therefore, there is an urge to find an alternative medicine from natural product with no side effect and maximum benefits (Betanabhatla et al. 2009). The plant '*Pericampylus glaucus*' commonly known as 'Akkar chupping', which is traditionally used in the remedy of kidney stone and other variety of diseases as reported in literature (Kifayatullah et al. 2017a). Despite of traditional use, plant was previously investigated scientifically against cancer cell (Zhao & Cui 2009), followed by hepatitis B, C, AIDS (Yan et al. 2008), and diabetes (Kifayatullah & Sengupta 2016). But, no scientific paper was reported on this plant for urolithiasis. Hence, the objective of the present research was to determine the antiurolithiatic effect of *Pericampylus glaucus* against ethylene glycol induced urolithiasis in male Sprague Dawley rats.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The whole plant of *Pericampylus glaucus* was collected from Kampung Jeram Kedah, Negeri Sembilan, Malaysia in the month of June 2014 and was authenticated by Forest Research Institute Malaysia (FRIM) with voucher specimen herbarium number (FRIM/394/490/5/18(118), deposited to the Faculty of Pharmacy, Lincoln University College, Malaysia.

CHEMICALS AND REAGENTS

Chemicals used in this study were ethylene glycol (Sigma Aldrich Germany), chloroform (Riedel-De-Haen, Germany), formaldehyde 37% solution (Merck Germany), xylene, ethanol (Merck, Germany), paraffin, adhesive material, potassium dihydrogen phosphate extra pure (Riedel-De-Haen, Germany), sodium dihydrogen phosphate (Merck, Germany).

EQUIPMENTS

Equipment used were Microscope labomed (USA), centrifuge rototax 32 A (Hettich Zentrifugen). Digital weighing balance, TE214 (Sartorius Germany) and GE4101 (Sartorius Germany) capacity 0.1-4200 g, pH meter (pH720, WTW) and Microtome HM310 Sakura (Germany).

EXPERIMENTAL DETAILS

Thirty male Sprague-Dawley rats weighing between 100 and 110 g were used in this study, which were supplied from Institute for Medical Research, Pahang. The animals

were kept in plastic cages and placed in animals holding facility centre of Lincoln University College where the standard laboratory condition ($26 \pm 2^\circ\text{C}$) with humidity ($50 \pm 4\%$) and the natural lighting 12 h light/12 h night were maintained. The study was conducted according to the standard guidelines for the animal's care, which was approved by the Animal Ethics Committee of Lincoln University College, Malaysia (LUC-AEC) with approval number PHARM/2013/MSM/02-AUGUST/11/SEPTEMBER2014-AUGUST 2016. Standard pellet diet and water *ad libitum* were provided to animals during the whole experiment in accordance to the CPSCEA guidelines.

EXTRACTION OF PLANT MATERIAL

The leaves were dried in shade for 20 days and converted into coarse powder with the help of grinder. After converting into coarse powder, an amount of 500 g of powder was subjected to extraction by continuous hot extraction method using ethanol (95%) as a solvent soxhlet in apparatus. The resultant ethanolic extract was then concentrated under reduced pressure ($40-45^\circ\text{C}$) in a rotary evaporator. The residue left in evaporator was kept in desiccator for further pharmacological activities (Yadav & Agarwala 2011).

PRELIMINARY PHYTOCHEMICAL SCREENING

The presence of various phytochemicals in ethanol extract of *Pericampylus glaucus* was examined earlier by using different types of chemical identification tests that show the presence of alkaloids, saponins, reducing sugar, phenol, tannins, flavonoid, terpenoids and sterol except anthraquinone and resins (Kifayatullah et al. 2017b), as reported previously in research paper.

ACUTE TOXICITY STUDY

OECD guideline 423 was followed for ethanol extract of *Pericampylus glaucus* on female Balb/c mice where the maximum single dose 2000 mg/kg (b.wt) by mouth was used. The administered dose was found safe and no mortality was observed in treated groups (Kifayatullah et al. 2015a). Therefore, $1/10^{\text{th}}$ of the highest dose 200 and 400 mg/kg (b.wt) was selected for investigation of anti-anti-urolithiatic activity of ethanol extract of *Pericampylus glaucus* in male Sprague Dawley rats.

EXPERIMENTAL DESIGN FOR ANTI UROLITHIATIC ACTIVITY

The anti-urolithiatic activity of ethanol extract of *Pericampylus glaucus* was screened by Ethylene Glycol Induced model (Shah et al. 2012). Two dose rate (200 and 400 mg/kg (b.wt) of ethanol extract was used against ethylene glycol induced calcium oxalate stone. Ethylene glycol 0.75% (v/v) solution in drinking water was fed to all rats groups for induction of hyperoxaluria except Group I. Animals were randomly distributed into 06 different groups comprising 05 rats in each. Group I was named

as control that received only normal laboratory diets and drinking water throughout the study. Group II were kept as lithiatic group that received 0.75% (v/v) ethylene glycol solution from day 1st - 30th. Groups III and IV were named as pretreatment groups that received 0.75% (v/v) ethylene glycol and ethanol extract of *Pericampylus glaucus* at dose rate of 200 and 400 mg/kg (b.wt) daily from day 1st - 30th. While, groups V and VI were named as treatment groups that received 0.75% (v/v) ethylene glycol only from day 1st - 14th and from 15th - 30th day along with 0.75% (v/v) ethylene glycol received ethanol extract of *Pericampylus glaucus* at dose of 200 and 400 mg/kg (b.wt).

EXAMINATION OF URINE FOR THE DETERMINATION OF IONIC CONCENTRATIONS

The rats were kept individually in metabolic cages and the urine samples from each animal group was collected on day 14th and at the end of the experiment (24 h) to find the magnesium and calcium ions in the urine samples. During collection of urine periods, each animal groups were allowed free access to drinking water. After diluting the urine sample with 10 mL of reverse osmosis water, a volume of 0.5 mL of urine sample was taken, acidified through 5M HCl. The acidified urine was subjected to centrifugation for 10 min in order to remove supernatants and debris and refrigerated at (-20°C) until determination of calcium and magnesium using commercial diagnostic kits in spectrophotometer at wavelength of 422 nm. The volume of urine sample of each treated group on day 14th and 30 day was recorded by measuring cylinder and the pH was determined with pH meter (Kaur et al. 2009).

DISSECTION OF RATS

At the end of experiment, all animal groups after keeping on overnight fasting were sacrificed under ether anesthesia and both of kidneys were collected, immediately fixed in freshly prepared 10% buffered formalin pH7.4. The kidney samples were stored immediately in refrigerator (Kaur et al. 2009).

PREPARATION OF SLIDES FOR HISTOPATHOLOGICAL EXAMINATION

Kidney tissues previously fixed in buffered formalin (pH7.4) were bisected with the help of sterilized blades for gross examination. The tissues were further treated with 10% formalin, alcohol, xylene and finally embedded with paraffin for dehydration to fix the tissues. Microtome was used for cutting and sectioning the tissues to prepare the slides. Firstly, the slides were treated with adhesive material that was the mixture of albumin, glycerin and thymol crystals, and then previously sliced kidney tissues were allowed to stick to the glass surface. The prepared slides were then arranged on the hot plate at temperature of 65 ± 1°C for 10-15 min to remove the wax materials. Dehydration was carried out by dipping the slides in 75% ethanol for 2 min and then washed with water for 4 min.

Pizzolato's stain was used for the staining of calcium oxalate stones. An equal volume of 5% silver nitrate and 30% hydrogen peroxide solutions were mixed. Hydrated slides were flooded with mixture of solutions and exposed to 60-watt tungsten-filament electric lamp. The light was kept at a distance of 15 cm above from the sections and an exposure time of 30 min was given. 30 min after slides were thoroughly rinsed in distilled water and counter stained with 0.1% safranin in 1% acetic acid for 3 min again rinsed in distilled water, dehydrated, cleared and finally covered with cover slip and examined microscopically for calcium oxalate stones using compound microscope Labomed (USA) (Kaur et al. 2009).

BIOCHEMICAL ASSAY

Each kidney from treated groups was taken and dried in oven at 80°C for a period of 24 h. Samples of 200 mg of the dried kidney were heated in 10 mL of 1N HCL and homogenized. The homogenate prepared was centrifuged at 2500×g for a period of 10 min. The supernatant was separated and examined for the presence of calcium, oxalate and phosphate (Mayee & Thosar 2011).

STATISTICAL ANALYSIS

Values were expressed as mean ± Standard Error of Means. Values were statistically performed by one way ANOVA followed with post hoc Neumen-kelus multiple comparison tests by using Graph Pad Prism (5 version). A probability level of * $p < 0.05$ was consider statistically significant.

RESULTS AND DISCUSSION

In present research work, male Sprague Dawley rat was used for induction of urolithiasis by administartion of 0.75% (v/v) ethylene glycol solution that leave toxic metabolites inside the body (Tzou et al. 2016). The metabolic acidosis occurs after ingestion of ethylene glycol due to the accumulation of acid metabolite and lactate in the body as documented previously in literature (Tzou et al. 2016). Administrations of ethylene glycol for a period of 14 days lead to the formation of kidney stones that mainly composed of calcium oxalate. In this study, male animal was used due to the close resemblance of their urinary system to human and deposition of the stones in female is less as reported previously in the literature (Katzung & Trevor 2004). The oxalate level increases with ethylene glycol due to increased availability of substrate for synthesizing oxalate enzymes (Ratkalkar & Kleinman 2011). Increase level of calcium in the urine results crystals growth by providing an environment favorable for the nucleation and precipitation of calcium phosphate and calcium oxalate (Sathish et al. 2010).

URINE pH

The pH of urine sample on 30th day of treatment was determined by using pH meter. In comparison to normal

control, a slightly acidic pH was observed in animal groups that received only ethylene glycol and treatment *Pericampylus glaucus* at dose rate of 200 and 400 mg/kg (b.wt). However, urine pH was almost the same as compared to normal control. The pH of urine plays an important role in formation of kidney stones; dissolution of formed stones can be achieved through alteration in pH as previously reported in the research paper (Bahuguna et al. 2009). The acidic pH of lithiatic urine provides favorable conditions for oxalate type of kidney stone. Hence the antiurolithiatic effect of *Pericampylus glaucus* in pretreatment (200 and 400 mg/kg (b.wt) and treatment 200 mg/kg (b.wt) may be due to the high pH value as compared to lithiatic control.

URINE CONCENTRATION OF ELECTROLYTES

The urine samples from all treated animals were collected on day 14 and 30th for the analysis of magnesium and calcium ions in spectrophotometer wavelength (422 nm). A slight significant ($p<0.05$) enhancement in the calcium levels were seen in the urine of group II (lithiatic control and *Pericampylus glaucus* extract treated group at dose rate of 200 and 400 mg/kg (b.wt) on day 14 sample as compared to control. Same there was slight significant ($p<0.05$) increase in calcium levels were also found on 30th day of treatment in urine sample of lithiatic and treatment with *Pericampylus glaucus* extract at dose of 200 mg/kg (b.wt), but there was slight significant ($p<0.05$) reduction in level of calcium was observed in 400 mg/kg (b.wt) dose

treatment group as compared to lithiatic control. A slight significant ($p<0.05$) increase in calcium level was observed in pretreatment group (200 mg/kg (b.wt) in urine sample that was collected on 30th day of treatment but the level was ($p<0.005$) low as compared to normal and lithiatic group. There was no significant ($p>0.05$) variation in calcium ion was noted in pretreatment group that received ethanolic extract at dose rate of 200 and 400 mg/kg (b.wt) as matched to normal control and variation was significant ($p<0.05$) as compared to lithiatic group. The level of magnesium was slight significant low ($p<0.05$), in the lithiatic and treatment group at dose of 200 and 400 mg/kg (b.wt) in the urine samples, which was collected on 14th day of treatment as compared to control group. The reduction in magnesium levels were observed more significant ($p<0.01$) in control (lithiatic group) and treatment 200 mg/kg dose groups on day 30th day urine collected sample, while the concentration was significantly ($p<0.01$) increased in pre treatment and treatment at dose 400 mg/kg (b.wt) dose group as compared to lithiatic control group (Table 1).

KIDNEY WEIGHT

There was slight significant ($p<0.05$) increase in kidney weight were observed in lithiatic and treatment group that received ethanolic extract at dose rate of 200 mg/kg (b.wt) as compared to control. While the weight of other treated groups (pretreatment 200 mg/kg (b.wt), 400 mg/kg (b.wt) and treatment 400 mg/kg (b.wt) were remain unchanged during study (Table 2).

TABLE 1. Levels of calcium and magnesium in the urine samples collected at day 14 and 30

Treatment	Concentration in day 14 sample		Concentration in day 30 sample		
	Urine pH	Calcium (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Magnesium (mg/dl)
Normal control	7.0	6.4±0.5	3.4±0.7	7.3±0.5	3.4±0.5
Lithiatic control	5.8	13.7±0.9	2.7±0.3**	14.2±0.3**	1.5±0.4**
Pretreatment 200 mg/kg	6.5	7.5±0.6*	3.2±0.5*	9.7±0.2*	3.0±0.1*
Pretreatment 400 mg/kg	7.2	6.8±0.1*	3.4±0.0*	8.2±0.3*	3.8±0.5*
Treatment 200 mg/kg	6.0	14.2±0**	2.8±0.5**	15.4±0.4*	2.1±0.1**
Treatment 400 mg/kg	6.8	13.0±0.2**	3.4±0.9**	9.1±0.1*	3.7±0.0*

Data are expressed as mean concentration of ions ± SEM, N=5, shows significant variation * $p<0.05$, from lithiatic control and normal control. Analysis was performed by one way of ANOVA with post hoc Neumen-kelus multiple comparison tests.

TABLE 2. Effect of ethanolic extract of *Pericampylus glaucus* on kidney parameters in ethylene glycol induced urolithiasis animals

Treatment	kidney weight (g)	calcium mg/dl	phosphate mg/dl	oxalate mg/dl
Normal control	0.7±0.1	1.8±0.1	2.2±0.6	0.8±0.1
Lithiatic control	0.8±0.1**	4.4±0.2**	5.6±0.1**	1.9±0.5**
Pretreatment 200 mg/kg(b.wt)	0.7±0.7	2.2±0.0**	3.0±0.9**	1.0±0.3**
Pretreatment 400 mg/kg(b.wt)	0.7±0.1	1.7±0.1*	2.4±0.1*	0.98±0.4**
Treatment 200 mg/kg (b.wt)	0.8±0.1**	3.4±0.5**	4.9±0.1**	2.0±0.8**
Treatment 400 mg/kg (b.wt)	0.7±0.9	1.8±0.6*	2.6±0.8**	1.0±0.0**

Data are expressed as Mean ± SEM, shows significant (* $p<0.05$, ** $p<0.01$) variation as compared to lithiatic and normal control groups. Test employed was one-way ANOVA followed by Neumen-kelus test for multiple comparisons

KIDNEY HOMOGENATE ASSAY

The kidney samples from each treated groups were homogenized and analyzed for the formation of any stone constituents. The result was compared with group I and II (control and lithiatic). A significant ($p < 0.001$) increase in calcium levels were found in animals group that received only ethylene glycol and treatment 200 mg/kg (b.wt) as compared to control. The levels of the phosphate and oxalate were slight significantly ($p < 0.05$) high in lithiatic and treatment 200 mg/kg (b.wt) dose groups. Statistically significant ($p < 0.05$) increase and decrease in phosphate and oxalate levels were also observed in pretreatment 200 and treatment 400 mg/kg (b.wt) dose groups while matched to normal and lithiatic control (Table 2). The increased levels of calcium in lithiatic and treatment 200 mg/kg (b.wt) dose may due to lowering of glomerular filtration rate that results an obstruction in urine flow along with deposition of calcium oxalate in the urinary system and renal tubules and caused development of kidney stone (Lakshmi et al. 2014). Increased in phosphorus levels along with oxalate stress provides favorable condition for the formation of stones by forming calcium phosphate crystals that converts into calcium oxalate crystals and deposits in the renal tubules (Ratkalkar & Kleinman 2011). Significant ($p < 0.01$) increased in urinary phosphate levels were examined in ethylene glycol induced urolithiasis animals. Increased in urinary phosphate excretion provide a suitable environment for the formation of stone by making phosphate calcium crystals and thus induced calcium oxalate deposition (Ratkalkar & Kleinman 2011). In treatment group receiving ethanolic extract of *Pericampylus glaucus* at dose rate of 200 and 400 mg/kg (b.wt) decreased the urinary phosphate excretion, returning back the level of urinary phosphate to normal and hence it may be assume to be helpful in reducing the probability of stone formation. Magnesium is one of the well-known inhibitor for the formation of stone in urethra and kidney. Low level of magnesium in lithiatic and treatment 200 mg/kg (b.wt) in ethylene glycol induced urolithic rats may provide an environment favorable for development of lithiasis. Magnesium diminished the super saturation of calcium oxalate by lessening the saturation of calcium oxalate and reduces the progression and nucleation values of calcium oxalate crystals (Touhami et al. 2007). Returning of magnesium level to normal in extract treated group indicates that it may contain certain phytochemicals that has ability for reducing super saturation of calcium oxalate by lowering the saturation of calcium oxalate (Touhami et al. 2007). Decrease level of antioxidant potential and increased lipid peroxidation in rat's kidney treated with ethylene glycol results the renal cells damage by reacting with polyunsaturated fatty acid in membranes of the cells (Bahuguna et al. 2009). Antioxidant potential and presence flavonoids, phenolic and other secondary metabolites in *Pericampylus glaucus* may be responsible for its antiurolithiatic action (Kifayatullah et al. 2015b).

Presence of organic and inorganic inhibitors inhibits the crystal growth such as magnesium being an inhibitor of calcium crystal prevents the growth and nucleation rate by forming complex with oxalate thus reduces oxalate super saturation in urine (Bahuguna et al. 2009). The mechanism behind suppressing high level of intracellular calcium with ethanolic extract of *Pericampylus glaucus* are not clear, however it may be assume due to the raised bioavailability of nitric oxide (NO), that in turns triggers cGMP (Lulat et al. 2016). Previous studies has confirmed the ability of nitric oxide (NO) donors in controlling of intracellular increase calcium levels (Lulat et al. 2016). Therefore, the plant extract of *Pericampylus glaucus* may possibly well decreases the level by the inhibiting oxalate or increasing the bioavailability of nitric oxide (NO) to appropriate calcium because of cGMP pathway (Pragasam et al. 2005).

HISTOPATHOLOGICAL EXAMINATION

Pizzolato's staining technique was used for kidney tissues slices stained that were examined under microscopically ($\times 40$) for the presence of calcium oxalate stones. Irregular stones of calcium oxalate were found only in lithiatic control and treatment 200 mg/kg (b.wt) of *Pericampylus glaucus* extract receiving groups. There were no visible stones found in kidney tissues of other treated animals group (Figure 1). Histopathological examination of the kidney portion of ethylene glycol induced lithiasis in lithiatic and 200 mg/kg (b.wt) of extract treated group showed the presence of abnormal calcium oxalate crystals while absence of visible stones formation in the kidney tissues in other treated group; this may be attributed to oxalate formation. Oral administration of ethanolic extract at different dose rate (200 and 400 mg/kg b.wt) decrease the formation of crystals in kidney that indicate the ability of *Pericampylus glaucus* to dissolve the preformed stones to some extent. Co treatment of *Pericampylus glaucus* extract at dose (200 and 400 mg/kg b.wt) diminished the amount and mass of calcium oxalate deposits in various portions of the renal tubules. The result showed the urolithiatic protective effect of leaves of *Pericampylus glaucus* in ethylene glycol induced urolithiasis model. This effect of *Pericampylus glaucus* may be due to maintaining the balance between stone promoters and inhibitors, reducing deposition and excretion of small particles of calcium oxalate and thus reduced the chances of crystal to retain in the urinary tract (Patel et al. 2016). A number of plant based drug and their phytochemicals have also shown a defensive result versus stone renal formation (Atmani et al. 2003). The result point out that dispensation of ethanolic extract of *Pericampylus glaucus* to the rat among ethylene glycol induced lithiasis, decreased and prevents the development of urine stones and maintains balance between stone promoters and inhibitors constituents. This may support the information concerning with antiurolithiatic activity of plant *Pericampylus glaucus*.

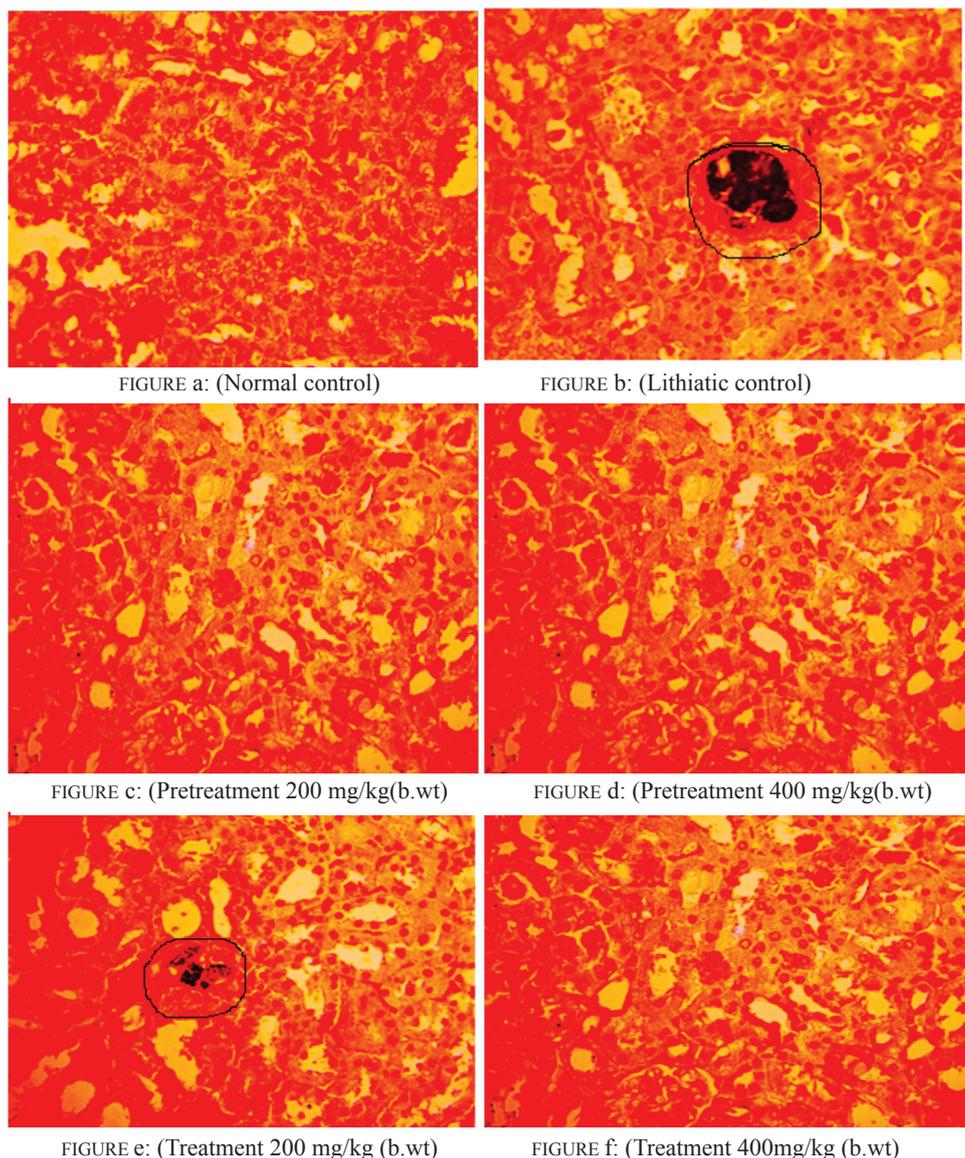


FIGURE 1. Examination of kidney tissue under microscope ($\times 40$) for the presence of calcium oxalate stones. Tissue slices stained by Pizzolato's staining technique.

CONCLUSION

This study for the first time established the antiurolithiatic effect of ethanolic *Pericampylus glaucus* extract against ethylene glycol induced urolithiasis and justified the traditional use of the investigated plant in the management and prevention of urinary and kidney stones. However, further studies on *Pericampylus glaucus* could contribute to discover a new antiurolithiatic agent. To continue this research work require the isolation of compound and evaluating the antiurolithiatic activity of the pure compound.

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REFERENCES

- Ashok, P., Koti, B.C. & Vishwanathswamy, A. 2010. Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats. *Indian Journal of Pharmacology* 42(6): 380-385.
- Atmani, F., Slimani, Y., Mimouni, M. & Hacht, B. 2003. Prophylaxis of calcium oxalate stones by *Herniaria hirsuta* on experimentally induced nephrolithiasis in rats. *BJU International* 92(1): 137-140.
- Awari, D.M., Vaishali, M., Babhale, S.P. & Chaudhari, S.P. 2009. Antilithiatic effect of *Achyranthus aspera* Linn. leaves extract on ethylene glycol induced nephrolithiasis. *Journal of Pharmacy Research* 2(5): 994-997.

- Bahuguna, Y., Rawat, M., Juyal, V. & Gnanarajan, G. 2009. Antilithiatic effect of grains of *Eleusine coracana*. *Saudi Pharmaceutical Journal* 17(2): 182-188.
- Betanabhatla, K.S., Christina, B.S., Sundar, S., Selvakumar, S. & Saravanan, S.K. 2009. Antilithiatic activity of *Hibiscus sabdariffa* Linn. on ethylene glycol-induced lithiasis in rats. *Indian Journal of Natural Products and Resources* 8(1): 43-47.
- Chitme, H.R., Alok, S., Jain, S. & Sabharwal, M. 2010. Herbal treatment for urinary stones. *International Journal of Pharmaceutical Sciences and Research* 1(2): 24-31.
- Choubey, A., Parasar, A., Choubey, A., Iyer, D., Pawar, R. & Patil, U. 2010. Potential of medicinal plants in kidney, gall and urinary stones. *International Journal of Drug Development and Research* 2(2): 431-447.
- Katzung, B.G., Masters, S.B. & Trevor, A.J. 2004. *Basic & Clinical Pharmacology*. 9th edition. New York: McGraw-Hill Publishing Co.
- Kaur, T., Bijarnia, R.K., Singla, S.K. & Tandon, C. 2009. *In vivo* efficacy of *Trachyspermum ammi* anticalcifying protein in urolithiatic rat model. *Journal of Ethnopharmacology* 126(3): 459-462.
- Kifayatullah, M. & Sengupta, P. 2016. Effect of *Pericampylus glaucus* on plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats. *Bangladesh Journal of Pharmacology* 11(1): 200-205.
- Kifayatullah, M., Mustapha, M.S., Sarker, M.M.R. & Amin, M. 2017a. Effect of compounds identified in the active fraction of *Pericampylus glaucus* on blood glucose and lipid profiles in streptozotocin-induced diabetic rats. *Egyptian Pharmaceutical Journal* 16(1): 8-15.
- Kifayatullah, M., Sarker, M.M.R. & Mustapha, M.S. 2017b. Phytochemical investigation of ethanolic extract of *Pericampylus glaucus* leaves from Malaysia by GC-MS analytical technique. *International Journal of PharmTech Research* 10: 234-240.
- Kifayatullah, M., Mustafa, M.S., Sengupta, P., Sarker, M.M.R., Das, A. & Das, S.K. 2015a. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. *Journal of Acute Disease* 4(4): 309-315.
- Kifayatullah, M., Senguptha, P., Mustafa, M.S., Das, S.K. & Sisugoswomi, M. 2015b. Evaluation of ethanolic extract of *Pericampylus glaucus* (Lamk.) Merr for total phenolic, total flavonoids contents and *in vitro* anti-oxidant activity. *International Journal of Pharmacognosy and Phytochemical Research* 7: 677-683.
- Lakshmi, K.S., Prabhakaran, V., Mallikarjuna, G. & Gowthami, A. 2014. Antilithiatic activity of *Trianthema portulacastrum* L. and *Gymnema sylvestre* R. Br against ethylene glycol induced urolithiasis. *International Journal of Pharmaceutical Sciences Review and Research* 25: 16-22.
- Lulat, S.I., Yadav, Y.C., Balaraman, R. & Maheshwari, R. 2016. Antiuro lithiatic effect of lithocare against ethylene glycol-induced urolithiasis in Wistar rats. *Indian Journal of Pharmacology* 48: 78-82.
- Mayee, R. & Thosar, A. 2011. Evaluation of *Lantana camara* Linn. (*Verbenaceae*) for antiuro lithiatic and antioxidant activities in rats. *International Journal of Pharmaceutical and Clinical Research* 3(1): 10-14.
- Pragasam, V., Kalaiselvi, P., Sumitra, K., Srinivasan, S. & Varalakshmi, P. 2005. Counteraction of oxalate induced nitrosative stress by supplementation of l-arginine, a potent antilithic agent. *Clinica Chimica Acta* 354(1-2): 159-166.
- Patel, P.K., Vyas, B.A., Joshi, S.V. & Gandhi, T.R. 2007. Evaluation of antiuro lithiatic effect of pedaliu murex fruit extract in ethylene glycol-induced nephrolithiasis in rat. *Indian Journal of Pharmaceutical Sciences* 78(2): 231-239.
- Prasad, K., Sujatha, D. & Bharathi, K. 2007. Herbal drugs in urolithiasis - A review. *Pharmacognosy Reviews* 1(1): 175-179.
- Ratkalkar, V.N. & Kleinman, J.G. 2011. Mechanisms of stone formation. *Clinical Reviews in Bone and Mineral Metabolism* 9(3-4): 187-197.
- Sathish, R., Natarajan, K. & Nikhad, M.M. 2010. Effect of *Hygrophila spinosa* T. anders on ethylene glycol induced urolithiasis in rats. *Asian Journal of Pharmaceutical and Clinical Research* 3(4): 61-63.
- Shah, J.G., Patel, B.G., Patel, S.P. & Patel, R.K. 2012. Antiuro lithiatic and antioxidant activity of *Hordeum vulgare* seeds on ethylene glycol-induced urolithiasis in rats. *Indian Journal of Pharmacology* 44(6): 672-677.
- Touhami, M., Laroubi, A., Elhabazi, K., Loubna, F., Zrara, I., Eljahiri, Y., Oussama, A., Grases, F. & Chait, A. 2007. Lemon juice has protective activity in a rat urolithiasis model. *BMC Urology* 7(1): 18-25.
- Tzou, D.T., Taguchi, K., Chi, T. & Stoller, M.L. 2016. Animal models of urinary stone disease. *International Journal of Surgery* 36: 596-606.
- Yadav, R. & Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology* 3(12): 10-14.
- Yan, M.H., Cheng, P., Jiang, Z.Y., Ma, Y.B., Zhang, X.M., Zhang, F.X., Yang, L.M., Zheng, Y.T. & Chen, J.J. 2008. Periglaucines A-D, anti-HBV and -HIV-1 alkaloids from *Pericampylus glaucus*. *Journal of Natural Products* 71(5): 760-763.
- Zhao, W. & Cui, C. 2009. Triterpenoidal constituents of *Pericampylus glaucus* and their antitumor activity *in vitro*. *Chinese Journal of Medicinal Chemistry* 19: 195-199.
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