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Assessment of Potential Antilithiatic Activity of *Cynodon dactylon, Emblica officinalis, Kalanchoe pinnata* and *Bambusa nutans* on Dietary Glycolic Acid Induced Nephrolithiasis

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- *Cynodon dactylon*
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- *Bambusa nutans*
- Glycolic acid
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Abstract

Cynodon dactylon, *Emblica officinalis*, *Kalanchoe pinnata* and *Bambusa* nutans is reported to have diuretic and antiurolithiatic potential against ethylene glycol and ammonium chloride, and glyoxalate induced urolithiasis along with in vitro calcium oxalate (CaOx) crystal growth inhibition property. The ethyl acetate fraction of these plants have a high content of antioxidative phytocompounds like polyphenols and flavonoids. This study aims at the exploration of antilithiatic potential of C. *dactylon*, *E. officinalis*, *K. pinnata,* and *B. nutans* ethyl acetate fraction on 3 % glycolic acid diet feeding induced nephrolithiasis in rats. On the 28th day of glycolic acid diet feeding, urine samples were collected to determine oxalate, calcium, phosphorus, sodium, and potassium concentration. On the 29th day, blood samples were collected after sacrifice to determine oxalate, calcium, phosphorus, and magnesium level and kidney tissue were observed for histopathological changes. Glycolic acid feeding resulted in a significant increase in serum oxalate, calcium, and phosphorus level with a decrease in magnesium. Urinary level of oxalate, calcium, and phosphorus was also increased with a decrease in sodium and potassium. C. *dactylon*, *E. officinalis*, *K. pinnata,* and *B. nutans* markedly ameliorates glycolic acid induced nephrolithiasis by reversing the abnormal serum and urinary levels of oxalate, calcium, and phosphorus. Ethyl acetate fraction of *E. officinalis* and *K. pinnata* showed decreased deposition of microcrystals in kidney tubules with a prominent reduction in glomerular and tubular damage. These observations indicate the prominent protective effect of *E. officinalis* and *K. pinnata* in the prevention of *Change Italic form to Normal form*.

INTRODUCTION

Nephrolithiasis is a common life threatening renal disease people get affected all over the world. Many factors can promote this disorder like some drugs, life style, climate, food habit etc. This disease is also associated with other disorder as chronic renal disease, bone loss, coronary disease, hypertension, type 2 diabetes, and other metabolic syndrome [1]. Nephrolithiasis is a complex process resulted from successive physicochemical events including supersaturation, nucleation, growth, aggregation, and retention within renal tubules [2]. Hyperoxaluria is one of the major risk factors of idiopathic calcium oxalate (CaOx) nephrolithiasis in human. The primary hyperoxaluria condition of the kidney was first time described by Lepoutre in 1925 [3]. Oxalate is a natural by-product of metabolism which is harmlessly excreted through the urine in normal individuals. However, hyperoxaluria or increased urinary excretion of oxalate can be dangerous due to its propensity to crystallize at physiologic pH and form CaOx crystals [4]. Epidemiological data showed that the majority of kidney stones are composed mainly of CaOx [5]. Most individuals have urine crystallization inhibiting capacity that does not allow nephrolithiasis to occur, but this natural inhibition is in deficit in stone formers [6].

Many remedies have been tried and tested to treat nephrolithiasis. In most cases, the management of nephrolithiasis involves both surgical and therapeutic approaches, which is relatively costly, painful, and require expert hands with the availability of appropriate equipment. Despite tremendous advances in the field of medicine, there is genuinely no satisfactory

drug available to treat renal calculi. Research in search of herbo-mineral preparations is still ongoing [7]. A large number of Indian medicinal plants are used in the treatment of nephrolithiasis, and they are reported to be active with no side effects [8]. The plants *C. dactylon* (Poaceae), *E. officinalis* (Euphorbiaceae), *K. pinnata* (Crassulaceae) and *B. nutans* (Graminae) have reported diuretic and antilithiatic properties against ethylene glycol and ammonium chloride, and glyoxalate induced urolithiasis in different animal models in our previous studies. We have reported rich presence of antioxidative phytocompounds like polyphenols and flavonoids in ethyl acetate fractions of *B. nutans* shoot, *E. officinalis* fruit, and *K. pinnata* leaf along with excellent in vitro CaOx crystal growth inhibition activity. We are exploring the whole spectrum of antiurolithiatic potential of the selected plants targeting the ethyl acetate fraction.

The study aims at the screening of *C. dactylon* whole plant, *E. officinalis* fruit, *K. pinnata* leaf and *B. nutan* shoot ethyl acetate fraction rich in flavonoid and polyphenol for antinephrolithiasis potential against glycolic acid rich diet feeding on the rat. *In-vivo* experimental induction of nephrolithiasis in the rat is commonly done with calcium oxalate and other oxalate metabolic intermediates like glycolate and ethylene glycol [9-11]. Ethylene glycol (EG) administration is a well-known model of nephrocalcinosis. EG metabolizes into glycolate, glyoxylate, and oxalate leading to CaOx crystal development in both urine and kidneys [12]. Glycolic acid and ethylene glycol are precursors of oxalic acid. Oxalic acid is produced by dehydrogenation of glycolic acid. Glycolate is oxidized to oxalate by lactate dehydrogenase enzyme [13,14]. Glycolic acid is an *in vivo* precursor of oxalate biosynthesis [15]. It is worth to study antiurolithiatic potential of *C. dactylon* whole plant, *E. officinalis* fruit, *K. pinnata* leaf and *B. nutan* shoot ethyl acetate fraction against urolithiasis induced by glycolic acid rich diet feeding.

MATERIALS AND METHODS

Collection and extraction of plant

The *C. dactylon* whole plant, *E. officinalis* fruit, *K. pinnata* leaf, and *B. nutan* shoot was collected in between October 2014 to April 2015 from Rewa, Raisen and Bhopal districts of M.P. Al the plants were authenticated by Dr. Zia Ul Hasan, Prof, and Head, Department of Botany, Saifia College of Science Bhopal, M.P., and voucher herbarium specimen (456/Bot/saifia/14) was maintained. Cleaned plant material was dried under the shade, pulverized to form a coarse powder and processed for hydro-methanolic extraction. Ethyl acetate fraction was enriched from hydro-methanolic extract following the method as described previously [16].

Experimental animal

In-vivo study was performed with due permission from the Institutional Animal Ethical Committee. Laboratory breed adult Wistar male rats (100-150gm) were housed in polypropylene cages with paddy husk bedding maintained at 22 ± 2C and 12 hr light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. All experimental procedures were conducted in accordance with the ethical guidelines of CPCSEA, New Delhi.

Study protocol

Based on the acute toxicity reported previously dose range selected for *E. officinalis*, *K. pinnata* and *B. nutans* is 25, 50 and 100 mg/kg, and 100, 300 and 500mg/kg p.o., for *C.* dactylon [17]. The 90 rats were divided into fifteen groups comprising of six animals in each group. All the animals were treated as per the protocol daily for 28 days to assess the effect of ethyl acetate fraction (EA) of *C. dactylon, E. officinalis, K. pinnata,* and *B. nutan*. Glycolic acid 3% (w/v) containing diet was used as nephrolithiasis inducing agent [10]. The animals were grouped as follows:

GROUP 1: Fed with the standard diet for 28 days (Vehicle control).

GROUP 2: Glycolic acid diet (Negative control).

GROUP 3: Glycolic acid diet + 750 mg/kg Cystone (Positive control).

GROUP 4: Glycolic acid diet + 100 mg/kg EA *C. dactylon*

GROUP 5: Glycolic acid diet + 300 mg/kg EA *C. dactylon*

GROUP 6: Glycolic acid diet + 500 mg/kg EA *C. dactylon*

GROUP 7: Glycolic acid diet + 25 mg/kg EA *E. officinalis*

GROUP 8: Glycolic acid diet + 50 mg/kg EA *E. officinalis*

GROUP 9: Glycolic acid diet + 100 mg/kg EA *E. officinalis*

GROUP 10: Glycolic acid diet + 25 mg/kg EA *K. pinnata*

GROUP 11: Glycolic acid diet + 50 mg/kg EA *K. pinnata*

GROUP 12: Glycolic acid diet + 100 mg/kg EA *K. pinnata*

GROUP 13: Glycolic acid diet + 25 mg/kg EA *B. nutan*

GROUP 14: Glycolic acid diet + 50 mg/kg EA *B. nutan*

GROUP 15: Glycolic acid diet + 100 mg/kg EA *B. nutan*

Vehicle control rats had fed with regular diet along with free access to drinking water *ad libitum*. Rats of groups 2 to 15 also had free access to the normal diet containing additional 3% glycolic acid (w/v) for 28 days in order to promote hyperoxaluria and CaO_x crystal deposition in the kidney. Animals of group 4 to 15 were treated with plant extracts per orally daily for 28 days.

Estimation of urine biochemical parameters: All the animals were kept in individual cages, and urine samples of 24 hr were collected on 28th day after the last dosing with free access to water. Urine samples were analyzed for the level of oxalate, calcium, phosphates, sodium, and potassium with the help of Autoanalyser (Star 21 Plus, Rapid Diagnostics, India) using diagnostic kits (Span Diagnostics Ltd, India).

Estimation of serum biochemical parameters: The animals were sacrificed on 29th day under ether anesthesia followed by cardiac puncture. Blood was collected in a clean and dry test tube immediately after sacrifice and allowed to coagulate for 30 min in room temperature. Serum was separated by centrifugation at 3000 rpm for 10 min, and the supernatant was collected and used for estimation of oxalate, magnesium, calcium, and phosphates using diagnostic kits (Span Diagnostics Ltd., India) and Autoanalyser (Star 21 Plus, Rapid Diagnostics, India).

Histopathology of kidney: Hematoxylin-eosin stained kidney tissue sections were observed for histopathological changes and photographed using an optical microscope at 10× magnification [18].

Statistical analysis

The values in tables were expressed as Mean ± SEM. Statistical analysis was performed using one-way analysis of variance ANOVA followed by Dunnett multiple comparison tests. A *P* value of less than 0.05 was considered statistically significant.

RESULT

Estimation of urine biochemical parameters

Nephrolithiasis induction by dietary glycolic acid is confirmed as it showed significant (P < 0.001) elevation of the urine biochemical parameters, i.e., oxalate, calcium, and Inorganic phosphates and decrease in sodium and potassium compared to the negative control group. The Ca/Ox ratio in the negative control group was found to be 0.372 compared to 0.362 of the vehicle control group. The ethyl acetate fraction of *C. dactylon* at 300 and 500 mg/kg dose showed significantly (P < 0.01 – 0.001) decreased urinary excretion of oxalate, calcium and inorganic phosphates with increased excretion of sodium and potassium. The Ca/Ox ratio is moderately increased as 0.507 and 0.487, respectively at 300 and 500 mg/kg dose. Urinary excretion of oxalate, calcium and inorganic phosphates was decreased significantly (P < 0.01 - 0.001) with an increase in sodium and potassium level by *E. officinalis* ethyl acetate fraction at 50 and 100 mg/kg dose. The Ca/Ox ratio is slightly increased as 0.415 and 0.427, respectively at 50 and 100 mg/kg dose.

K. pinnata leaf ethyl acetate fraction at 50 and 100 mg/kg dose significantly (P < 0.05 – 0.001) decreased urinary excretion of oxalate, calcium, and inorganic phosphates along with an increase in sodium and potassium level. Ca/Ox ratio are also slightly increased with a value of 0.442 and 0.462, respectively at 50 and 100mg/kg dose of *K. pinnata* ethyl acetate fraction. *B. nutan* ethyl acetate fraction at 50 and 100 mg/kg dose showed significant (P < 0.05-0.01) decrease in urinary excretion of oxalate, calcium, and inorganic phosphates with an increase in sodium and potassium excretion. *B. nutan* ethyl acetate fraction does not show a measurable difference in Ca/Ox ratio as compared to vehicle and negative control groups (Table 1).

Effect on serum biochemical parameters

The serum sample of glycolic acid induced nephrolithiatic negative control group animals showed significant (P < 0.01-0.001) increase in oxalate, calcium, and phosphorus with a decrease in magnesium concentration as compared to the vehicle control group. *C. dactylon ethyl acetate fraction* at 300 and 500 mg/kg dose showed significant (P < 0.01–0.001) reduction in elevated levels of oxalate, calcium, and phosphorus, and also increased magnesium level compared to the negative control group. *E. officinalis* ethyl acetate fraction at 50 and 100 mg/kg dose has significantly (P < 0.05 - 0.001) decreased oxalate, calcium, and phosphorus, and increased magnesium compared to the negative control group. Ethyl acetate fraction of *K. pinnata* at 50mg/kg dose has significantly (P < 0.05 - 0.01) decreased oxalate and calcium level of blood with an increase in magnesium level compared to the negative control group. At 100 mg/kg *K. pinnata* ethyl acetate fraction showed significant (P < 0.05 - 0.001) reversal of all four blood parameters. *B. nutan* ethyl acetate fraction at 50mg/kg dose has significantly (P < 0.05 - 0.01) decreased oxalate, calcium, and phosphorus, but with non-significant effect on magnesium level, whereas at 100 mg/kg dose *B. nutan* ethyl acetate fraction has normalized all the disturbed blood parameters (Table 2).

Histopathology of kidney

The tissue section of rat kidney treated with glycolic acid rich diet showed heavy deposition of CaOx microcrystals in tubules, moderate glomerular and tubular damage along with tubular dilation. Damage of the epithelial cell lining occurred with degeneration and thickening. Crystal deposition resulted in glomerular and peritubular congestion, blood vessel congestion, epithelial desquamation, and cellular inflammation.

Cystone moderately reduced CaOx microcrystals deposition, glomerular and tubular damage, epithelial cell damage, and inflammation. *C. dactylon ethyl acetate fraction* treated groups at 500 mg/kg dose showed an effective reversal of these symptoms and very less deposition of microcrystal in tubules with the presence of inflammatory damage. Ethyl acetate fraction of *E. officinalis* at 50 and 100mg/kg dose showed decreased deposition of microcrystals in kidney tubules with a noticeable reduction in glomerular and tubular damage. Tubular dilation and damage have also been reversed effectively by *E. officinalis* but with the presence of some inflammatory cell infiltration. The kidney tissue sections of *K. pinnata* ethyl acetate fraction treated group at 50, and 100mg/kg dose showed a moderate reduction in glomerular and epithelial damage and tubular dilation, though microcrystal deposition and inflammatory infiltration was not reversed effectively. The kidney tissue section of different doses of *B. nutan* ethyl acetate fraction treated groups showed a nominal reversal of glycolic acid induced histological damages with deposition of microcrystals in tubules, but a reduction in glomerular and tubular damage (Figure 1).

DISCUSSION

In the modern system of medicine treatment for urolithiasis is expensive and dissatisfactory as proper drug therapy is not available for clearing the kidney stones. Patients usually depend on alternative systems of medicine for getting relief of kidney stone [19]. Plant based phytotherapeutic agents are used for treating urolithiasis as they are efficacious and have lesser side effects and reduce the recurrence rate of renal stone [20]. Our study aims at exploration whole profile of antiurolithiatic potential of *C. dactylon, E. officinalis, K. pinnata* and *B. nutan* ethyl acetate fraction against all possible mechanisms of nephrolithiatic complications. The beneficial effects of *C. dactylon, E. officinalis, K. pinnata* and *B. nutan* ethyl acetate fraction against EG and ammonium chloride, and glyoxalate induced nephrolitisaia has already been reported along with in vitro CaOx crystal dissolution efficacy.

In human nephrolithiasis, most stones contain calcium and are located within urinary cavities as monohydrate calcium oxalate, dihydrate calcium oxalate and/or calcium phosphates in various proportion. Oxalate urinary calculi can be experimentally

Table 1: Urine biochemical parameter of glycolic acid induced nephrolithiatic rats treated with ethyl acetate fraction of *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutan.*

Treatment group (mg/kg)	Oxalate mg/24h	Calcium mg/24h	Inorganic Phosphorus mg/24h	Sodium mg/24h	Potassium mg/24h	Ca/Ox ratio
Vehicle control	14.68 ± 1.13	5.32 ± 0.35	0.98 ± 0.02	11.23 ± 1.31	12.88 ± 1.31	0.362
Negative control	43.96 ± 2.64***	16.36 ± 1.21***	4.27 ± 0.85**	4.67 ± 0.81***	7.23 ± 0.44***	0.372
Cystone (750)	19.87 ± 1.35^{ns,c}	7.65 ± 0.42^{ns,c}	1.87 ± 0.54^{ns,b}	10.96 ± 1.24^{ns,c}	11.49 ± 1.30^{ns,c}	0.385
EACD (100)	36.43 ± 1.91^{**,a}	14.47 ± 1.33^{***,ns}	2.82 ± 0.61^{**,a}	6.55 ± 0.81^{***,a}	9.87 ± 0.92^{**,a}	0.397
EACD (300)	25.63 ± 1.83^{*,b}	13.01 ± 1.34^{**,b}	2.35 ± 0.24^{**,b}	8.36 ± 0.62^{**,b}	10.01 ± 0.66^{ns,b}	0.507
EACD (500)	21.01 ± 1.41^{ns,c}	10.24 ± 1.03^{*,c}	1.91 ± 0.13^{*,b}	9.42 ± 0.79^{ns,c}	10.65 ± 0.79^{ns,c}	0.487
EAEO (25)	27.21 ± 1.22^{*,b}	12.98 ± 1.04^{***,a}	3.11 ± 0.56^{**,a}	6.32 ± 0.26^{***,ns}	8.21 ± 0.98^{*,ns}	0.477
EAEO (50)	25.33 ± 1.58^{*,b}	10.52 ± 0.71^{**,c}	2.89 ± 0.26^{*,b}	8.96 ± 0.61^{**,b}	9.98 ± 0.57^{ns,b}	0.415
EAEO (100)	20.97 ± 1.62^{ns,c}	8.96 ± 0.41^{ns,c}	2.16 ± 0.34^{ns,b}	9.28 ± 0.41^{ns,b}	10.14 ± 0.68^{ns,b}	0.427
EAKP (25)	39.43 ± 2.03^{***,ns}	15.23 ± 1.32^{***,ns}	3.67 ± 0.47^{**,ns}	5.21 ± 0.30^{***,ns}	8.69 ± 0.45^{**,a}	0.386
EAKP (50)	28.88 ± 1.01^{**,a}	12.78 ± 1.10^{***,a}	2.62 ± 0.31^{*,a}	7.88 ± 0.74^{*,b}	9.36 ± 0.68^{*,b}	0.442
EAKP (100)	24.22 ± 1.13^{*,b}	11.21 ± 1.07^{**,b}	2.13 ± 0.23^{ns,b}	8.33 ± 0.95^{ns,c}	10.66 ± 0.80^{ns,c}	0.462
EABN (25)	38.66 ± 1.84^{**,a}	13.12 ± 1.02^{***,a}	3.74 ± 0.51^{***,ns}	5.96 ± 0.33^{***,ns}	8.43 ± 0.97^{**,a}	0.339
EABN (50)	31.21 ± 1.34^{**,b}	11.98 ± 1.11^{**,b}	3.16 ± 0.41^{***,a}	7.98 ± 0.37^{**,b}	9.28 ± 0.89^{*,b}	0.383
EABN (100)	27.98 ± 1.27^{*,b}	9.22 ± 1.06^{*,b}	2.91 ± 0.37^{*,b}	8.65 ± 0.20^{*,b}	9.88 ± 0.84^{n*,b}	0.329

All the values are Mean ± SEM of six animals per group. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ and ns = not significant compared to vehicle control values. $^{c}P< 0.001$,$^{b}P< 0.01$, $^{a}P< 0.05$ and ns = not significant compared to negative control values. EACD = ethyl acetate fraction of *C. dactylon*, EAEO = ethyl acetate fraction of *E. officinalis,* EAKP = ethyl acetate fraction of *K. pinnata*, EABN = ethyl acetate fraction of *B. nutan.*

Table 2: Serum biochemical parameter of glycolic acid induced nephrolithiatic rats treated with ethyl acetate fraction of *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutan.*

Treatment group (mg/kg)	Oxalate mg/dl	Magnesium mmol/L	Calcium mg/dl	Phosphorus mg/dl
Vehicle control	1.97 ± 0.20	2.96 ± 0.31	8.49 ± 0.65	5.65 ± 0.62
Negative control	4.66 ± 0.36***	1.21 ± 0.04**	12.89 ± 1.03**	8.47 ± 0.84**
Cystone (750)	2.04 ± 0.34^{ns,c}	2.48 ± 0.27^{ns,b}	8.91 ± 0.79^{ns,c}	6.14 ± 0.78^{ns,b}
EACD (100)	3.95 ± 0.17^{**,ns}	2.03 ± 0.23^{*,ns}	10.42 ± 1.11^{**,ns}	7.97 ± 0.91^{*,a}
EACD (300)	2.87 ± 0.17^{*,b}	2.65 ± 0.28^{ns,a}	9.71 ± 0.72^{*,a}	7.27 ± 0.73^{*,a}
EACD (500)	2.25 ± 0.20^{ns,b}	2.98 ± 0.24^{ns,b}	9.15 ± 0.63^{ns,b}	6.64 ± 0.64^{ns,b}
EAEO (25)	3.98 ± 0.51^{**,a}	1.95 ± 0.18^{*,a}	10.75 ± 1.14^{*,a}	8.15 ± 0.64^{**,ns}
EAEO (50)	2.74 ± 0.30^{*,b}	2.26 ± 0.38^{ns,b}	9.47 ± 0.82^{ns,b}	7.53 ± 0.57^{*,a}
EAEO (100)	2.14 ± 0.24^{ns,c}	2.37 ± 0.28^{ns,b}	9.98 ± 0.61^{ns,c}	6.80 ± 0.54^{ns,b}
EAKP (25)	3.14 ± 0.41^{**,a}	1.94 ± 0.25^{**,ns}	11.23 ± 1.21^{**,ns}	8.13 ± 0.74^{***,ns}
EAKP (50)	2.96 ± 0.21^{*,b}	2.14 ± 0.21^{*,a}	10.97 ± 1.11^{**,b}	7.98 ± 0.57^{**,ns}
EAKP (100)	2.47 ± 0.22^{ns,c}	2.28 ± 0.18^{ns,a}	9.99 ± 0.75^{*,c}	7.32 ± 0.48^{*,a}
EABN (25)	3.54 ± 0.22^{**,a}	1.48 ± 0.05^{**,ns}	11.87 ± 0.63^{**,ns}	8.05 ± 0.52^{**,ns}
EABN (50)	2.70 ± 0.23^{*,b}	1.85 ± 0.07^{*,ns}	10.28 ± 0.54^{*,b}	7.48 ± 0.46^{*,a}
EABN (100)	2.44 ± 0.24^{ns,b}	2.08 ± 0.18^{*,a}	9.25 ± 0.37^{ns,b}	6.95 ± 0.41^{ns,b}

All the values are Mean ± SEM of six animals per group. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ and ns = not significant compared to vehicle control values. $^{c}P< 0.001$,$^{b}P< 0.01$, $^{a}P< 0.05$ and ns = not significant compared to negative control values. EACD = ethyl acetate fraction of *C. dactylon*, EAEO = ethyl acetate fraction of *E. officinalis,* EAKP = ethyl acetate fraction of *K. pinnata*, EABN = ethyl acetate fraction of *B. nutan.*

induced in male rats by single large glycolic acid oral dosing [21], or by diet feeding for 4 to 6 weeks [10,15,22,23]. Administration of glycolic acid diet for four weeks leads to hyperoxaluria and CaOx tubular crystal deposition not only within cortex and medulla but also in pelvic cavities [22]. Glycolic acid administration increases the urinary calcium level. It has been stated that hypercalciuria favors precipitation of CaOx from urine [24]. Glycolic acid diet provokes hyperoxaluria, which is due to the fast conversion of glycolic acid to oxalate by the oxalate synthesizing enzyme, glycolic acid oxidase and lactate dehydrogenase in the liver.

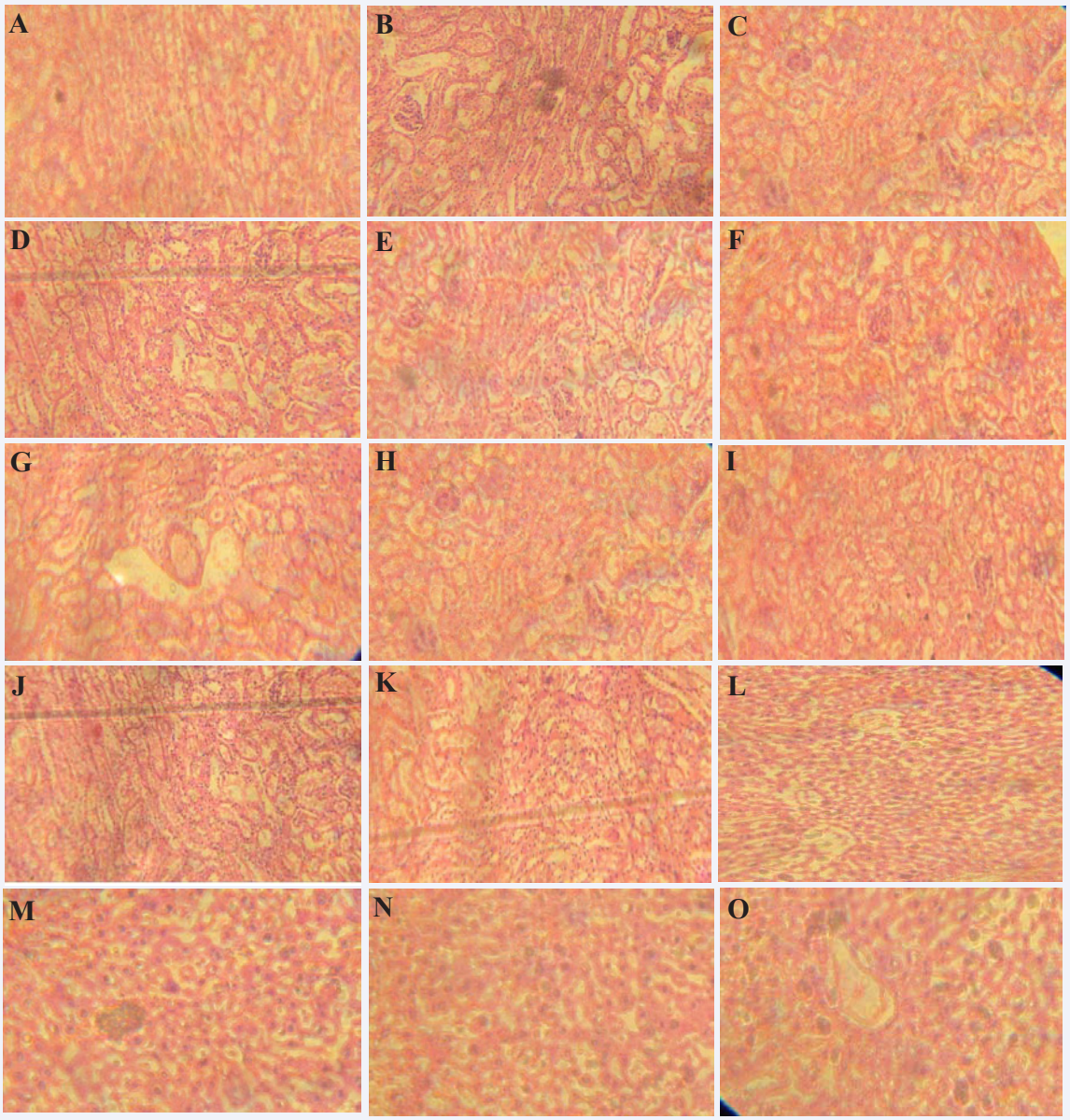

Figure 1 Kidney histopathology of glycolic acid induced nephrolithiatic rats treated with ethyl acetate fraction of *C. dactylon, E. officinalis, K. pinnata,* and *B. nutan*.
Vehicle control (A); Glycolic acid diet (B); Cystone 750 mg/kg (C); EACD 100 mg/kg (D); EACD 300 mg/kg (E); EACD 500 mg/kg (F); EAEO 25 mg/kg (G); EAEO 50 mg/kg (H); EAEO 100 mg/kg (I); EAKP 25 mg/kg (J); EAKP 50 mg/kg (K); EAKP 100 mg/kg (L); EABN 25 mg/kg (M); EABN 50 mg/kg (N); EABN 100 mg/kg (O). EACD = *C. dactylon* ethyl acetate fraction, EAEO = *E. officinalis* ethyl acetate fraction, EAKP = *K. pinnata* ethyl acetate fraction and EABN = *B. nutan* ethyl acetate fraction.

Glycolic acid is known to increase the incidence of oxalate lithiasis, and our results are in concord with these reports [25].

Cystone has reduced serum, and urinary oxalate levels may be due to its inhibitory action on oxalate synthesizing liver enzyme indicating that cystone can play an essential role in the prevention of disorders associated with kidney stone formation [26]. Ethyl acetate fractions of *E. officinalis, K. pinnata, C. dactylon,* and *B. nutan* have significantly reversed elevated serum and urinary calcium, oxalate and phosphorous levels. *E. officinalis, K. pinnata,* and *C. dactylon* has moderately increased Ca/Ox ratio of urine. Glycolic acid rich diet feeding showed heavy deposition of CaOx microcrystals in tubules along with tubular dilation and tubular damage. *E. officinalis* showed decreased deposition of microcrystals in kidney tubules with a prominent reduction in glomerular and tubular damage whereas *K. pinnata* and *C. dactylon* showed a moderate reduction in glomerular and epithelial damage and tubular dilation, though microcrystal deposition and inflammatory infiltration was not reversed effectively. Ethyl acetate fraction of *E. officinalis* and *K. pinnata* showed most potential antilithiatic effect against glycolic acid induced urolithiasis.

Phytocompounds exert antilithogenic effect by altering the ionic composition of urine, decreasing the calcium ion concentration or increasing magnesium and citrate excretion

[27]. The plants with additional diuretic activity also have good lithotriptic activity [4]. Herbal medicine contains groups of phytocompounds that are capable of minimizing tissue injury enabled with multiple mechanisms of protective action [28]. Among the four plants, *K. pinnata* ethyl acetate fraction had the highest content of total flavonoid and polyphenol whereas *B. nutans* had the lowest flavonoid content through its polyphenol content was rich [16]. Ethyl acetate fractions of *K. pinnata*, *C. dactylon* and *E. officinalis* showed potential diuretic activity [17]. Ethyl acetate fraction of *K. pinnata* and *E. officinalis* was most effective against ethylene glycol induced nephrolithiasis on rats [29], whereas *K. pinnata* and *B. nutan* were highly effective on mice against glyoxylate nephrolithiasis [30].

Acquired and genetic kidney disorders are associated with excessive oxalate excretion, in particular with primary hyperoxaluria types I [31]. Oxalate is a useless metabolic end product, formed as a byproduct of the metabolism of glyoxylate and ascorbate, excreted in the urine where it constitutes a hazard because of the insolubility as the calcium salt. Glycolic acid (or hydroxyacetic acid) is the smallest α-hydroxy acid. Determination of oxalate, as well as glycolic acid, is essential for the diagnosis of primary hyperoxaluria type I (glycolic aciduria), caused by the low or absent activity of the liver-specific peroxisomal alanine:glyoxylate aminotransferase [32]. Hyperoxaluria induction with glyoxylate and glycolic acid treatment produced an increase in glycolic acid oxidase and lactate dehydrogenase enzyme level [26,33,34]. High content of glycolic acid can modify normal tubular epithelium into a crystal-binding epithelium [35]. The observed antiurolithiasis activity of E. officinalis and K. pinnata ethyl acetate fraction can be correlated to the concerted effect of flavonoids and polyphenolic compounds. Amla (E. officinalis) containing tannins, flavonoids, phenolic compounds, saponins, terpenoids, especially chebulinic acid, chebulagic acid, emblicanin, gallic acid, ellagic acid, and quercetin is highly valued in traditional Indian medicine [36]. Bogucka-Kocka et al., reported rich quantity of ferulic and caffeic acid in K. pinnata leaf [37]. All these phenolic and flavonoid constituents are well known for antioxidant and anti-inflammatory activity. Ethyl acetate fraction of all the four plants had shown a highly significant decrease in lactate dehydrogenase level of kidney tissue of mice treated with glyoxalate [30]. The results signify that E. officinalis and K. pinnata have modulating effect on down regulation of oxalate synthesis in rat kidney followed by glycolic acid dietary intake may be via antioxidant and antiinflammatory activity along with inhibition of oxalate synthesizing enzymes.

CONCLUSION

This study substantiates the excellent antiurolithiasis potential of *E. officinalis* and *K. pinnata* against urolithiasis induced by glycolic acid feeding for 28 days. *K. pinnata* and *E. officinalis* ethyl acetate fractions exerted crystal growth inhibition properties along with the noteworthy reversal of urine and serum abnormal parameters. The observed potent antiurolithiatic activity of *K. pinnata* and *E. officinalis* ethyl acetate fraction with rich presence of flavonoid and polyphenol may be due to antioxidant and bioprotective mechanisms on the renal epithelium.

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