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Protective Effect of Propolis in Proteinuria, Crystaluria, Nephrotoxicity and Hepatotoxicity Induced by Ethylene Glycol Ingestion

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Background and Aims. Propolis is a natural honeybee product with wide biological activities and potential therapeutic properties. The aim of the study is to evaluate the protective effect of propolis extract on nephrotoxicity and hepatotoxicity induced by ethylene glycol in rats.

Methods. Five groups of rats were used. Group 1 received drinking water, group 2 received 0.75% ethylene-glycol in drinking water, group 3 received 0.75% ethylene-glycol in drinking water along with cystone 500 mg/kg/body weight (bw) daily, group 4 received 0.75% ethylene-glycol in drinking water along with propolis extract at a dose of 100 mg/kg/bw daily, and group 5 received 0.75% ethylene-glycol in drinking water along with propolis extract at a dose of 250 mg/kg/bw daily. The treatment continued for a total of 30 d. Urinalyses for pH, crystals, protein, creatinine, uric acid and electrolytes, and renal and liver function tests were performed.

Results. Ethylene-glycol increased urinary pH, urinary volume, and urinary calcium, phosphorus, uric acid and protein excretion. It decreased creatinine clearance and magnesium and caused crystaluria. Treatment with propolis extract or cystone normalized the level of magnesium, creatinine, sodium, potassium and chloride. Propolis is more potent than cystone. Propolis extract alleviates urinary protein excretion and ameliorates the deterioration of liver and kidney function caused by ethylene glycol.

Conclusions. Propolis extract has a potential protective effect against ethylene glycol induced hepatotoxicity and nephrotoxicity and has a potential to treat and prevent urinary calculus, crystaluria and proteinuria.

Key Words: Propolis, Cystone, Ethylene glycol, Liver, Kidney, Toxicity, Crystal, Proteinuria.

Introduction

Ethylene glycol (EG) is a synthetic chemical liquid used in almost all radiator fluid products and used as solvent, emulsifier or surfactant. Its metabolites include glycolic acid, glyoxylic acid and oxalic acid. EG is a common cause of overdose and toxicity and also commonly used to induce nephrolithiasis (1,2). Ingestion of EG causes renal injury and exposure to EG in industries causes impairment in liver and kidney functions (3,4).

Toxicity occurs after EG is converted to its metabolites, glycolic acid and oxalic acid, which cause central nervous system and cardiovascular dysfunction, severe metabolic acidosis, and acute kidney failure (5–7). Calcium oxalate crystals accumulate in blood and other tissues including the renal cortex that result in kidney injury (8). In addition to supportive care, treatment of EG toxicity includes intravenous fomepizole, which inhibits alcohol dehydrogenase or hemodialysis (6,9–11). Reduction of urinary oxalate and other crystal levels can decrease calcium oxalate depositions and stone formation. There is no effective treatment that targets oxalate biosynthesis.

Propolis or bee glue is a resinous hive product that honeybees (*Apis mellifera L.*) collect from various plant species. Honeybees collect propolis from cracks in the bark

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of trees and leaf buds and enrich it with their salivary enzymes and beeswax. Propolis contains highly complex and variable chemical compositions, which are directly related to that of bud exudates. Basically, propolis is composed of 30% wax, 10% essential and aromatic oils, 50% resin and vegetable balsam, 5% pollens and 5% various other substances that include organic compounds and minerals (12).

Cystone is a polyherbal formulation, which is used for antilithic activity in traditional medicine at doses of 500 and 750 mg/kg/bw. It has a protective effect against experimentally induced urolithiasis in rats (13). Cystone is used to prevent and facilitate passage of cysteine kidney stones (13).

Propolis has antimicrobial, antifungal, antioxidant, anti-inflammatory, antitumor, radioprotective, and anti-ulcer activities as well as wound healing properties (14–20). These properties make propolis a candidate to be tested in inflammatory or pathological conditions such as toxicity challenges as well as due to a pathological entity resulting from the high oxidative process. Therefore, the objective of the present study was to assess the effects of hydro-alcoholic extract of propolis (HAEP) as a preventive agent in EG-induced nephrotoxicity and hepatotoxicity in rats.

Materials and Methods

Experimental Animals

Adult male Wistar rats (150–220 g) were obtained from the Animal Housing Breeding Center, Department of Biology, Faculty of Sciences, Fés, Morocco and were used for the experiments. Animals were housed under standard environmental conditions (25 ± 1°C, 55 ± 5% humidity and 12 h/12 h light/dark cycle) and were maintained with free access to water and laboratory rat chow. All experiments were conducted in accordance with the internationally accepted principles for the care and use of laboratory animals. Approval from the ethics committee at the Faculty of Sciences, Fés, Morocco was obtained.

Collection and Extraction of Propolis

HAEP was prepared from propolis obtained from colonies of honeybees in the region of Salé (Morocco). The collected propolis was frozen at −20°C and ground in a chilled mortar. The ground powder (30 g) was then extracted with the use of 100 mL of ethanol 70% at ambient temperature and maceration under agitation for 1 week. The solution was then filtered through a Whatman filter paper and concentrated in a rotary evaporator under reduced pressure to get a solid residue. The residue was dissolved in a minimal volume of ethanol and stored at −20°C until use. During the experiment, distilled water was added to obtain the required propolis concentration that was given to the animals daily by gavage for 30 d.

Experimental Design

Animals were housed in metabolic cages 3 days prior to the start of the experiment for adaptation and they were divided into five groups, each containing six animals. Group I (control group) animals were maintained on regular food and received only drinking water ad libitum for 30 d. Group 2 (EG group) animals received 0.75% EG in drinking water ad libitum for 30 d. Group 3 (EG-cystone group) animals received 0.75% EG in drinking water ad libitum along with cystone 500 mg/kg/body weight (bw) daily by gavage for 30 d. Group 4; (EG-propolis 100 mg group) animals received 0.75% EG in drinking water ad libitum along with HAEP at a dose of 100 mg/kg/bw daily by gavage for 30 d. Group 5 (EG-propolis 250 mg group) animals received 0.75% EG in drinking water ad libitum along with HAEP at a dose of 250 mg/kg/bw daily by gavage for 30 d.

Collection and Urinalysis

Animals were kept in metabolic cages individually for the collection of 24-h urine samples on days 0, 7, 14, 21 and 30 of treatment. Urine pH and urinary volume were measured immediately after collection. Urine samples collected on day 30 were acidified by the addition of concentrated hydrochloric acid and stored at −20°C for determination of various parameters. Urine was analyzed for calcium, magnesium, inorganic phosphorus, sodium, potassium, chloride, creatinine, protein and uric acid. Urinary oxalate was not measured because the test was not available in Morocco.

Urinary Crystal Study

Urine was collected from all groups after 30 d of the interventions and microscopic examination was done to identify urinary crystals.

Blood Tests

After 30 d of the experiment, blood samples were collected from the anaesthetized animals in all groups by cardiac puncture. Blood was analyzed for creatinine, urea, uric acid, potassium, sodium, and magnesium. Creatinine clearance as a measure of renal function was calculated from serum and urinary creatinine levels. Hepatic function was evaluated by measuring serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Statistical Analysis

All data expressed are mean ± SEM for six rats in each group. All statistical comparisons between groups were done by one-way analysis of variance (ANOVA) followed by post hoc Tukey's Multiple Comparison Test using Graph Pad Prism 5 software.

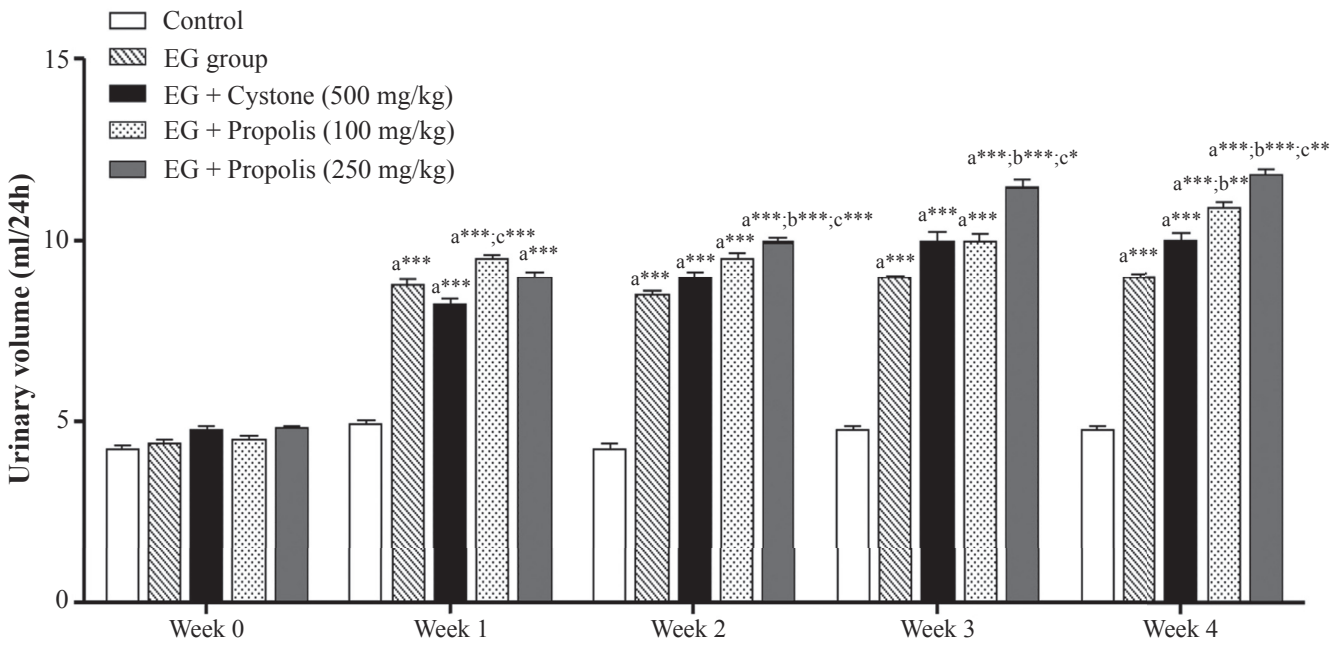


Figure 1. Effect of hydro-alcoholic extract of propolis on urinary volume in control and experimental animals during the 30-d treatment period. [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups. [c]Comparison between EG + cystone group and EG + propolis 100 and EG + propolis 250 groups. [d]Comparison between EG + propolis 100 group and EG + propolis 250 group. *p <0.05; **p <0.01; ***p <0.001. EG, ethylene glycol.

Results

Effect of the Interventions on Urinary Volume and pH

Chronic administration of 0.75% (v/v) EG aqueous solution caused a significant increase in urinary volume (p <0.001) in rats. On day 30, urinary output was 4.75 ± 0.35 mL/d in the control group and 9 ± 1.21 mL/d in the EG group. HAEP further increased urinary output when compared to the EG group during the 2nd, 3rd and 4th week of the experiment and when compared to the cystone group during the 3rd and 4th week of the experiment (Figure 1).

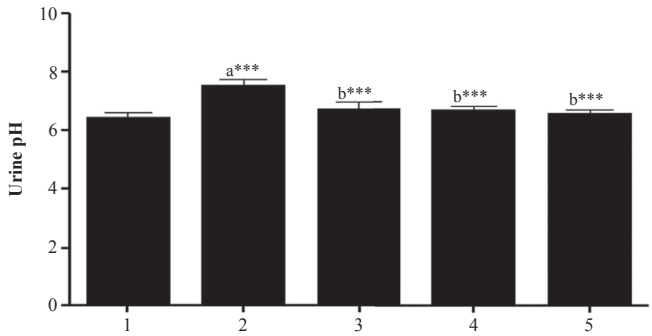
The effect of HAEP on urine pH level is presented in Figure 2. Urine pH level in the EG group was higher than the control group. On the other hand, cystone and HAEP did not cause significant changes in urine pH as compared to the control group when they were used along with EG.

Effects of the Interventions on the Urinary Excretion of Calcium, Phosphate and Magnesium

Administration of 0.75% (v/v) EG in drinking water to the male Wistar rats increased urinary excretion of calcium and phosphate and decreased urinary excretion of magnesium (Figure 3). However, HAEP and cystone decreased urinary excretion of calcium and phosphate as compared to the control group and increased urinary excretion of magnesium as compared to the EG group.

Effects of the Interventions on the Urinary Excretion of Creatinine, Uric Acid and Protein

EG significantly decreased the urinary excretion of creatinine (p <0.001). However, HAEP increased urinary excretion of creatinine at both doses as compared to the



1 Control
2 EG group
3 EG + Cystone (500 mg/kg)
4 EG + Propolis (100 mg/kg)
5 EG + Propolis (250 mg/kg)

Figure 2. Effect of hydro-alcoholic extract of propolis on urinary pH in control and experimental animals at day 30 of treatment. [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG +cystone, EG +propolis 100, and EG + propolis 250 groups. ***p <0.001. There is no significant difference in the urine pH among EG + cystone, EG + propolis 100, and EG + propolis 250 groups. EG, ethylene glycol.

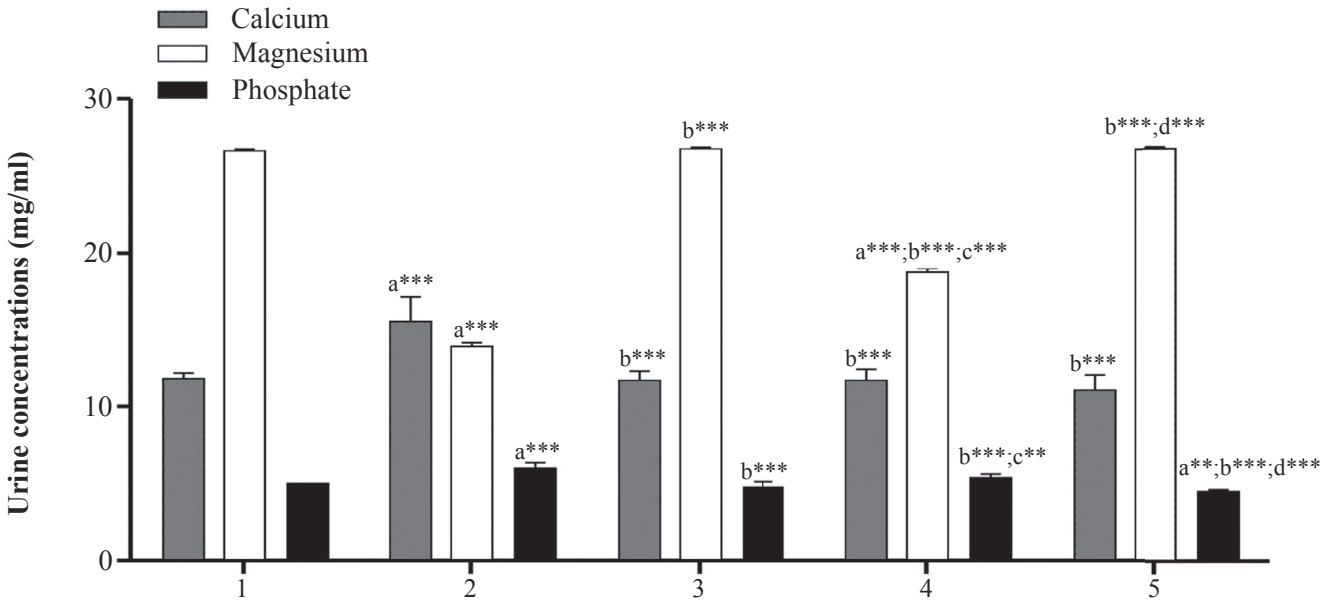


Figure 3. Effect of hydro-alcoholic extract of propolis on the concentration of urine calcium, phosphate and magnesium in control and experimental animals at the end of 30-d treatment period (mg in 24-h urine sample). [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups. [c]Comparison between EG + cystone group and EG + propolis 100 and EG +propolis 250 groups. [d]Comparison between EG + propolis 100 group and EG + propolis 250 group. $^{**}p < 0.01$. $^{***}p < 0.001$. EG, ethylene glycol.

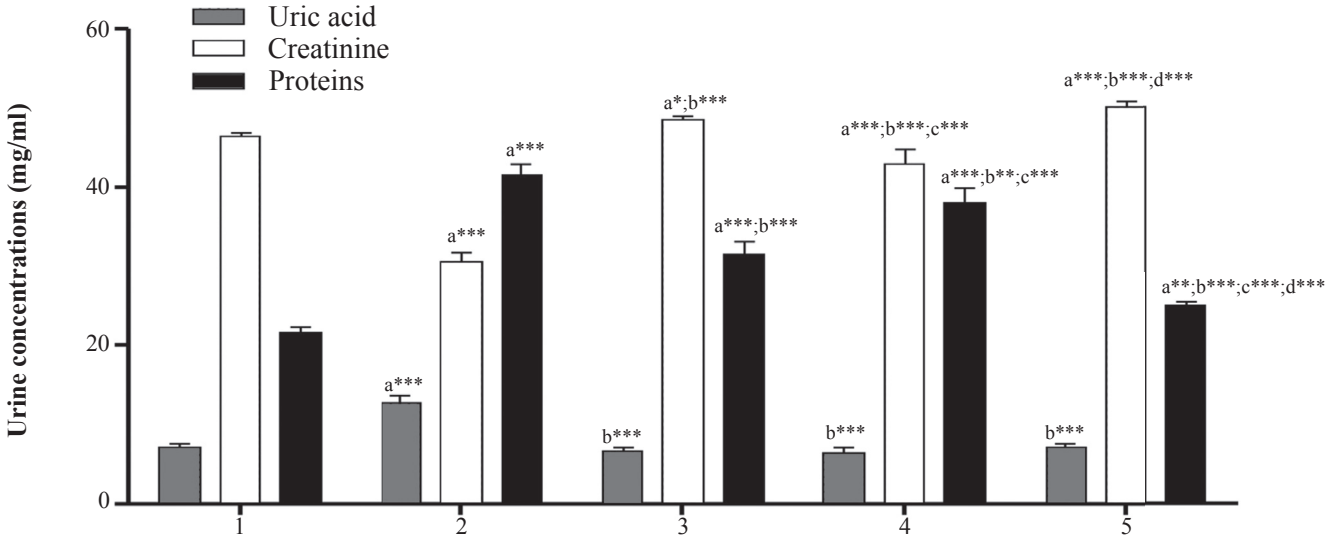
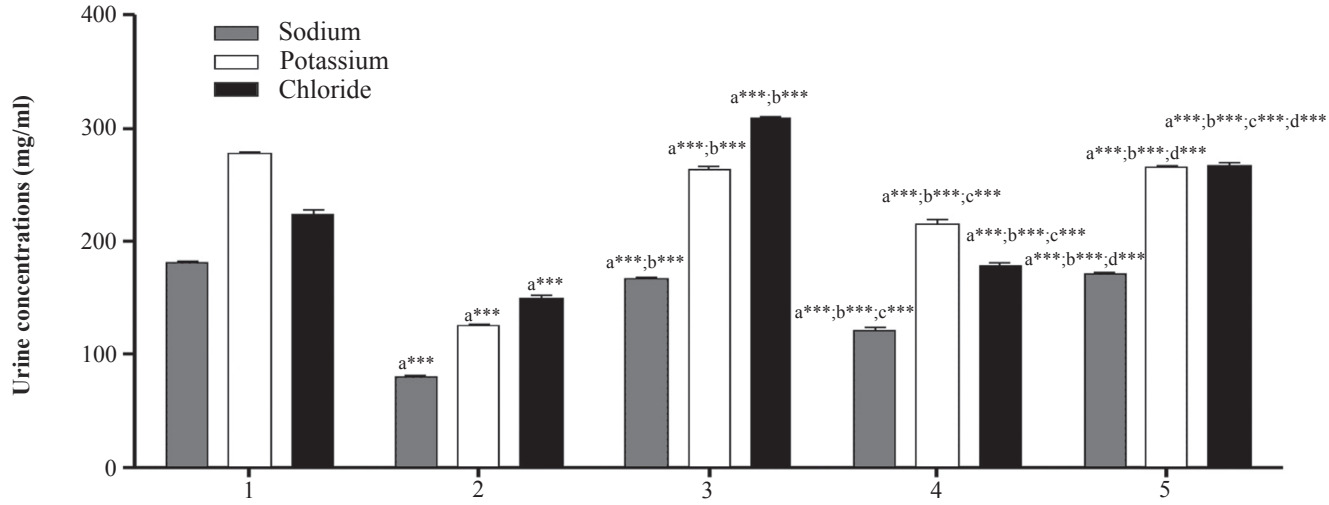


Figure 4. Effect of hydro-alcoholic extract of propolis on the concentration of creatinine, uric acid and proteins in normal and experimental animals at the end of 30-d treatment period (mg in 24-h urine sample). [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG +cystone, EG + propolis 100, and EG + propolis 250 groups. [c]Comparison between EG + cystone group and EG + propolis 100 and EG +mpropolis 250 groups. [d]Comparison between EG + propolis 100 group and EG + propolis 250 group. $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$. EG, ethylene glycol.



1 Control
2 EG group
3 EG + Cystone (500 mg/kg)
4 EG + Cystone (500 mg/kg)
5 EG + Cystone (500 mg/kg)

Figure 5. Effect of hydro-alcoholic extract of propolis on the concentration of urinary potassium, sodium and chloride in normal and experimental animals at the end of the 30-d treatment period (mg in 24-h urine sample). [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups. [c]Comparison between EG + cystone group and EG + propolis 100 and EG + propolis 250 groups. [d]Comparison between EG + propolis 100 group and EG + propolis 250 group. ***$p < 0.001$. EG, ethylene glycol.

EG group and the control. Higher dose of HAEP showed a higher effect than cystone (Figure 4). EG significantly increased urinary excretion of uric acid, whereas HAPE and cystone decreased it. Regarding urinary protein excretion, EG increased urine protein excretion, whereas cystone and HAPE decreased urinary protein excretion. The higher dose of HAEP has the highest effect on urinary protein and was more effective than cystone.

Effects of the Interventions on Urinary Electrolytes and Creatinine Clearance

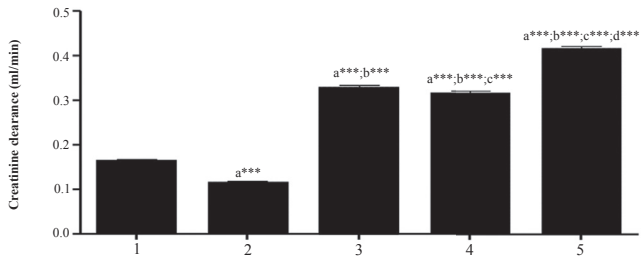
The effects of the interventions on urinary excretion of sodium, chloride and potassium are summarized in Figure 5. EG significantly decreased the urinary excretion of potassium, sodium and chloride. Cystone and HAPE counteracted the effect of EG and elevated the excretion of potassium, sodium and chloride as compared to the EG group. Furthermore, EG significantly decreased the creatinine clearance, whereas cystone and HAEP increased creatinine clearance. The effect was more pronounced with a higher dose of the propolis extract (Figure 6).

Urinary Crystal Study

Light microscopic study of the urinary crystals in the urine samples revealed that the crystals were absent in the urine of control animals, whereas in the EG treatment group, large numbers of various crystals were observed in the urine samples. Treatment with HAEP or cystone reduced the crystal number as well as the crystal size (Figure 7).

Effect of HAPE on Biochemical Parameters

Effects of the interventions on serum levels of creatinine, urea, uric acid and electrolytes are shown in Table 1. EG



1 Control
2 EG group
3 EG + Cystone (500 mg/kg)
4 EG + Propolis (100 mg/kg)
5 EG + Propolis (100 mg/kg)

Figure 6. Effect of hydro-alcoholic extract of propolis on creatinine clearance in control and experimental animals at the end of the 30-d treatment period. [a]Comparison between normal group and all groups. [b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups. [c]Comparison between EG + cystone group and EG + propolis 100 and EG + propolis 250 groups. [d]Comparison between EG + propolis 100 group and EG + propolis 250 group. ***$p <$ 0.001. EG, ethylene glycol.

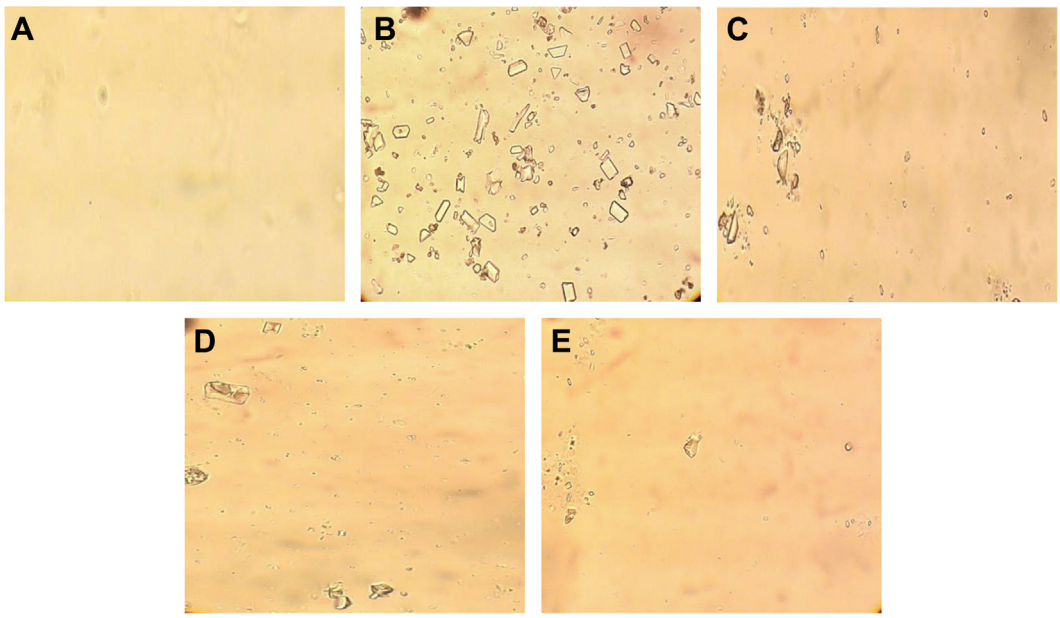


Figure 7. Light microscopic examination of the urine samples collected at the end of the 30-d control and treatment period showing multiple crystals. [a]Normal group. [b]EG group. [c]EG and cystone group. [d]EG + propolis at 100 mg/kg/bw. [e]EG + propolis at 250 mg/kg/bw. (A color figure can be found in the online version of this article.)

increased serum creatinine, urea and uric acid level as compared to the normal control. However, simultaneous administration of HAEP or cystone significantly decreased serum creatinine, urea and uric acid as compared to the EG group; HAPE at a dose of 250 mg/kg/bw decreased the parameters more than cystone. EG elevated serum potassium, sodium and magnesium. These changes were significantly normalized with the use of HAEP or cystone.

Effect of HAPE on Liver Enzymes

EG increase serum levels of AST, ALT and ALP as compared to the control group ($p < 0.001$). Cystone and HAEP decreased liver enzymes as compared to the EG group, and HAPE at a higher dose almost normalized the liver enzymes level (Table 2).

Discussion

The results of the present study showed that HAEP has a powerful protective effect against EG-induced nephrotoxicity and hepatotoxicity and potentially prevent urinary calculus risk factors. The higher dose of the HAEP is more effective than cystone to increase the urinary excretion of creatinine and creatinine clearance and to decrease the urinary protein excretion. These results are important because

Table 1. Effect of the interventions on serum level of various parameters at the end of the 30-d treatment period

	Normal group	EG group	EG + cystone 500 mg/kg^{-1}	EG + propolis 100 mg/kg^{-1}	EG + propolis 250 mg/kg^{-1}
Urea (mg/dL)	38.35 ± 2.03	44.66 ± 1.34[a,***]	39.03 ± 1.95[b,***]	40.15 ± 2.16[b,**]	38.75 ± 2.75[b,***]
Creatinine (mg/dL)	0.925 ± 0.02	1.65 ± 0.06[a,***]	1.02 ± 0.01[b,***]	1.03 ± 0.13[b,***]	0.99 ± 0.02[b,***]
Uric acid (mg/dL)	0.9 ± 0.15	1.7 ± 0.2[a,***]	1.25 ± 0.16[a,*,b,**]	1.13 ± 0.21[b,***]	1.03 ± 0.20[b,***]
Potasium (mmol/l)	4.2 ± 0.22	6.48 ± 0.67[a,***]	4.39 ± 0.21[b,***]	4.21 ± 0.32[b,***]	4.24 ± 0.44[b,***]
Sodium (mmol/l)	150.1 ± 2.53	159.76 ± 2.05[a,***]	152.72 ± 2.39[b,***]	152.25 ± 2.83[b,***]	151.34 ± 3.06[b,***]
Magnesium (mg/dL)	3.75 ± 0.04	4.6 ± 0.15[a,**]	4.21 ± 0.52	4.13 ± 0.57	3.94 ± 0.67
Proteins (g/l)	68.5 ± 0.49	76.81 ± 3.32[a,***]	71.83 ± 2.41[b,**]	73.65 ± 1.38[a,**]	71.3 ± 2.56[b,**]

EG, ethylene glycol.
There is no significant difference in the variables among EG + cystone, EG + propolis 100, and EG + propolis 250 groups.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a]Comparison between normal group and all groups.
[b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups.

Table 2. Effect of the interventions on liver enzymes at the end of the 30-d treatment period

	Normal group	EG group	EG + cystone 500 mg/kg^{-1}	EG + propolis 100 mg/kg^{-1}	EG + propolis 250 mg/kg^{-1}
AST (U/L)	145.5 ± 5.95	170.83 ± 5.68[a,***]	148.33 ± 6.06[b,***]	148.16 ± 6.44[b,***]	146.66 ± 3.85[b,***]
ALT (U/L)	61.5 ± 3.85	81 ± 2.98[a,***]	68.5 ± 4.40[a,*,b,***]	66.33 ± 2.90[b,***]	63.41 ± 2.16[b,***]
ALP (U/L)	392 ± 7.53	556 ± 9.72[a,***]	413.16 ± 4.19[a,***,b,***]	420.58 ± 4.95[a,***,b,***]	400.58 ± 6.44[b,***,c,*,d,***]

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; EG, ethylene glycol.
*p <0.05.
***p <0.001.
[a]Comparison between normal group and all other groups.
[b]Comparison between EG group and EG + cystone, EG + propolis 100, and EG + propolis 250 groups.
[c]Comparison between EG + cystone group and EG + propolis 100 and EG + propolis 250 groups.
[d]Comparison between EG + propolis 100 group and EG + propolis 250 group.

HAEP demonstrated hepatorenal protective effects and, therefore, could be a significant intervention for future testing in kidney failure and hepatic toxicity. Propolis and honey, another bee product, might alleviate proteinuria and renal failure in animals with diabetes and in animals treated with cytotoxic medications (Al-Waili 2016; unpublished).

The effects of EG demonstrated in the present study are similar to its effects reported elsewhere (21–27). Chronic administration of 0.75% (v/v) EG aqueous solution to male rats increased urinary calcium, phosphate, uric acid, magnesium, urea, and oxalate (22,24). Glycolic acid, the precursor of oxalic acid and a product of EG metabolism, significantly increases the incidence of oxalate lithiasis (7). Another study showed that EG also elevated serum levels of creatinine, uric acid, and BUN that indicates renal injury (22).

In the present study, chronic administration of EG in drinking water significantly increased urinary volume in rats. Use of HAEP also increased urinary output but more than EG. Increased urine volume reduces urinary calcium oxalate concentration and improves supersaturation and crystallization, ultimately preventing stone formation. However, in urolithiasis or excessive crystal deposition, it is expected that the glomerular filtration rate should be decreased due to the obstruction of urinary outflow. This causes accumulation of waste products in the blood, increasing blood urea, creatinine and uric acid. EG increased waste products in the blood despite the increased urinary output. However, with the use of HAPE, a pronounced increase in urinary volume was noticed without any increase in waste products in the blood. Therefore, HAEP prevents kidney damage caused by EG.

EG increased urinary calcium and phosphorus levels and decreased magnesium excretion in the 24-h urine samples. Furthermore, EG increased urinary phosphorus excretion, which facilitates formation of calcium phosphate stones and ultimately calcium oxalate stones. Interestingly, HAEP reduced the urinary excretion of calcium and phosphorus and increased urinary excretion of magnesium. This will prevent stone formation because calcium and phosphorus play a major role in renal stone formation. Cystone treatment also reduced the level of calcium and phosphorus. Increased urinary calcium causes nucleation and precipitation of calcium oxalate and calcium phosphate (28). In addition, increased urinary inorganic phosphate and oxalate excretion induce stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition. It was found that magnesium is a potent inhibitor of calcium oxalate crystallization and reduces the precipitation potential (29,30). In our experiment, EG decreased magnesium that was alleviated by the use of HAPE or cystone.

Regarding uric acid, another factor in stone formation (31), EG increased urinary excretion of uric acid and protein, plasma uric acid, and significantly decreased creatinine excretion in urine. Uric acid crystal adsorbs organic compounds and participates in calcium oxalate stone formation.

Protein excretion in the urine indicates a proximal tubular dysfunction. Furthermore, proteinuria may cause supersaturation of urinary colloids that result in precipitation and crystal initiation particle (26). Interestingly, treatment with HAEP or cystone significantly lowered the elevated level of urinary excretion of uric acid and protein and increased excretion of creatinine. HAPE (250 mg/kg/bw) was more effective than cystone. The effect of HAEP on urinary protein is very important. The ability of HAEP to decrease urinary excretion of uric acid might help dissolving the preformed stones and also help in the prevention of new calculus formation (22,31).

Hepatic function has been monitored by the measurement of the serum levels of ALT, AST and ALP. EG increased liver enzymes, which have been normalized by HAPE or cystone. It has been found that propolis has a protective effect against 2,3,7,8-tetrachlorodibenzo-p-dioxin, acute septic shock, and carbon tetrachloride-induced hepatic toxicity. The effect was most likely due to anti-inflammatory and antioxidant properties (32–34).

The mechanism of action of HAPE might be due to its antioxidant and anti-inflammatory effects. It was observed

that administration of EG increased MDA content of kidneys and decreased activity of the antioxidant enzymes (24). EG Hdismutase, and catalase levels (22,24). Furthermore, EG increases lipid peroxidation and decreases levels of antioxidant potential in the kidneys of rats (22). It was shown that the effect of high levels of oxalate has an impact on intracellular oxidative stress (35). In hyperoxaluric rat kidney, oxidative stress is a common feature that includes increased superoxide and H_2O_2-generating enzymes and lipid peroxidation products (36). Simultaneously, there is a major defect in the antioxidant system, which includes decreased superoxide dismutase, vitamin E, ascorbic acid, catalase, glutathione peroxidase, glucose-6 phosphate dehydrogenase, protein thiol, and glutathione (36).

Antioxidants have been shown to possess a protective effect against nephrotoxicity and nephrolithiasis induced by EG (22,36−38). Phenolic compounds present in *M. elengi* may prevent the lipid peroxidation-induced renal damage caused by calcium oxalate crystal deposition in renal tissue (22). Antioxidant therapy with vitamin E, glutathione monoester, methionine, lipoic acid, or fish oil normalized the cellular antioxidant system and prevented calcium oxalate precipitation in the rat kidney and reduced oxalate excretion in stone patients (36). Recently, it was found that L-arginine, which has antioxidant properties, significantly restored alteration in serum and urine biochemical parameters, urinary output, urinary density, urinary pH, and water intake induced by EG (39). Data showed that treatment with pyridoxamine, which has an antioxidant activity, resulted in 50% lower urinary glycolate and oxalate excretion in rats associated with a significant reduction in calcium oxalate crystal formation in renal tissues (40).

It was shown that flavonoids have powerful antioxidant properties that prevent the development of papillary and intratubular calcification of the kidney; therefore, preventing the development of papillary calculi (41). Propolis contains flavonoids (flavones, flavonols, and flavanones) and various phenolic compounds (42). The antioxidant activity of HAEP may have contributed to its preventive effect in EG-treated rats.

It is well known that propolis contains hundreds of substances and molecules that might have an active role in its protective effect; caffeic acid phenethyl ester (CAPE) is the most studied substance among the components of propolis. CAPE has antioxidant properties and has a favorable effect on several nephropathies due to various toxic materials (43−46). Therefore, it might play a role in the protective effect of propolis in EG toxicity.

The next step will be to study the effect of CAPE in EG toxicity compared to whole propolis. Future studies will include measuring body weight before and after treatment with propolis or CAPE, which might help to explain the effect of propolis or the toxic substances in urine and blood electrolytes. Interestingly, the present findings will pave the way for the use of propolis in nephropathy and hepatic toxicity in clinical settings. However, further experimentations are needed to identify the mechanism of action and to confirm the present results.

In conclusion, EG showed hepatorenal toxicity and increased urinary excretion of crystals and minerals involved in urinary calculus formation. These toxicities were markedly prevented with the use of HAEP or cystone. HAEP is more potent than cystone in amelioration of proteinuria. Therefore, the results might pave the way to use propolis in prevention and/or management of urinary calculus, proteinuria, renal damage and chronic EG toxicity.

Conflict of Interest

The authors have no conflicts of interest to declare.

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