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Original Article

Antilithiatic Activity of Arbutus Pavarii (Shemeri) Extract on Ethylene Glycol Induced Lithiasis in Rats

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ABSTRACT

***Objectives**. The objective of this study was to investigate the antilithiatic activity of shemeri (Arbutus Pavarii) on ethylene glycol-induced urolithiasis in male albino rats. **Methods.** 24 healthy male albino rats were divided into 4 groups. The rats in group A served as the normal control group. Group B (EG-treated group) rats received 0.75% ethylene glycol for three weeks followed by drinking water for the next 3 weeks. Group C (preventive group) rats received 0.75% ethylene glycol along with shemeri for six weeks. Group D (treatment group) rats received 0.75% ethylene glycol for three weeks then supplemented with shemeri aqueous solution. After six weeks, various levels of oxalate, calcium, and phosphate in urine and serum levels of creatinine, urea, uric acid, phosphate, calcium, and sodium were measured. Histopathological examination for kidney samples was also conducted. **Results.** In comparison to the EG-treated group, oral administration of Arbutus Pavarii (shemeri) significantly lowered elevated levels of creatinine, urea, and uric acid while, significantly increased levels of phosphate, calcium, and sodium in the serum. Shemeri also reduced elevated levels of urinary oxalate, calcium, and phosphate. Histopathological examination showed noticeable kidney lesions in group (B), but these lesions improved in groups (C, and D). **Conclusion**. This study suggests that shemeri is a promising and effective plant in prevention and treatment of Ethylene glycol-induced urolithiasis in male albino rats.*

***Keywords:** Ethylene Glycol, Urotithiasis, Kidney Stone, Arbutus Pavarii (Shemeri), Rats.*

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INTRODUCTION

Urolithiasis refers to the multifaceted process of stone formation in the urinary tract. Renal stones are composed of crystallized minerals that supersaturate the urine [1]. The global prevalence of renal stones has risen to around 14% in the last 30 years, with a higher incidence in males than females (3:1 ratio) [2].

Kidney stones are formed through a complex process that is not fully understood and a range of factors are considered important during the formation process, including the concentrations of calcium, sodium, oxalate, urate, urine volume, and low urine pH [3]. The most common type of kidney stone is made of calcium oxalate (CaOx), but other types of stones can also develop such as uric acid stones and ammonium-magnesium phosphate stones [4].

Safer alternatives are needed for the management of urolithiasis due to the likelihood

of stone recurrence [5]. Medical professionals are exploring natural antilithiatic agents to improve outcomes [6]. Plant phytochemical compounds such as flavonoids and phenolic agents have shown prophylactic and curative effects against kidney stones and offer health benefits [7].

Phytochemicals present in plants play a crucial role in preventing and treating urolithiasis through diverse mechanisms. These mechanisms include antioxidants, anti-inflammatory and antimicrobial activities, along with the capability to hinder the formation and growth of crystals [8]

Arbutus pavarii locally known as (Shemeri) is an endemic medicinal plant in El-Jabal El-Akhdar, Libya. The local community utilizes the plant for various purposes, including honey production, as a food source, and for treating gastritis and renal diseases. A chemical analysis of Arbutus pavarii (Shemeri) revealed the presence of several phytochemical agents, including flavonoids, tannins, glycosides, simple phenolics, free-reducing sugars, triterpenes, and sterols [9].

Ethylene glycol (EG) has been used to induce calcium oxalate urolithiasis in rats due to hyperoxaluria. EG's toxic metabolites, such as glycolic acid and oxalic acid, raise blood pH and precipitate calcium oxalate, and this leads to kidney stone formation [10][11]. Despite medical advancements, no satisfactory medication exists for treating kidney stones, necessitating the exploration of alternative agents. Thus, it was thought worthwhile to investigate the effect of Arbutus Pavarii extract on an experimentally induced lithiasis in male albino rats because of the traditional uses of Arbutus Pavarii for the treatment of kidney stones, as well as urinary insufficiency [9]. As a result, the objective of the present research was to investigate the antilithiatic activity of Arbutus Pavarii (shemeri) on ethylene glycol-induced nephrolithiasis in male albino rats.

METHODS

Animals: Twenty-four male mature albino rats (Wister strain) weighing 130-150g, 7 months of age, were randomly housed in 4 groups in stainless steel wire bottom cages and kept under constant environmental conditions (temperature 27°C ± 1°C, humidity 60% ± 4%, and natural lighting). All rats were fed on standard pellets and given tap water ad libitum.

Plant collection and identification

Arbutus Pavarii plant from El-Jabal El-Khdar was collected during the spring seasons 2009-2010 and authenticated by the Department of Botany, Faculty of Science, Tripoli University, Libya. Aqueous extraction of Arbutus Pavarii (shemeri) was conducted using the decoction method. Fresh shemeri leaves were cleaned, dried, and finely powdered. 10 grams of the powder boiled in 100 ml water for 1-2 hours, cooled and refrigerated overnight. The resulting liquid was filtered and stored at 4ºC [12].

Experimental protocol

Normal rats were randomly divided into four groups; each group consisted of six rats. Group A (control group): rats were received standard pellet and tap water ad libitum. Group B (EG-treated group): rats were received 5ml of 0.75% ethylene glycol (Merck, Germany) in drinking water for the first 3 weeks, followed by drinking water alone for the next 3 weeks. Group C (preventive group): rats were received 0.75% ethylene glycol with 2ml of 10% of Arbutus Pavarii (shemeri) filtrate in drinking water for 6 weeks. Group D (treatment group): rats were received 0.75% ethylene glycol in drinking water for 3 weeks, then supplemented with 2ml of 10% of Arbutus Pavarii (shemeri) filtrate in drinking water for another 3 weeks.

Urine samples

Rats were kept separately in metabolic cages designed for the collection of rat urine. Twenty-four-hour urine were collected on days 0, 7, 14, 21, 28, 35, 42. The urine samples were quickly kept frozen at -20°C until analyzed.

Serum samples

On day 42, rats were sacrificed and blood was collected in centrifuge tubes. After standing for an hour, serum was separated by centrifugation at 1500 rpm for 15 min. and frozen at -20°C. Serum levels of sodium, calcium, phosphate, creatinine, urea, and uric acid were measured.

Determination of biochemical parameters

Urinary levels of oxalate, calcium, and phosphate were evaluated with a spectrophotometer (Jenway, England) at a wavelength of 1000nm. Serum levels of creatinine, urea, uric acid, phosphate, calcium, and sodium were determined using a semi-auto chemistry analyzer (Microlab 300) and Spinlab from the Spinreact S.A model (2013).

Histopathological examination

After sacrifice, the abdomen was opened to extract the kidneys. The kidneys were preserved in 80% formalin and then dehydrated, embedded in wax, and finally sliced using a microtome. All sections were stained with H&E. Photographs were captured with a light microscope equipped with a camera.

Statistical analysis

The data were presented as mean ± SEM using SPSS software (version 11.0.1, SPSS Inc.). The impact of ethylene glycol and shemeri was assessed using ANOVA, and differences between means were compared using the LSD procedure.

RESULTS:

Biochemical Analysis

Urine analysis

In this study, oral administration of 0.75% ethylene glycol (EG) for 3 weeks in male albino rats of group B (EG-treated group) led to a significant increase in urine levels of oxalate, calcium, and inorganic phosphate compared to the control group ($p<0.05$). However, both treatment and preventive groups showed a significant decrease in urine concentrations of oxalate, calcium, and phosphate compared to the EG-treated group (Table 1).

Serum analysis

The levels of serum creatinine, urea, and uric acid were found to be increased significantly ($p<0.05$) in the calculi-induced rats (EG-treated group). However, the serum concentrations of creatinine, urea, and uric acid in both preventive and treatment groups were lower than the detected values in the EG-treated group (Table 1).

Additionally, the serum levels of creatinine, and urea were decreased non-significantly in the treatment group in comparison to the control group. Moreover, the serum level of uric acid in the treatment group was higher than that in the control and EG-treated group (Table 1).

Rats in the EG-treated group showed a significant decrease ($p<0.05$) in serum calcium, phosphate, and sodium levels compared to the control group. On another hand, the serum levels of calcium, phosphate, and sodium were significantly increased in the treatment and preventive groups compared to the EG-treated group (table 1). However, serum levels of phosphate in the treatment group were non-significantly increased while; the concentrations of serum calcium in both treatment and preventive groups were decreased significantly in comparison with the control group (table 1).

In compression to the control group, the concentrations of serum sodium in rats of the treatment group showed a non-significantly decrease. While the decrease in the sodium levels in the serum was significant in the

preventive group (table 1).

Kidney histopathology

Histopathological examination of kidneys from rats of the control group revealed normal histological appearance (Figure A). However, rats treated with EG showed lytic necrotic damage in the renal tubules, with loss of tubular architecture, which was replaced by edema mixed with inflammatory cells. In addition, renal glomeruli of the EG-treated group revealed frequent endothelial cell necrosis of glomerular tufts (Figure. B).

In the preventive group, the kidney showed congested renal blood vessels and perivascular edema mixed with aggregates of mononuclear inflammatory cells mainly lymphocytes and fewer macrophages (Figure. C). similar abnormal changes in the glomeruli and renal tubules that have been reported in EG-treated rats were also observed in the preventive group, but the severity and frequency were mild, and large numbers of glomeruli in the renal cortex showed normal histology. Mild degenerative changes were observed in the kidney of rates from treatment group and Glomerular tufts revealed vacuolization of lining endothelial cells with hypereosinophilic cytoplasm and pyknotic nuclei (Figure D). Moreover, the treatment group showed Infrequent Segmental necrosis of renal tubules and few renal cortex degenerations.

Table 1. Effect of Arbutus Pavarii (shemeri) on urine and serum parameters of experimental groups

Parameters	Groups			
	Control	EG treated	Preventive	Treatment
Urine parameters Oxalate*	1.19± 0.15 [a]	3.55± 0.5 [b]	1.6 ± 0.12 [c]	1.79 ± 0.12[c]
Ca^{2+*}	1.03±0.07 a	2.68 ±0.37[b]	1.31± 0.1[c]	1.66 ± 0.14[c]
Ph*	2.1 ± 011 [a]	4.51±0 71 [b]	2.45 ± 0.13 [c]	3.11± 0.25[c]
Serum parameters Creatinine*	1.17 ±0.01 [a]	3.20±0.58[b]	1.76 ± 0.68 [c]	1.01 ± 0.09 [a]
Urea*	28.67± 1.48[a]	62.00± 2.12[b]	42.33 ± 2.45[c]	26.67 ± 4.17[a]
U.A*	3.95±0.13[a]	6.32±0.57[b]	4.07 ± 0.21 c	4.26 ± 0.25c
Ph*	3.36±0.32[a]	1.71±0.57[b]	3.38 ± 0.17 [c]	3.50 ± 0.28[a]
Ca^{2+*}	7.19±0.05[a]	3.72±0.83[b]	5.99 ± 0.07 [c]	5.54 ±0.43[c]
Na^{2+*}	146.33 ±3.34[a]	103.67 ±11.78[b]	125.67 ± 8.01 [c]	141.67± 30.19[a]

Values are expressed as mean ± SEM, with six animals in each group. Within columns means with different small alphabets superscripts are significantly different at least at (P<0.05).
**The measurement unit for all parameters is mg/dl except for Na2+ is mEq/L. mEq/L: Milli equivalents per liter.*
Ca2+: Calcium, Ph: Phosphate, Na 2+: Sodium, U.A: uric acid

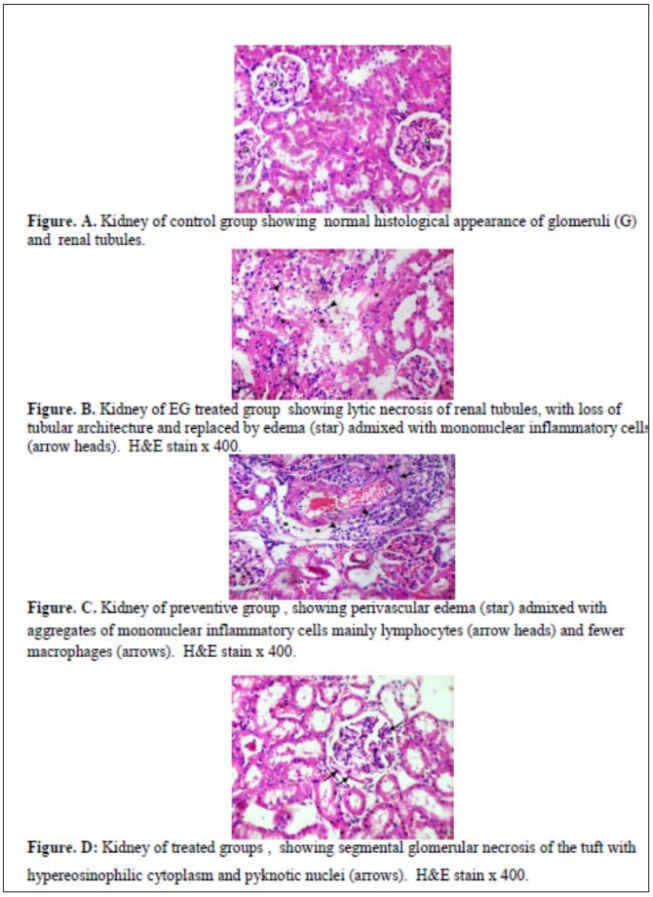

Figure 1: (a) Control group (H and E, ×400); (b) stone-induced group (H and E, ×400); (c) preventive group (H and E, ×400); (d) treatment group (H and E, ×400)

DISCUSSION

Plants with high oxidative activity have shown antiurolithiatic effects and are used in both the treatment and prevention of kidney stones [14]. *Arbutus Pavarii* (shemeri) is a plant that used to treat kidney stones in many cultures [16]. This study examined the anti-urolithiatic activity of Arbutus Pavarii (shemeri) against ethylene glycol-induced renal calculi in male albino rats.

Male rats were used in this study due to the similarity of their urinary system to humans [18]. Male albino rats developed renal stones when given ethylene glycol (0.75%, v/v) for 28 days and the stones were primarily composed of calcium oxalate CaOX, as well as small amounts of calcium phosphate and uric acid [17]. Daily oral administration of EG for 3 weeks in male albino rats resulted in increased concentrations of oxalate, calcium, and phosphate in urine which indicates the formation of kidney stones. This aligns with findings from another study [17]. EG supplementation increases the risk of urolithiasis through two mechanisms: elevating calcium, oxalate, phosphate, and uric acid levels, and creating a favorable environment for stone formation. Moreover, the excessive tubular damage in the kidney results in excessive excretion of calcium and phosphorus in the urine. The increased phosphorus leakage besides hyperoxaluria seems to provide a suitable environment for stone formation by developing calcium phosphate crystals, which encourage nucleation and precipitation of calcium oxalate, leading to the formation of kidney stones [18]. Additionally, Ethylene glycol enhances oxalate production by increasing the activity of oxalate-synthesizing liver enzymes [17-18].

EG treatment increases serum levels of creatinine, urea, and uric acid, likely due to their accumulation. This accumulation might occur due to urine flow obstruction and decreased GFR that resulted from stone formation in the renal tubule [19]. Uric acid also acts as a stone former by affecting calcium oxalate CaOX crystallization and reducing its solubility [20].

In calculi-induced rats, serum calcium levels decreased and this was consistent with previous study [21]. Additionally, serum phosphorus and sodium concentrations were reduced, possibly due to increased renal deposition and excretion of calcium, which disrupts tubular reabsorption and leads to phosphate and sodium loss (hyponatremia and hypophosphatemia) [22].

Histopathological examination of EG-treated rats' kidneys demonstrated notable infiltration of inflammatory cells and necrotic damage in endothelial and glomerular cells. An evident connection exists between severe inflammatory renal damage and the formation of calcium oxalate crystals [19].

Administration of Arbutus Pavarii (shemeri) in the preventive and treatment groups significantly reduced urinary oxalate, calcium, and phosphate levels compared to the EG-treated group. It also had a similar effect on serum creatinine, urea, and uric acid levels. However, these findings follow the same trend as previous studies using the same treatment protocol [23]. This finding suggests that shemeri supplementation may impede the formation of EG-induced stones. Histopathological analysis of the kidneys from the preventive and treatment group further supports the antilithiatic effect of shemeri, as it showed fewer severe histological changes and a larger number of glomeruli with normal criteria compared to EG-induced calculi.

Arbutus Pavarii (shemeri) may have a protective effect due to its high content of phenolic compounds and flavonoids, which have showed anti-inflammatory and antioxidant properties. These compounds could potentially exert their effects in preventing epithelial cell damage caused by crystals through various levels of cellular and molecular processes, including the reduction in the binding between crystals and cells and the decrease in crystal aggregation [8] [9].

Similar actions have been observed in other plants containing similar phytochemical agents, such as Kigelia pinnata fruit. Additionally, Arbutus Pavarii (shemeri) may regulate oxalate metabolism by inhibiting the activity of endogenous oxalate synthesizing enzymes in the liver, as seen in the effect of alcoholic extracts of the root wood of Moringa oleifera Lam on oxalate synthesis. [7] [24]

CONCLUSION

The study's findings indicate that *Arbutus Pavarii* (shemeri) provides protective and treatment properties against EG-induced urolithiasis. The potential explanation for these observed effects might attributed to the antioxidant and anti-inflammatory properties of Arbutus Pavarii, as well as its ability to reduce the presence of stone-forming constituent. More studies are needed to validate the safety, efficacy, and toxicity profiles of *Arbutus Pavarii (shemeri)* in humans

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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