



## The Beneficial Effect of Hydro-alcoholic Extract of *Punica Granatum* L. Leaves & Flower on Ethylene Glycol-Induced Kidney Calculi in Rats

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### ABSTRACT

**Background & Aim:** *Punica granatum* L. (pomegranate) is a widely used plant that has high nutritional value. This study evaluated prevention effect of hydro alcoholic extract of *Punica granatum* flower and leaves ethylene glycol-induced Nephrolithiasis in Wistar rats.

**Experimental:** 20 male Wistar rats were randomly divided into 4 equal groups. Group I served as positive control and received distilled water for 28 days. Group II to IV received 1% (V/V) ethylene glycol (EG) in distilled water for 28 days. Group II served as EG or negative control. Group III and IV (preventive groups) received hydroalcoholic extract of *Punica granatum* flower and leaves orally for 28 days in dose of 400 mg/kg respectively. 24-hour urine samples were collected on day 0 and 28. Urine volume and urine oxalate levels were measured. On day 28, blood was collected for biochemical parameters (creatinine, urea and uric acid). Animals were sacrificed and kidneys were harvested, histopathologically evaluated for calcium oxalate (CaOx) crystals.

**Results:** EG significantly increased urine oxalate and calcium, serum creatinine, urea and uric acid levels. It also significantly decreased urine citrate level in group III and IV resulted in significantly lower levels of urine oxalate and serum creatinine, urea and uric acid as compared to Group II ( $P < 0.001$ ).

**Recommended applications/industrie:** Hydro alcoholic extracts of *Punica granatum* flowers and leaves are effective in prevention of EG-induced nephrolithiasis in Wistar rats.

### 1. Introduction

Urinary stone is the third most common condition affecting the urinary tract. (Saeidi *et al.*, 2012). The physicochemical mechanism of stone formation is not fully understood yet. It is agreed that urolithiasis

generally involves events such as crystal nucleation as well as aggregation and growth of insoluble particles (Baumann, 1998). Urine is supersaturated with common stone-forming minerals; however the crystallization inhibiting capacity of urine does not allow urolithiasis in most of individual, whereas, this natural inhibition capacities deficient in stone forming individuals

(Tiselius, 2003). It has multi-factorial etiopathogenesis, involving anatomic, environmental, genetic, infections, metabolic, nutritional and socio-economics major factors (Alessandra and Elvino, 2003). The medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheapest source of medicine for majority of world population, which are considered as quite safe, with minimal or no side effects (Bashir and Gilani, 2009). *Punica granatum* is used as antiparasitic agent, a blood tonic, and to heal aphtae, diarrhoea and ulcers. In the Unani system, practiced in the Middle East and India, and according to the same review, pomegranate was described also a remedy for diabetes (Julie, 2008). *Punica granatum* have been extensively studied for potential uses including, Anti-inflammatory effects (Lee et al., 2010), antioxidant (Celik et al., 2009), protective role in atherosclerosis and thyroid dysfunctions (Singh and Kar, 2007) and cardiovascular protection (Shiraishi et al., 2002).

Today, surgical endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of urolithiasis but does not prevent the likelihood of new stone formation (Kalayan et al., 2009). The recurrence of stone formation is also very high (50–80%) and no suitable medical therapy is available for such stone disorders. The various therapies including thiazide as diuretic and alkali-citrate are being used to prevent the recurrence of hypercalciuria and hyperoxaluria, which induce calculi formation but evidence for their efficacy is less (Pay, 1989). Urinary calculi may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly cost-effective and may cause severe complications. According to the importance of medicinal plants, the aims of this study were to assess the effect of hydroalcoholic extract of *Punica granatum* flower and leaves on ethylene glycol-induced Nephrolithiasis in Wistar rats.

## 2. Materials and Methods

### 2.1. Plant material

Leaves and flowers of pomegranate were collected in natural habitat in Dorak Anari, Chaharmahal-va-

Bakhtiari province, Iran. The identity of plant was confirmed by V. Mozaffarian (Research Institute of forests and Rangelands, Tehran) using Flora Iranica (Rechinger, 1982). The Leaves and flowers of *Punica granatum* were shade-dried. Shade drying was carried out under natural air flow and ambient temperature (mean temperature =24°C). Then, 1000 g of the prepared dried and powdered sample was mixed with a sufficient volume of 96% ethanol and extracted with a soxhlet apparatus for 12 to 14 hours. After removing the solvent in vacuum, the extract was dried in an oven with the temperature of 50°C to 60°C. Then the residues were weighed (20 g) and kept.

### 2.2. Animals

The animal procedure was conducted in conformity with institutional guidelines and national laws, and the study was approved by Shahrekord University of Medical Sciences. Thirty-two male Wistar rats weighed  $200 \pm 10$  g were housed at  $21 \pm 2^\circ\text{C}$  on a standard diet and tap water. Animals were acclimatized in institutional animal house and were exposed to 12 h day and night cycle. They were randomly divided into 4 equal groups and treated according to the experimental protocol for 28 days.

### 2.3. Ethylene glycol-induced urolithiasis

The animals were divided into 4 groups, each group containing 5 animals.

Group I (Control: Cont) normal rats received distilled water for 28 days. Group II; (negative control: EG) received ethylene glycol (EG) 1% v/v in drinking distilled water for 28 days. Group III and IV (preventive groups) fed hydroalcoholic extract of *Punica granatum* flower (P.G.F) and leaves (P.G.L) orally for 28 days in dose of 400 mg/kg, respectively. The groups III and IV, received ethylene glycol (EG) 1% v/v in drinking water for 28 days.

### 2.4. Outcome measures

**2.4.1. Urinary parameters.** Twenty-four hours urine specimens were collected on day 0 and 28 of the study by keeping each rat in separate metabolic cage. Urine volume was measured. It was acidified and kept under refrigeration (2- 8°C). Urinary oxalate was measured by spectrophotometer.

**2.4.2. Biochemical parameters.** On day 28, blood was collected for biochemical parameters (creatinine, urea and uric acid).

The 2 ml blood samples were collected by puncturing the retro orbital venous plexus from each animal in centrifuge tubes without anticoagulant and allowed to clot at room temperature. The serum was separated by centrifugation at 1500 rpm for 15 min in refrigerated research centrifuge and used for estimation of serum creatinine, urea and uric acid using commercially available kits and Star-21 plus semi-auto analyzer.

**2.4.3. Histopathological analysis.** The animals were sacrificed soon after blood collection under the continued effect of anesthesia. Both kidneys were removed and kept in formaldehyde (10% V/V) for at least 24 hours. Then 5 mm thick sections were taken and enclosed in paraffin. They were cut into 5  $\mu$ m thin sections, stained with hematoxylin-eosin (H & E) and evaluated under optical light binocular microscope and changes e.g. necrosis, leukocyte infiltration and tubular dilatations were also noted (Mandavia, 2013).

### 2.5. Statistical analysis

Analyses of variances were conducted for all characters. Significant differences or combination of main effects were stated based on the Duncan Multiple Range Test at a probability level of 5%. Excell 2010 software was used to draw the figures and histograms.

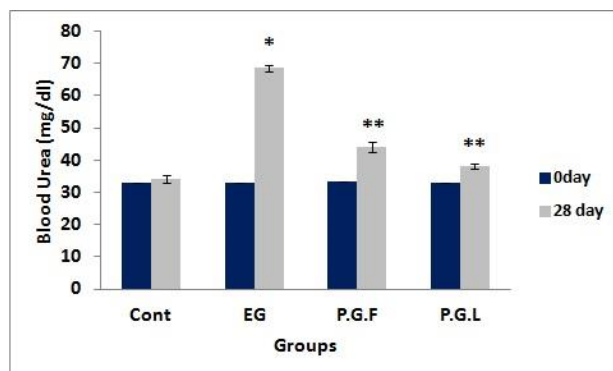
## 3. Results and discussion

Ethylene glycol administration increased the values of blood urea and serum creatinine and uric acid significantly ( $p < 0.05$ ) as compared to control group. *Punica granatum* flower and leaves extracts showed the significant improvement in renal parameters in experimental groups. The maximum decrease in serum creatinine was found in 400 mg/kg, showing dose dependent activity and at 400 mg/kg concentration, reduced the urea and uric acid levels ( $P < 0.05$ ) (Fig.1-3).

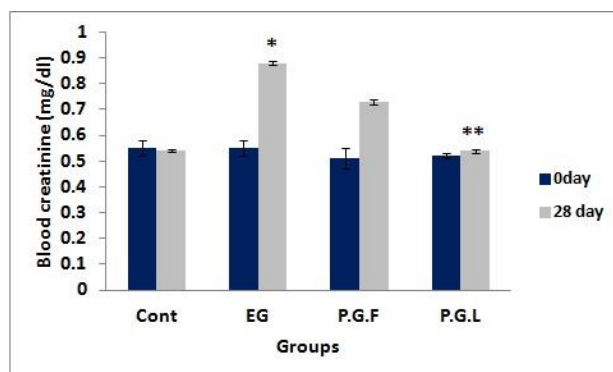
There was no statistically significant difference in urinary volume and oxalate in all the groups on day 0. Urinary oxalate was significantly increased after ethylene glycol administration ( $P < 0.001$ ). *Punica granatum* flowers and leaves was show significant effect on urinary oxalate ( $P < 0.05$ ) (Fig. 4). Urine volume was significantly increased after *Punica granatum* flowers and leaves administration ( $P < 0.05$ ) (Fig. 5).

Histopathological examination of kidney in control group revealed the normal glomeruli and tubular region

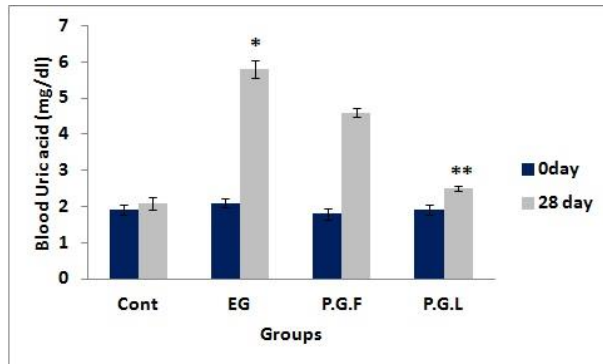
with the absence of any pathologic lesions. EG control group showed the maximum renal damages. In glomeruli, dilation of urinary space occurred and some of glomeruli were atrophied. In the tubules eosinophilic protein accumulation and formed hyaline casts. Some of hyaline casts were basophilic due to deposition of calcium. Proximal and distal tubules had degenerative changes including vacuolation and hydropic degeneration of the epithelium. Some epithelial cells were desquamated. Mononuclear cells especially lymphocytes were infiltrated in the renal interstitium. The most of kidneys were congested and fewer had hemorrhage. The histopathologic lesions in the kidneys of treated groups with 200 and 400 mg/kg of *Punica granatum* flowers and leaves extracts were reduced but no significant difference were observed between them ( $P < 0.05$ ) (Fig. 6).



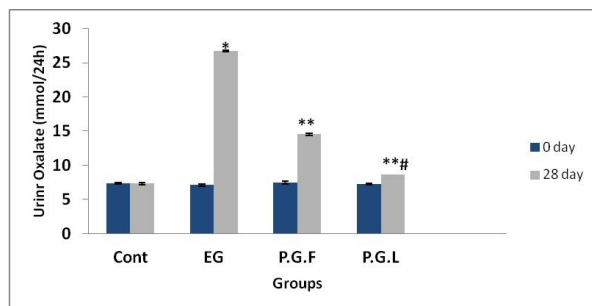
**Fig.1.** Effects of hydro alcoholic extracts of *Punica granatum* flowers and leaves (400 mg/kg) on blood urea. \* $P < 0.05$  as compared to group I, \*\* $P < 0.01$  as compared to group II. P.G.F: *Punica granatum* flowers extract; P.G.L: *Punica granatum* leaves extract; EG: Ethylene glycol.



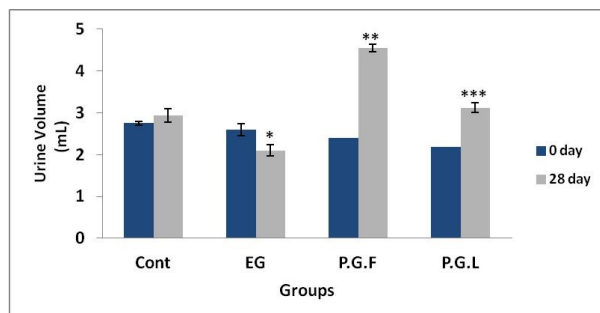
**Fig.2.** Effects of different hydro alcoholic extracts of *Punica granatum* flower and leaves (400 mg/kg) on blood cratinine. \* $P < 0.05$  as compared to group I, \*\* $P < 0.01$  as compared to group II. P.G.F: *Punica granatum* flowers extract; P.G.L: *Punica granatum* leaves extract; EG: Ethylene glycol.



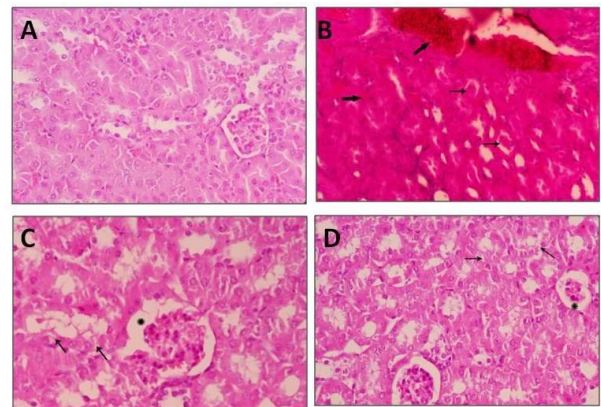
**Fig.3.** Effects of hydro alcoholic extracts of *Punica granatum* flowers and leaves (400 mg/kg) on blood uric acid. \*P < 0.05 as compared to group I, \*\*P < 0.01 as compared to group II. P.G.F: *Punica granatum* flowers extract; P.G.L: *Punica granatum* leaves extract; EG: Ethylene glycol.



**Fig. 4.** Effects of hydro alcoholic extract of *Punica granatum* flowers and leaves (400 mg/kg) on urine oxalate. \*P < 0.05 as compared to group I, \*\*P < 0.01 as compared to group II, #P < 0.01 as compared to group III. P.G.F: *Punica granatum* flowers extract; P.G.L: *Punica granatum* leaves extract; EG: Ethylene glycol.



**Fig.5.** Effects of hydro alcoholic extract of *Punica granatum* flowers and leaves (400 mg/kg) on urine volume. \*P < 0.05 as compared to group I, \*\*P < 0.01 as compared to group II, \*\*\*P < 0.001 as compared to group III. P.G.F: *Punica granatum* flowers extract; P.G.L: *Punica granatum* leaves extract; EG: Ethylene glycol.



**Fig. 6.** A) Normal medullary and papillary tubules are shown in a rat's kidney. B) Multiple tubular calculi (arrows) and dilation of urinary space in an ethylene glycol treated rat. C&D) The histopathologic lesions in the kidneys of treated groups were reduced.

These results indicate that the hydro alcoholic extract of *Punica granatum* flowers and leaves orally improves renal function in III and IV groups as compared to ethylene glycol group.

Our data demonstrated that hydro alcoholic extract of *Punica granatum* flowers and leaves orally had a preventive effect on CaOx calculus formation in the kidney of rats.

It was reported earlier that ethylene glycol causes hypercalciuria, hyperphosphaturia and hyperoxaluria leading to urolithiasis (Verma et al., 2009). We also were found the increased levels of oxalates in the urine of ethylene glycol treated control rats. In other studies it was reported that response in 28 days ethylene glycol (1% V/V) administration in rats; forms renal calculi composed of mainly calcium oxalate (Bahuguna et al., 2009). The mechanism for this process may be due to an increase in the urinary concentration of oxalates. The increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate from urine and subsequently crystal growth (Lemann et al., 1991).

A recent study reported the chemical constituents of diverse parts of *P. granatum* as well as their potential for prevention and treatment of inflammation and cancer. The authors claimed that phenols (flavonoids and tannins) can be detected in pericarp, leaf and flowers being some of them unique. Complex polysaccharides have also been detected and characterized in the peels (Lansky and Newman, 2007). Many alkaloids can cause

smooth muscle relaxation specifically to the urinary and biliary tract which could facilitate the expulsion of stones from both kidneys (Calixto *et al.*, 1984). It has been reported that CaOx calculi such as struvite calculi may have a bacterial origin such as nanobacteria (Kramer, 2000). It seems antibacterial hydro alcoholic extract of *punica granatum* flower and leaves orally effects and therefore, may be effective in this mechanism of CaOx calculus formation. Pomegranate is a polyphenol rich fruit, which showed potential as an anti-inflammatory agent in several experimental models (Lee *et al.*, 2010).

#### 4. Conclusion

According to our results it was found that EG significantly increased urine oxalate, serum creatinine, urea and uric acid levels ( $P < 0.05$ ). In groups III and IV, EG resulted in significantly lower levels of urine oxalate, calcium and serum creatinine, urea and uric acid as compared to Group II. Hydro alcoholic extract of *Punica granatum* flowers and leaves is effective in prevention of EG- induced nephrolithiasis in Wistar rats. Hydro alcoholic extracts of *Punica granatum* flowers and leaves are effective in prevention of EG- induced nephrolithiasis in Wistar rats. Further studies on larger animal models and on human are warranted to draw final conclusions.

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