

# Aqueous extract of *Parkinsonia aculeata* has an anti-urolithiatic effect in rats

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## Research Article

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# Abstract

**Introduction:** Many antioxidant agents have been studied for prevention and treatment of the kidney stone disease in animals and humans. *Parkinsonia aculeate* (*P. aculeate*) has a wide range of pharmacological and biological activities, particularly antioxidant properties. The goal of this research was to investigate the anti-urolithiatic effect of *P. aculeate* extract on ethylene glycol induced renal calculi.

**Materials and methods:** A total of 42 male Wistar rats were randomly divided into six equal groups with seven rats in each group: Group A served as healthy control group and received distilled water for 30 days. Group B served as ethylene glycol control group and received 1% v/v ethylene glycol in distilled water for 30 days. Group C, D, and E animals (preventive groups) concurrently received 1% v/v ethylene glycol in distilled water along with extract of *P. aculeata* orally for 30 days in doses of 100 mg/kg (low dose), 200 mg/kg (moderate dose), and 300 mg/kg (high dose), respectively. Group F (treatment groups) received 1% v/v ethylene glycol in distilled water for the first 30 days along with extract of *P. aculeata* 300 mg/kg orally from 15th to 30th days. At the end of study period, 24-hour urine and serum samples were collected and analyzed. All rats were sacrificed and both kidneys were removed, weighed and histopathologically evaluated for calcium oxalate crystals.

**Results:** Ethylene glycol significantly decreased serum calcium, urinary levels of creatinine, calcium and uric acid. On the contrary, it significantly increased 24-hour urine volume, kidney weight, and calcium oxalate deposits. The highest number of calcium oxalate crystal depositions were in the high dose preventive group (76.6%), which is statistically significant difference with other groups including ethylene glycol control (70%), low dose preventive (72%), treatment (38%), and moderate dose preventive (42%) groups (P value < 0.05). Administration of extract of *P. aculeata* significantly reduced the production of calcium oxalate stones in moderate dose preventive and treatment group. However, *P. Aculeata* extract was not effective in reducing the formation of calcium oxalate stones in low dose and high dose preventive groups. Interestingly, the number of calcium oxalate deposits in the renal tubules of high dose preventive group was significantly more than the ethylene glycol control group.

**Conclusions:** Extract of *P. aculeata* has preventive and therapeutic effects in ethylene glycol induced renal calculi in Wistar rat. Further studies are necessary to clarify the mechanism underlying this effect.

## 1. Introduction

Kidney stone is a common urological disease, which can seriously affect health and quality of life in populations worldwide. It affects approximately 1 in 11 people in the United States [1]. The incidence and prevalence of kidney stones is increasing globally and is seen across sex, race, and age. In Iran, urolithiasis is slightly more frequent and persisted in males (6.1%) than females (5.3%) giving a male-to-female ratio of 1.15:1 [2]. Iran, Japan, and the United States have stone incidence reports stratified by age. The peak incidence is age 40 to 49 years for all 3 countries [3]. Urolithiasis is a multifactorial disease

caused by complex interactions between many genetic and environmental factors. Environmental factors, such as dietary habits, obesity, dehydration, and lifestyle have been involved in urolithiasis development, whereas hormonal, genetic or anatomical factors might also affect its pathogenesis [4].

More than 80% of patients with renal stones suffer from urolithiasis caused by calcium oxalate [5]. Some investigators have suggested that calcium oxalate kidney stones form while attached to Randall plaques, the subepithelial deposits on renal papillary surfaces, and that this process is triggered by reactive oxygen species and the development of oxidative stress [6]. The different theories of the kidney stone pathogenesis suggest that stone formation is a complex process. The pathogenesis of calcium oxalate stone formation is a multistep process that includes nucleation, crystal growth, crystal aggregation and crystal retention. Although many aspects of the mechanism of kidney stone formation are unknown, it is certain that reactive oxygen species, oxidative stress and renal tubular cell injury are important factors of lithogenesis [4]. It seems that the process of renal tubular cell injury is considered as a major risk factor for the initial formation of kidney stones [7]. Renal tubular cell injury, particularly mitochondrial damage, and oxidative stress induce the early stage of calcium oxalate crystal formation [8]. In addition, exposure to calcium oxalate crystals induces oxidative stress, as shown by: (I) an increase in free radical generation; (II) an increase in lipid peroxidation; and (III) an increase in levels of arachidonic acid [9]. It should be noted that reducing oxidative stress with antioxidant compounds is associated with decreased cellular injury and calcium oxalate urolithiasis [10].

*Parkinsonia aculeata* (*P. aculeata*) is a small, spiny deciduous tree, grows up to 4–10 m high with a short and often crooked trunk up to 40 cm in diameter. *P. aculeata* is native to tropical America, extending from Mexico to South America, and, now, it has been introduced into many regions worldwide, including South Africa, Israel, Uganda, India, Pakistan, the Middle East, Italy, Cyprus, and Australia. *P. aculeata* has been widely used in various traditional system of medicine. Leaf, fruit and stem decoctions are taken orally to treat fever, malaria and as an abortifacient. Flower and leaf extractions in alcohol are applied as a poultice to treat rheumatism. *P. aculeata* has a wide range of pharmacological and biological activities, including antibacterial, antidiabetic, antioxidant, antirabies, amoebicidal, hepatoprotective, antispermatogenic, and antimalarial properties [11]. Since, the aqueous extract fraction of *P. aculeata* was found to have a high antioxidant activity. The antioxidant activity of the 70% hydroalcoholic extract of *P. aculeata* was evaluated in vitro by various experimental parameters such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide scavenging,  $\beta$ -carotene-linoleic acid module system, hydroxyl radical scavenging activity and lipid peroxidation. The extract successfully reduced ferric ions and its total phenolic content was determined [11, 12].

Animal studies have demonstrated the likely role of oxidative stress in the pathophysiology of kidney stone formation, and many antioxidant agents have been studied for prevention and treatment of the kidney stone disease in animals and humans [13, 14]. Therefore, antioxidant strategy might be logical in the treatment of kidney stone disease. In this study, we investigated the possible antilithiatic effects of aqueous extract of *P. aculeata* on calcium oxalate urolithiasis in male Wistar rats.

## 2. Materials And Methods

### 2.1 Animals and ethics

In this study, 42 male Wistar rats, 6-8 weeks old and weighing an average of  $230 \pm 30$  gr, were used and housed under standard laboratory conditions: temperature  $25 \pm 2^\circ\text{C}$ , humidity 65–70%, and 12-h light/12-h dark cycle. The animals were supplied with acidified water and standard pelleted diet (Lactamin R36, Stockholm, Sweden) ad libitum throughout the entire experiment. All procedures in this study were approved by the Guidelines of Animal Ethics Committee of Bushehr University of Medical Sciences and institutional ethics committee with approval code IR.BPUMS.REC.1391.209, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council Committee.

### 2.2 Plant material

The leaves of plant *P. Aculeata* were collected from the garden of Agriculture, Bushehr University of Medical Sciences, Bushehr, Iran, during May 2007. After identification, the scientific name of the plant was approved by the Department of Pharmacognosy, Faculty of Pharmacy, Bushehr University of Medical Sciences.

### 2.3 Preparation of aqueous extract

All parts of the plant were cleaned and chopped in to small pieces and dried under shade. The dried plant material was powdered. Powder of dried aerial parts of *P. Aculeata* (500 g) were macerated with sterile distilled water for 24 hours and was heated 12 hours at  $40^\circ\text{C}$ . The supernatants obtained of extracts were centrifuged and concentrated using a rotary evaporator (Rotavapor, BÜCHI Labortechnik AG, Flawil, Switzerland). The extract was stored at  $-20^\circ\text{C}$  until use.

### 2.4 Ethylene glycol induced nephrolithiasis model and experimental design

Formation of kidney stone was induced in rats using 0.75% ethylene glycol in drinking water [15]. A total of 42 rats were randomly divided into six equal groups with seven rats in each group:

Group A (Healthy control group): received distilled water for the first 30 days;

Group B (Ethylene glycol control group): received 1% v/v ethylene glycol in distilled water for the first 30 days;

Group C (Low dose preventive group): concurrently received 1% v/v ethylene glycol in distilled water for the first 30 days, along with aqueous extract of *P. aculeata* 100 mg/kg body weight via oral gavage for the first 30 days;

Group D (Moderate dose preventive group): concurrently received 1% v/v ethylene glycol in distilled water for the first 30 days, along with aqueous extract of *P. aculeata* 200 mg/kg body weight via oral gavage for

the first 30 days;

Group E (High dose preventive group): concurrently received 1% v/v ethylene glycol in distilled water for the first 30 days, along with aqueous extract of *P. aculeata* 300 mg/kg body weight via oral gavage for the first 30 days;

Group F (Treatment group): received 1% v/v ethylene glycol in distilled water for the first 30 days, along with aqueous extract of *P. aculeata* 300 mg/kg body weight via oral gavage from 15th to 30th days.

## **2.5 Biochemical assays**

Ethylene glycol was purchased from Merck (Darmstadt, Germany). All urine and serum biochemical parameters were measured with the use of an automatic biochemical analyzer (Hitachi, Ltd., Tokyo, Japan) and enzyme-based kits (Alpha Laboratories, Hampshire, United Kingdom).

## **2.6 Collection and analysis of urine**

All rats were individually housed in metabolic cages (Tecniplast, Buguggiate, Varese, Italy) and had free access to drinking water during the urine collection period. 24-hour urine samples were collected on the 30<sup>th</sup> day of the calculi induction treatment period. The urinary volume and pH were measured. Urinary parameters include calcium, phosphate, uric acid, and creatinine were analyzed using Mindray BS-200 Chemistry Analyzer machine (Mindray, Shenzhen, China). Urinary crystals were examined by a light microscope (Olympus Optical Co., Lake Success, NY, USA).

## **2.7 Collection and analysis of serum**

At the end of the study period, rats were anesthetized with diethyl ether and 5 mL of venous blood was collected from the retro-orbital sinus. Serum was separated by centrifugation at 10,000 × g for 10 min and analyzed for creatinine, phosphate, calcium, uric acid, albumin, and urea using Mindray BS-200 Chemistry Analyzer machine (Mindray, Shenzhen, China).

## **2.8 Histopathological studies**

After blood collection, all rats were sacrificed and the abdomen was opened by midline incision and both kidneys were quickly removed, weighed and fixed at 10% neutral buffered formalin. Thin slices with a thickness of 5 µm were taken and stained with haematoxylin-eosin (H&E) and evaluated under a light microscope (Olympus Optical Co., Lake Success, NY, USA). Then in each section, 20 microscopic fields with magnification of 40×10 were randomly selected in equal numbers in the cortex and medulla, and numbers of calcium oxalate crystals (number of tubules containing calcium oxalate deposits) were counted.

## **2.9 Statistical analysis**

The statistical analysis of the data was done using the SPSS software for Windows, version 21 (SPSS Inc., Chicago, IL, United States). Statistical evaluation was done using one-way analysis of variance (ANOVA) test followed by Tukey-Kramer multiple comparison test as data were normally distributed. Charts were drawn using Microsoft Excel 2015 software. The results were expressed as means  $\pm$  standard deviations. A P-value of  $< 0.05$  was considered statistically significant.

## **3. Results**

### **3.1 Body weight of rats before and after the study**

Mean body weight in rats of different groups before and after the study is shown in Table 1. Only in the healthy control group, mean body weight after study ( $251.14 \pm 15.68$  gr) was significantly higher than the mean body weight before study ( $232.14 \pm 19.85$  gr) ( $p$ -value  $< 0.001$ ). There was not statistically significant in mean body weight before and after the study among the other groups ( $p$ -value  $> 0.05$ ).

### **3.2 Kidney weight**

As shown in Table 2, mean kidney weight was significantly higher in the ethylene glycol control group ( $1.00 \pm 0.23$  gr) compared to the healthy control group ( $0.77 \pm 0.13$  gr) ( $p$ -value  $< 0.05$ ), whereas there was not statistically significant in mean kidney weight between the other groups ( $p$ -value  $> 0.05$ ).

### **3.3 Urine 24-hour volume**

As shown in Table 2, 24-hour urine volume was significantly higher in the ethylene glycol control group ( $10.60 \pm 3.71$  mL) compared to the healthy control group ( $6.90 \pm 1.86$  mL), moderate dose preventive group ( $6.60 \pm 0.96$  mL), and treatment group ( $7.00 \pm 3.26$  mL) ( $p$ -value  $< 0.05$ ), whereas there was not statistically significant in 24-hour urine volume among the low dose and high dose preventive groups ( $p$ -value  $> 0.05$ ).

### **3.4 Urine analysis**

The results of the 24-hour urine parameters in rats of different groups on day 30 are shown in Table 3. At the end of the study period, 24-hour urine calcium and uric acid levels were significantly lower in the ethylene glycol control group compared to the healthy control group ( $p$  value  $< 0.001$ ), whereas there were no significant differences in the 24-hour urine calcium and uric acid levels between ethylene glycol control group and groups of preventive and treatment.

In ethylene glycol control group, 24-hour urine creatinine level was significantly lower than the healthy control group ( $p$ -value =  $0.028$ ). In addition, in moderate and high dose preventive groups, 24-hour urine creatinine level was significantly lower than ethylene glycol control group ( $p$ -value  $< 0.05$ ), whereas there was no significant difference in the 24-hour urine creatinine level between ethylene glycol control group and low dose preventive and treatment groups.

Furthermore, there were not statistically significant in 24-hour urine volume, phosphorus, and PH levels among all the groups.

### 3.5 Serum analysis

The results of blood biochemical analyses in rats of different groups on day 30 are shown in Table 4. At the end of the study period, serum calcium level was significantly lower in the ethylene glycol control group compared to the healthy control group (p value = 0.015). In addition, in moderate and high dose preventive groups, serum calcium level was significantly lower than ethylene glycol control group (p-value < 0.05), whereas there was no significant difference in the serum calcium level between ethylene glycol control group and low dose preventive and treatment groups.

In all preventive groups (C, D, and E), serum albumin level was significantly lower than ethylene glycol control group (p-value < 0.05), while there was no significant difference in the serum albumin level between ethylene glycol control and treatment group (p-value > 0.05).

Moreover, there were not statistically significant in serum levels of creatinine, uric acid, phosphorus, and urea among all the groups (p-value > 0.05).

### 3.6 Analysis of crystalluria

Microscopic examination of 24-hour urine revealed large amounts of calcium oxalate crystals in rats of different groups. Most of the calcium oxalate crystals were observed in the moderate dose preventive and treatment groups, so that in these groups, the number of calcium oxalate crystals in urine was significantly higher than other groups (p-value < 0.05).

### 3.7 Renal calcium oxalate crystal deposition

**Figure 1** shows the number of calcium oxalate crystals in renal tissue in rats of different groups. The number of calcium oxalate crystals in renal tissue in the moderate dose preventive and treatment groups was less in comparison to the other groups, and there was a significant difference between them (p-value < 0.05).

The highest number of calcium oxalate crystal deposition was in the high dose preventive group (76.6%), which is statistically significant difference with other groups, including treatment (38%), and moderate dose preventive (42%) groups (P value < 0.05). The lowest number of calcium oxalate crystal deposition was in the treatment group (38%), which is statistically significant difference with other groups, including high dose preventive (76.6%), ethylene glycol control (70%) and low dose preventive (72%) groups (P value < 0.05).

### 3.8 Kidney histopathological analysis

Histopathological analysis of the kidneys of healthy control group revealed normal size tubules with single epithelial lining along the margin without any calcium oxalate deposits or other abnormalities in

the nephron segments. On the other hand, in the ethylene glycol control group, all parts of the kidney showed the presence of calcium oxalate deposits, marked dilation of the tubules, and interstitial inflammation. Similarly, in all preventive groups, histology showed calcium oxalate deposition in all parts of the kidney. Interestingly, the number of calcium oxalate deposits in the renal tubules of high dose preventive group was significantly more than the ethylene glycol control group. Treatment with *P. Aculeata* extract significantly reduced the number of calcium oxalate crystal depositions that the reduction was statistically significant compared to other groups (P value < 0.05). Compared with other groups, in the treatment group, the crystal size was smaller and lower parts of the kidney were involved.

## 4. Discussion

Some researchers have reported that various phytotherapeutic agents may show beneficial effects in ethylene glycol induced nephrolithiasis model. To the best of our knowledge, no studies have been done to assess the effect of *P. Aculeata* on ethylene glycol-induced nephrolithiasis in rats, and this is the first report that evaluates *P. Aculeata* as an antiurolithiatic agent. This study showed that *P. Aculeata* extract was able to reduce the production of calcium oxalate stones in moderate dose preventive and treatment group. However, *P. Aculeata* extract was not effective in reducing the formation of calcium oxalate stones in the low dose preventive group, which this dose of extract may be insufficient in reducing calcium oxalate stone formation. In addition, *P. Aculeata* extract had no beneficial effect in reducing the formation of calcium oxalate stones in the high dose preventive group, perhaps because of cumulative and toxic dose of extract of *P. Aculeata*.

In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly urea, uric acid and, creatinine get accumulated in blood [16]. In addition, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet [17]. Oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane [18]. Many phytochemical compounds are known to support antioxidant activities. Antioxidants may prevent ethylene glycol-induced renal calcium oxalate crystal deposition in Wistar rats [19]. It has been reported that the flavonoids and polyphenols found in the extract of *P. Aculeata* might have a high antioxidant and free radical scavenging activity [20]. Indeed, the extract of *P. Aculeata* may reduce the deposition of calcium oxalate in the kidneys of rats through its anti-inflammatory and antioxidant effects. This is probably because of preventing the release of pro-inflammatory mediators and kidney stone formation factors through interference in the process of cell damage caused by crystals.

In a research by Shukla et al. [21], the anti-urolithiatic effect of cow urine ark on ethylene glycol induced renal calculi in 36 male rats was investigated. Administration of cow urine ark significantly reduced the levels of urine oxalate, blood urea, serum creatinine, and calcium oxalate depositions as compared to ethylene glycol control group. It also significantly restored kidney weight. Cow urine ark inhibited 40% and 35% crystallization of calcium oxalate and calcium phosphate, respectively. Their results showed that

Cow urine ark has preventive and therapeutic effects in ethylene glycol induced renal calculi in rat [21]. These findings are not consistent with the results of our study, because in our study the extract of *P. aculeata* had no effect on blood and urine biochemical parameters, but in line with their study, the extract of *P. aculeata* had preventive and therapeutic effects in ethylene glycol induced renal calculi. Additionally, in another study by Shukla et al. [22], the anti-urolithiatic effect of aqueous extract of *Bryophyllum pinnatum* leaves using ethylene glycol-induced renal calculi were evaluated. They found that administration of aqueous extract of leaves of *B. pinnatum* significantly reduces urine oxalate level, kidney weight and calcium oxalate depositions. They suggested that *B. pinnatum* is effective in prevention and treatment of ethylene glycol-induced urolithiasis [22]. Our results also are not consistent with this study, because in our study the extract of *P. aculeata* had no effect on blood and urine biochemical parameters, but caused a reduction in the production of calcium oxalate stones in kidney.

In a study conducted by Mehrabi et al. [23], the effect of hydrophilic extract of *Nasturtium officinale* on prevention of ethylene glycol induced renal stone in 32 male Wistar rats was studied. In this study, the highest percentage of calcium oxalate crystal depositions were in negative control groups (75%), followed by preventive groups with high dose (57.1%) and low dose (28.6%), and healthy control group (12.5%). At the end of the study period, urinary oxalate level in preventive and negative control groups were more than healthy control group. The results of this study was not in consistent with the result of our study, because in contrast with this study, in our study the highest number of calcium oxalate crystal deposition was in the high dose preventive group (76.6%), followed by ethylene glycol control (70%), low dose preventive (72%), treatment (38%), and moderate dose preventive (42%) groups. Also, our results are not consistent with this study, because the extract of *P. aculeata* significantly reduced the production of calcium oxalate stones in moderate dose preventive and treatment group. In addition, Mehrabi et al. [23] showed the *Nasturtium officinale* extract has no significant effects on urinary and chemical parameters efficient in calcium oxalate stone crystals in rat but its extract in low dose has some preventive effect on renal stone formation. These findings are consistent with the results of our study, because in our study the extract of *P. aculeata* had no effect on blood and urine biochemical parameters, but caused a reduction in the production of calcium oxalate stones in kidney.

In order to evaluate the antiurolithiatic effect of methanolic extract of *Hygrophila spinosa* in ethylene glycol induced nephrolithiasic rats, Ingale et al. [24] reported that ethylene glycol feeding results in increased levels of urinary oxalate, urinary calcium and serum uric acid along with increased levels of calcium and oxalate in kidney. On the other hand, treatment with methanolic extract of *Hygrophila spinosa* significantly results in reduced levels of urinary oxalate, urinary calcium and serum uric acid along with decreased levels of calcium and oxalate in kidney. They concluded that methanolic extract of *Hygrophila spinosa* possesses significant antilithiatic activity [24]. Also in a study by Jarald et al. [25], the effect of *Unex* on ethylene glycol-induced urolithiasis in 30 rats was studied. They found that the treatment with *Unex* restores all the elevated biochemical parameters (creatinine, uric acid, and blood urea nitrogen), restores the urine pH to normal, and increases the urine volume significantly. The findings of this study showed that the administration of *Unex* to rats with ethylene glycol-induced lithiasis reduces the growth of the urinary stone [25]. The results of these two studies were not in consistent with the result

of our study, because in our study the ethylene glycol significantly results in decreased serum calcium, urinary levels of creatinine, calcium and uric acid along with increased 24-hour urine volume, kidney weight, and calcium oxalate deposits.

## 5. Conclusion

Our data demonstrate that administration of aerial parts of *P. aculeate* has preventive and therapeutic effects in ethylene glycol induced renal calculi in Wistar rats. The mechanism underlying this effect is still unknown, but it probably exerts its effect through its antioxidant properties and also reducing the oxalate excretion and crystallization inhibition. However, further studies are necessary to clarify the mechanism underlying this effect.

## Declarations

### Compliance with Ethical Standards

- **Conflict of Interest:** We declare that there is no conflict of interest in our article.
- **Funding:** We declare that there is no any internal or external source of funding.
- **Ethical approval:** All procedures in this study were approved by the Guidelines of Animal Ethics Committee of Bushehr University of Medical Sciences and institutional ethics committee with approval code IR.BPUMS.REC.1391.209.
- **Authors' contributions:** AB, SA, and AY conceived and designed the experiments. SA, AB performed experimental procedures. SSE produced all histological figures and data. KM analyzed the data. AB and NM wrote the paper. All authors read and approved the final version of the manuscript.
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## Tables

Table 1: Mean body weight in rats of different groups before and after the study.

Body weight after study (gr)	Body weight before study (gr)	Groups
<b>251.14 ± 15.68*</b>	232.14 ± 19.85*	<b>A</b>
<b>233.80 ± 24.02</b>	232.20 ± 29.45	<b>B</b>
<b>244.40 ± 34.22</b>	236.00 ± 21.71	<b>C</b>
<b>208.40 ± 33.32</b>	224.60 ± 26.94	<b>D</b>
<b>222.00 ± 19.34</b>	231.17 ± 17.07	<b>E</b>
<b>229.20 ± 23.90</b>	222.80 ± 11.43	<b>F</b>

All values are expressed as mean ± SEM,

\*p< 0.001, significant difference between the body weight before and after the study in the healthy control group.

Table 2: Kidney weight and 24-hour urine volume in rats of different groups.

24-hour urine volume (mL)	Kidney weight (gr)	Groups
6.90 ± 1.86	0.77 ± 0.13	A
10.60 ± 3.71*	1.00 ± 0.23*	B
12.40 ± 3.42	1.16 ± 0.15	C
6.60 ± 0.96**	0.94 ± 0.28	D
9.17 ± 5.27	1.05 ± 0.30	E
7.00 ± 3.26**	1.02 ± 0.40	F

All values are expressed as mean ± SEM,

\*p< 0.05 when the ethylene glycol control group (B) compared to healthy control group.

\*\*p< 0.05 when the ethylene glycol control group (B) compared to groups of D and F.

Table 3: The results of the 24-hour urine parameters in rats of different groups (mg/dL).

Creatinine	Uric acid	Phosphorus	Calcium	PH	Groups
7.40 ± 4.68	1.17 ± 0.32	1.77 ± 0.55	1.62 ± 0.32	7.57 ± 1.81	<b>A</b>
1.12 ± 0.36*	0.16 ± 0.06*	1.62 ± 0.37	0.67 ± 0.21*	8.50 ± 0.86	<b>B</b>
0.94 ± 0.46	0.16 ± 0.12	2.22 ± 0.94	0.62 ± 0.39	8.33 ± 0.58	<b>C</b>
0.50 ± 0.14**	0.21 ± 0.31	1.34 ± 0.09	0.62 ± 0.18	8.00 ± 1.32	<b>D</b>
0.63 ± 0.11**	0.19 ± 0.08	2.48 ± 1.16	0.24 ± 0.11	7.50 ± 0.70	<b>E</b>
0.98 ± 0.42	0.19 ± 0.37	1.75 ± 0.77	0.75 ± 0.69	8.33 ± 0.58	<b>F</b>

All values are expressed as mean ± SEM,

\*p< 0.05 when the ethylene glycol control group (B) compared to healthy control group.

\*\*p< 0.05 when the ethylene glycol control group (B) compared to groups of D and E.

Table 4: The results of blood biochemical analyses in rats of different groups (mg/dL).

Creatinine	Uric acid	Phosphorus	Albumin	Calcium	Urea	Groups
0.61 ± 0.20	1.03 ± 0.18	6.51 ± 1.28	3.67 ± 0.31	9.37 ± 0.40	36.86 ± 9.63	<b>A</b>
0.82 ± 0.21	0.90 ± 0.35	5.33 ± 1.65	3.45 ± 0.22	8.58 ± 0.53*	52.45 ± 8.46	<b>B</b>
0.89 ± 0.13	0.83 ± 0.05	5.69 ± 0.51	2.99 ± 0.25**	8.06 ± 0.81	76.50 ± 22.81	<b>C</b>
0.69 ± 0.19	0.63 ± 0.21	4.55 ± 1.64	2.66 ± 0.40**	6.60 ± 1.37**	47.25 ± 11.32	<b>D</b>
0.63 ± 0.26	0.73 ± 0.31	4.61 ± 0.72	2.47 ± 0.45**	6.36 ± 1.38**	52.20 ± 18.06	<b>E</b>
0.77 ± 0.23	0.95 ± 0.37	7.99 ± 2.05	3.50 ± 0.07	9.00 ± 0.52	55.50 ± 22.65	<b>F</b>

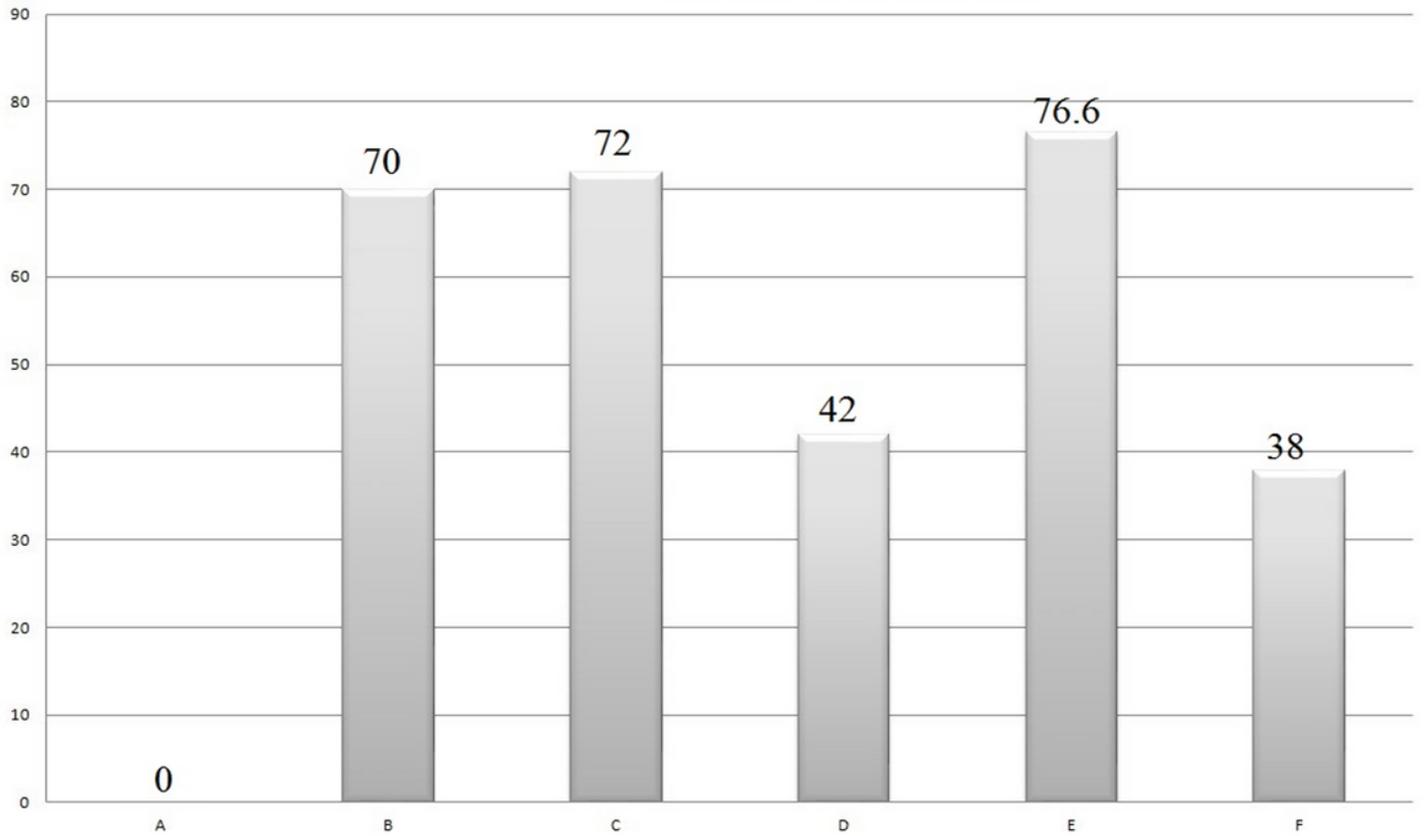
All values are expressed as mean ± SEM,

\*p< 0.05 when the ethylene glycol control group (B) compared to healthy control group.

\*\*p< 0.05 when the ethylene glycol control group (B) compared to groups of C, D and E.

## Figures

## Calcium oxalate crystal deposition



**Figure 1**

showing the number of calcium oxalate crystals in renal tissue in rats of different groups