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Effects of hydroalcoholic extract of Devil's claw on ethylene glycol and ammonium chloride-induced urinary tract stones in male rats

Hossein Kargar Jahromi[1], Mohammad Mohajer[2], Hossein Hakimelahi[3], Abdolreza Sotoodeh Jahromi[4], Hassanali Abedi[5], Amirashkan Mahjour[6], Bahareh Ebrahimi[7,*]

[1]*Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran*
[2]*Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran*
[3]*Assistant Professor of Urology, Jahrom University of Medical Sciences, Jahrom, Iran*
[4]*Zoonoses Research center, Jahrom University of Medical Sciences, Jahrom, Iran*
[5]*Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran*
[6]*Department of Pathobiology, Kazeroun branch.Isalamic azad university, Kazeroun, Iran*
[7]*Shiraz geriatric research center, Shiraz University of Medical Sciences, Shiraz, Iran*

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**** Corresponding Author:***
Ebrahimi_b@sums.ac.ir

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Abstract

Objective: Despite advances in conventional kidney stone treatments, limitations such as recurrence and side effects of the treatments necessitate the investigation of alternative treatments. Devil's claw (*Harpagophytum procumbens*), a medicinal plant known for its anti-inflammatory and antioxidant properties, may offer therapeutic benefits in the management of urinary tract stones. This study investigates the effects of hydroalcoholic extract of Devil's claw on urinary stones induced by ethylene glycol and ammonium chloride in male rats and evaluates its effect on biochemical, histological, and inflammatory markers.

Materials and Methods: A total of 40 male Wistar rats were divided into eight experimental groups including a control group, a kidney stone-induced control group, a kidney stone-induced group, and groups receiving Devil's claw extract (150 or 300 mg/kg) or Cystone® (750 mg/kg) via oral gavage for prevention (28 days) or treatment (days 14-28). Urinary, serum, and histological parameters were evaluated to determine the efficacy of the extract.

Results: Devil's claw extract significantly reduced urinary calcium and phosphorus levels, improved markers of kidney function, and reduced oxidative stress-related damage. The high dose showed better effects in reducing tubulointerstitial changes and minimizing kidney stone formation. The extract also modulated cytokine levels, reducing inflammatory markers such as Interleukin 1 beta and Tumor necrosis factor alpha while increasing IL-10.

Conclusion: The findings indicate that Devil's claw extract has nephroprotective and anti-kidney stone properties, demonstrating its potential as a natural alternative for kidney stone management.

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Introduction

Kidney stones, among the most common urinary tract disorders, represent a significant global health challenge, accounting for approximately 30% of urology consultations (Khan et al. 2016). Composed predominantly of calcium oxalate crystals, oxalate kidney stones are a frequent variant, often forming as mineral deposits in the renal pelvis or attached to the renal papilla (Khan et al. 2016; Leslie et al. 2024; Stamatelou and Goldfarb 2023). The formation of these stones is multifactorial, involving metabolic factors like hyperoxaluria and hypercalciuria, alongside inflammatory and immune responses (Nazarian et al. 2025; Peerapen and Thongboonkerd 2023; Stamatelou and Goldfarb 2023). Key inflammatory mediators including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are implicated in the pathophysiology of stone formation (Nazarian et al. 2025; Sun et al. 2024; Taguchi et al. 2021). These cytokines activate macrophages which while attempting to clear calcium oxalate crystals through phagocytosis, can exacerbate renal tissue damage and oxidative stress, perpetuating a cycle of inflammation and stone recurrence (Sun et al. 2024; Taguchi et al. 2021).

Recurrent kidney stones substantially diminish quality of life and kidney function. Despite advancements in medical and surgical interventions, challenges such as high costs, side effects, and limited efficacy persist. This has spurred interest in alternative therapeutic approaches including the use of herbal medicines rooted in traditional knowledge (Arya et al. 2017; Azarfar et al. 2020; Patel 2019). Cystone®, a well-known herbal formulation, has demonstrated efficacy in reducing stone size, preventing crystal aggregation, and promoting stone excretion, though further research is warranted to enhance its application (Azarfar et al. 2020; Erickson et al. 2011).

Devil's claw (*Harpagophytum procumbens*), a medicinal plant native to dry regions of Africa, is a promising alternative due to its potent anti-inflammatory, antioxidant, and analgesic properties (Gxaba and Manganyi 2022; McGregor et al. 2005). Its active compounds, particularly harpagoside, exert their effects by modulating inflammatory signaling pathways (such as NF-κB and MAPK), reducing cytokine production, and inhibiting proinflammatory enzymes such as Cyclooxygenase-2 (COX-2) and Lipoxygenases (LOX) (Gyurkovska et al. 2011; McGregor et al. 2005). These mechanisms suggest its potential application in reducing renal inflammation and oxidative stress associated with kidney stones. Previous studies on Devil's claw have highlighted its therapeutic effect in various inflammatory conditions and its protective effects on kidney and liver cells (Brendler 2021; Gyurkovska et al. 2011). However, its specific role in the prevention and treatment of urinary stones remains unknown.

This study investigates the effect of hydroalcoholic extract of Devil's claw on urinary tract stones induced by ethylene glycol and ammonium chloride in male rats. These findings aim to shed light on its potential as an effective, economical, and patient-friendly treatment option for the management of urinary stones.

Materials and Methods

Animals

In this study, 40 healthy male Wistar rats, 10 to 12 weeks old, weighing approximately 200 to 220 g, were used. The rats were randomly divided into eight groups (5 per group) with ethical considerations regarding animal care and use. For adaptation, the rats were kept for one week in controlled conditions at Jahrom University of Medical Sciences, Jahrom, Iran with a 12-hr light/dark cycle, ambient temperature of 21-25°C, and 50-60% humidity. All animals had free access

to standard laboratory chow and fresh drinking water throughout the study period.

Group assignments

Forty male Wistar rats were randomly assigned to eight experimental groups according to the administration of Cystone® (Cys) and Devil's Claw extract (DC).

1. Control Group: No treatment throughout the 28-day study (n=5).
2. Kidney Stone Group: Administered 8.98 mL/L ethylene glycol and 2.5 g/L chloride ammonium in drinking water ad libitum for the entire 28 days (n=5).
3. Prevention Group DC-150 (PG1): Received 8.98 mL/L ethylene glycol, 2.5 g/L ammonium chloride in drinking water ad libitum for the entire 28 days, and devil's claw extract (150 mg/kg) via gavage for 4 weeks (n=5).
4. Prevention Group DC-300 (PG2): Received 1% ethylene glycol, 0.25% ammonium chloride in drinking water ad libitum for the entire 28 days, and devil's claw extract (300 mg/kg) via gavage for 4 weeks (n=5).
5. Prevention Group Cys (PG3): Received 1% ethylene glycol, 0.25% ammonium chloride in drinking water ad libitum for the entire 28 days, and Cystone® (750 mg/kg/day) via gavage for 4 weeks (n=5).
6. Treatment Group DC-150 (TG1): Received 1% ethylene glycol, 0.25% ammonium chloride in drinking water ad libitum for the entire 28 days, and devil's claw extract (150 mg/kg) via gavage starting on day 14 for 14 days (n=5).
7. Treatment Group DC-300 (TG2): Received 1% ethylene glycol, 0.25% ammonium chloride in drinking water ad libitum for the entire 28 days, and devil's claw extract (300 mg/kg) via gavage starting on day 14 for 14 days (n=5).
8. Treatment Group Cys (TG3): Received 1% ethylene glycol, 0.25% ammonium chloride in drinking water ad libitum for the entire 28 days, and Cystone® (750 mg/kg/day) via gavage starting on day 14 for 14 days (n=5).

The selected doses of Devil's Claw and Cystone® were based on our previous studies which demonstrated their efficacy and safety in experimental models (Haghshenas et al. 2023; Jahromi 2018). To induce kidney stone, the animals received drinking water containing 8.98 mL/L ethylene glycol and 2.5 g/L ammonium chloride in drinking ad libitum for the entire 28-day study period (Pourahmadi et al. 2023; Saremi et al. 2015).

Hydroalcoholic extract preparation

Harpagophytum procumbens (Burch.) DC. aerial parts (leaves, stems, and branches) collected from Yasuj, Iran was powdered. The plant was taxonomically identified and authenticated at Jahrom Azad University of Medical Sciences. A voucher specimen was deposited in the Herbarium of Jahrom Azad University of Medical Sciences under voucher number 1042. The extract was prepared by soaking 300 g of the aerial parts with 4 L of 70% ethanol (Focused Photonics Inc. (China)) at 80°C using a rotary apparatus (Ebara (Japan)). After filtration (0.45 µm, Millipore), the extract was freeze-dried to a yellow powder. The final yield of the hydroalcoholic extract was approximately 42.6 g, corresponding to 14.2% w/w of the initial dry plant material. Based on previous studies, doses of 150 and 300 mg/kg were used (Haghshenas et al. 2023; Jahromi 2018).

Urinalysis parameter and serum biochemistry assessments

Body weight was recorded at the beginning and end of the experiment using a digital laboratory scale. Twenty-four-hour urine samples were collected on day 28 using individual metabolic cages designed for small laboratory animals to ensure sample purity and accurate volume measurement. Urinary parameters including volume, pH, uric acid (UA),

creatinine (Cr), calcium (Ca), phosphorus (P), and total protein (TP) were analyzed using an autoanalyzer (Mindray BS-430, SN: YW-01000242T, China). Urine pH was measured using a digital pH meter (SANA SL-901, Biorex, Iran). Calcium oxalate crystal deposition was assessed histologically in hematoxylin and eosin (H&E) stained kidney sections and incorporated into the semi-quantitative scoring system for tubulointerstitial damage.

On day 29, animals were anesthetized with ketamine (100 mg/kg) (Alfasan-Netherland) and xylazine (20 mg/kg) (Alfasan-Netherland) administered intraperitoneally, and then their cardiac blood was collected (Pourahmadi et al. 2023; Saleh et al. 2023). Serum biochemical parameters including phosphorus, total protein, uric acid, blood urea nitrogen (BUN), creatinine, calcium, and liver enzymes (Aspartate aminotransferase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP)) were measured using an autoanalyzer (Mindray BS-430, China) and Biorex diagnostic kits (Iran).

Inflammatory factors results

Serum levels of IL-10, IL-1β, and TNF-α were determined using enzyme-linked immunosorbent assay (ELISA) kits (Awareness Technology, Stat Fax 2100 Microplate Reader, USA) according to the manufacturer's instructions. The ELISA method was performed as described by Pourahmadi et al. (Pourahmadi et al. 2023), ensuring specificity and sensitivity for rat cytokines.

Histopathology evaluations

Following sacrifice by intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine, the rats were placed in dorsal recumbency and the abdominal cavity was opened after shaving and disinfection of the skin. Both kidneys were carefully dissected free from surrounding perirenal adipose and connective tissue, and the renal pedicles.

Renal index was calculated by dividing kidney weight (g) by body weight (g) multiplied by 100. Removed Kidneys were cleaned, weighed, and fixed in 10% formalin for histological evaluation. Tissue sections (5 µm) were stained with hematoxylin and eosin (H&E). Histopathological grading was performed independently by a qualified pathologist, using a semiquantitative scale (0–4).

Tubulointerstitial changes were assessed using a semiquantitative scoring system (0–4) based on the severity of histopathological changes (Pourahmadi et al. 2023; Taguchi et al. 2021). The score indicates: score 0, no detectable damage and normal renal histology; score 1, minimal changes such as mild tubular dilation or interstitial edema; Score 2, moderate alterations including focal tubular degeneration and mild inflammatory infiltration; Score 3, marked damage characterized by widespread tubular injury, moderate inflammation, and crystal deposition; and Score 4, severe histopathological changes including extensive tubular necrosis, severe inflammatory response, and dense crystal accumulation (Figure 1).

Analyze

Statistical calculations were performed using IBM SPSS software, version 21. Kolmogorov-Smirnov test was used to determine the normality. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with $p<0.05$ considered statistically significant.

Results

Urinalysis parameter and Serum Biochemistry results

Urinalysis results showed significant differences in parameters among the study groups. Devil's claw extract resulted in a decrease in urine pH compared to the

control group. All study groups, except prevention group Cys, showed an increase in urine creatinine. Devil's claw extract caused a decrease in 24-hr urine volume, which was partially compensated by prevention groups DC-300 and Cys and treatment group Cys, as these groups increased urine volume relative to the others ($p<0.05$). Urinary calcium levels were higher in most groups except for prevention group Cys, with both prevention and treatment groups showing a reduction in calcium levels compared to Devil's claw extract. The high dose generally resulted in greater reductions in urinary calcium. All study groups showed increased urinary phosphorus levels compared to the control group, but the prevention and treatment groups showed lower phosphorus levels than the kidney stone group ($p<0.05$) (Figure 2).

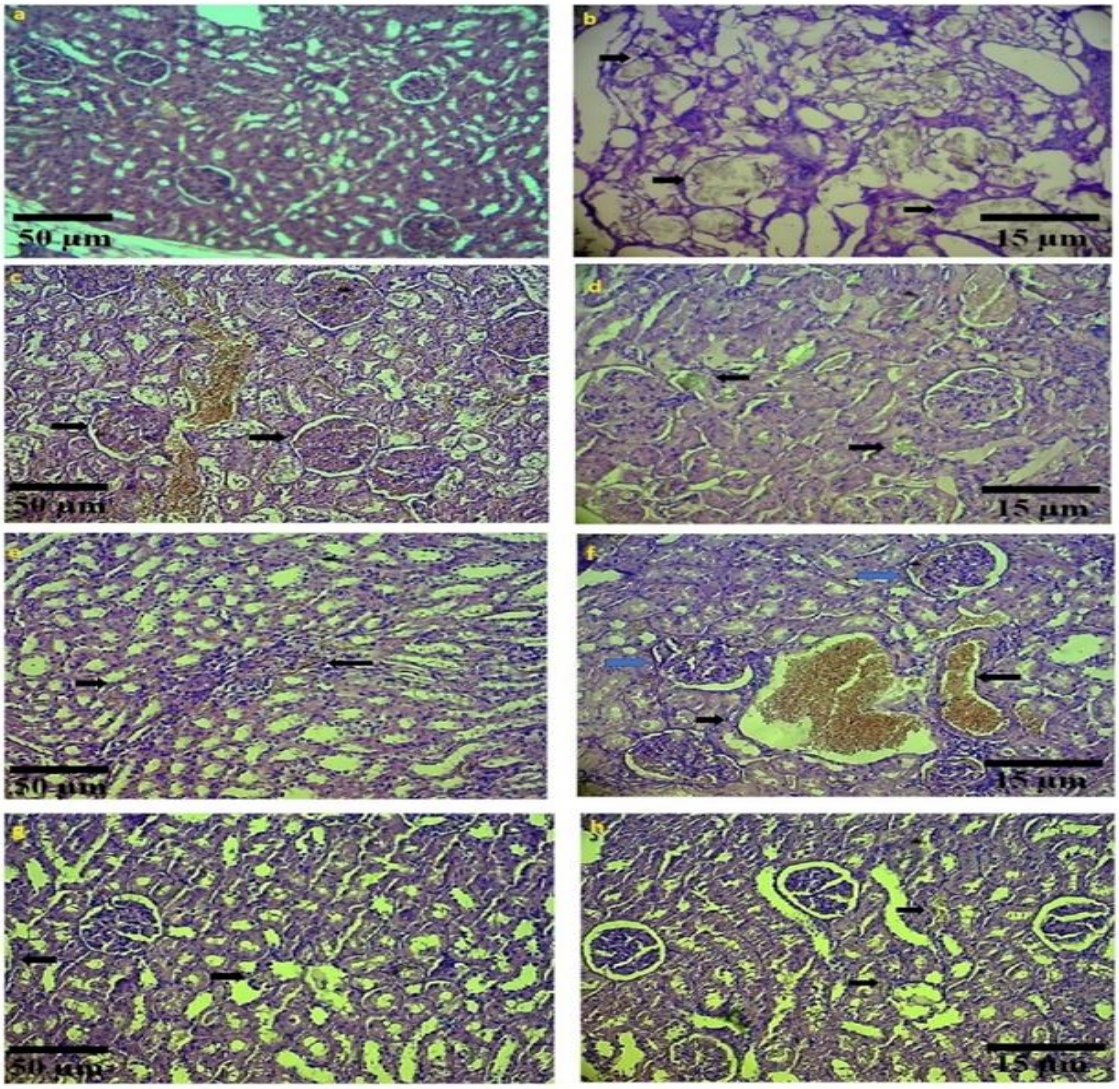

Figure 1. Representative histological images of hematoxylin and eosin (H&E) stained kidney tissue sections in the experimental groups. (a) Control group (×10) showing normal renal architecture with healthy glomeruli and tubules. (b) Stone-induced group (EG+AC; ethylene glycol + ammonium chloride) (×40) showing extensive tubular damage, vacuolation, and disrupted tissue structure. (c–e) Prevention groups treated with Devil's Claw extract (150 and 300 mg/kg) or Cystone® (750 mg/kg) (×40), showing reductions in tubulointerstitial damage and crystal deposition. (f – h) Treatment groups that received devil's claw extract or Cystone® (×40) after stone induction showed partial restoration of renal histology and reduction of inflammatory changes. Black arrows indicate areas of histopathological alteration including glomerular disruption, tubular dilation, and interstitial inflammation.

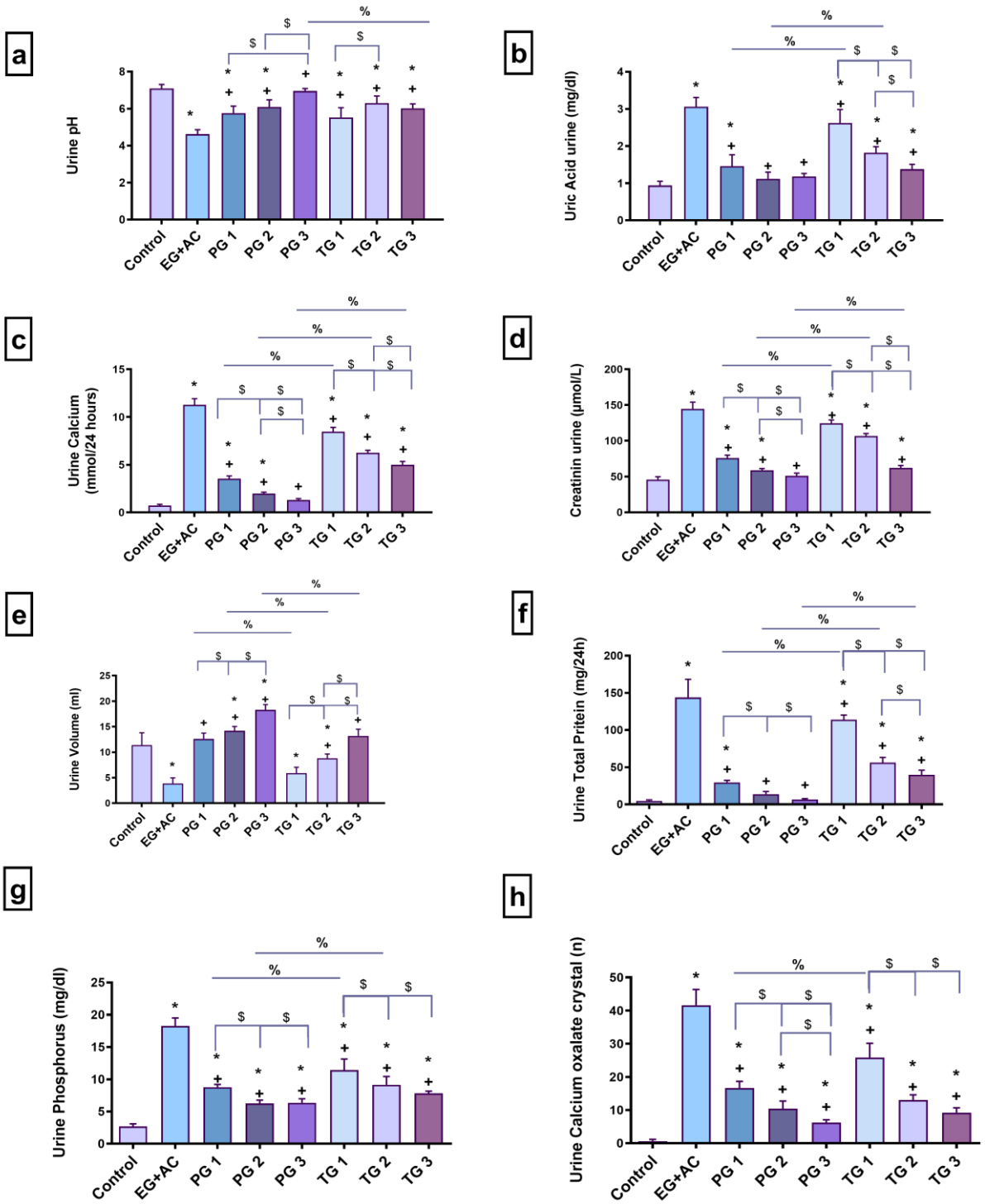

Figure 2. Urinary biochemical changes in kidney stone-induced rats following herbal treatments. (a) Urine pH; (b) Uric acid (mg/dL); (c) Calcium (mmol/24h); (d) Total protein (mg/24h); (e) Urine volume (mL); (f) Creatinine (µmol/L); (g) Calcium oxalate crystals (n); (h) Phosphorus (mg/dL). Data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Group abbreviations: EG+AC (ethylene glycol + ammonium chloride); PG1 (prevention group 1, Devil's claw 150 mg/kg); PG2 (prevention group 2, Devil's claw 300 mg/kg); PG3 (prevention group 3, Cystone® 750 mg/kg); TG1 (treatment group 1, Devil's claw 150 mg/kg); TG2 (treatment group 2, Devil's claw 300 mg/kg); TG3 (treatment group 3, Cystone® 750 mg/kg); mg/dl, milligram per deciliter; µmol/l, micromole per liter; ml, mililiter; mg, milligram; and n, number. Symbols indicate significant differences between specific groups at $p<0.05$: *: Significant difference with Control; +: Significant difference with EG+CA group; $: significant difference within prevention or treatment groups; %: significant difference between prevention and treatment groups.

Serum analysis results showed significant changes among the study groups in key biochemical parameters. Kidney stone resulted in significant increases in serum phosphorus, total protein, uric acid, blood urea nitrogen, creatinine, and calcium levels compared with the control group ($p<0.05$). In comparison, the prevention and treatment groups showed effects in modulating these parameters, the high dose typically showing significant adjustments to the normal range (Figure 3).

As shown in Figure 3, blood urea nitrogen and serum creatinine levels increased in kidney stone, but these levels decreased significantly in the prevention and treatment groups ($p<0.05$). Serum calcium which showed a sharp increase in kidney stone group, was relatively controlled in the prevention and treatment groups, the high dose of DC and Cys helping to better regulate calcium levels.

Inflammatory factors result

Serum cytokine levels and liver enzyme analysis showed significant differences among the study groups. Kidney stone caused a significant increase in AST, ALT, and ALP levels, indicating a significant impact on liver function. In contrast, the prevention and treatment groups showed reductions in their levels, the high dose of both DC and Cys showing better regulation levels ($p<0.05$). Cytokine analysis in Table 1 showed marked changes in inflammatory and anti-inflammatory markers. Kidney stone resulted in decreased levels of IL-10, an important anti-inflammatory cytokine, while levels of IL-1β and TNF-α were significantly increased. The prevention groups managed to return IL-10 levels closer to control values. Similarly, IL-1β and TNF-α levels were reduced in both prevention and treatment groups, with DC-300 and Cys achieving the lowest levels among all groups (Table 1).

Histopathology results

Histopathological and morphological kidney assessment, Figure 4 reveals significant differences between study groups. Kidney stone resulted in the most severe tubulointerstitial changes, while both the prevention and treatment groups showed reductions in these changes ($p<0.05$).

The renal index in Figure 4 shows a significant increase in kidney stone group compared to the control group, indicating significant renal dysfunction. Both prevention and treatment groups showed progressive improvements in renal index values, the high dose providing increased protective effects ($p<0.05$). The number of kidney stones was highest in the kidney stone group, while the prevention and treatment groups showed significant reductions (Figure 4).

Body weight analysis revealed distinct patterns among the study groups. The kidney stone group showed the least weight gain; in contrast, the prevention and treatment groups showed varying degrees of body weight change, the high dose of DC and Cys contributing to better weight maintenance ($p<0.05$) (Figure 5). Analysis of kidney weight showed that the kidney stone group had the highest kidney weight and the prevention and treatment groups showed reductions in kidney weight ($p<0.05$). The high dose of DC and Cys were particularly effective in normalizing kidney weight (Figure 5).

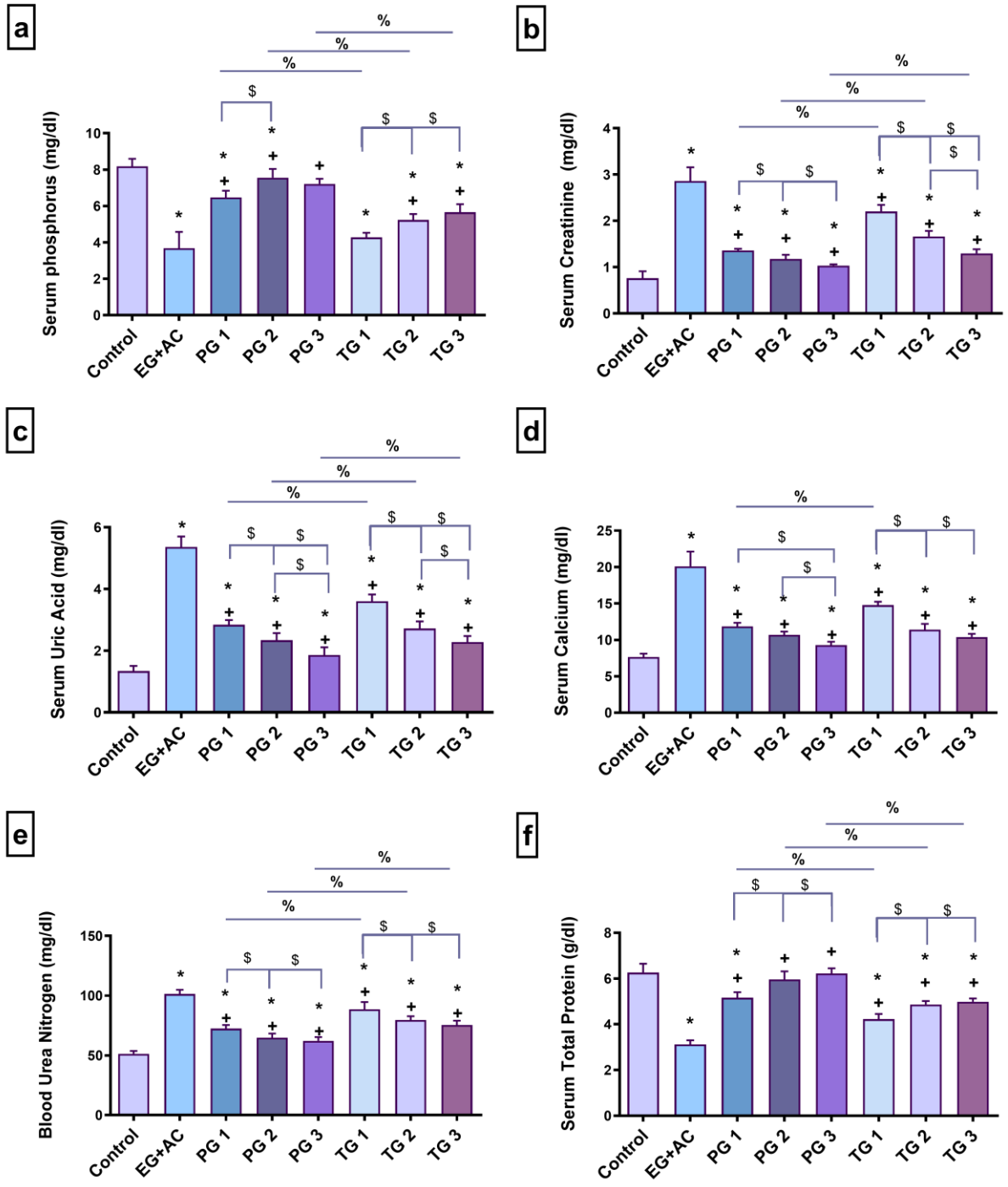

Figure 3. Serum biomarker modulation by *Harpagophytum procumbens* (Devil's Claw) extract and Cystone® in kidney stone prevention and treatment. Parameters measured include serum: (a) phosphorus (mg/dl); (b) creatinine (mg/dl); (c) uric acid (mg/dl); (d) calcium (mg/dl); (e) blood urea nitrogen (BUN, mg/dl); (f) total protein (g/dl). Data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Abbriviatin: EG+AC (ethylene glycol + ammonium chloride); PG1 (prevention group 1, Devil's claw 150 mg/kg); PG2 (prevention group 2, Devil's claw 300 mg/kg); PG3 (prevention group 3, Cystone® 750 mg/kg); TG1 (treatment group 1, Devil's claw 150 mg/kg); TG2 (treatment group 2, Devil's claw 300 mg/kg); TG3 (treatment group 3, Cystone® 750 mg/kg); mg/dl, milligram per deciliter; and g/dl, gram per deciliter. Symbols indicate significant differences between specific groups at $p<0.05$: *: Significant difference with Control; +: Significant difference with EG+CA group; $: significant difference within prevention or treatment groups; %: significant difference between prevention and treatment groups.

Table 1. Effects of hydroalcoholic extract of Devil's claw and Cystone® on serum cytokine levels and liver enzymes in kidney stone-induced rats.

	Control	**EG+AC**	**Prevention groups**			**Treatment groups**		
			DC 150mg/kg	**DC** 300mg/kg	Cystone® 750mg/kg	**DC** 150mg/kg	**DC** 300mg/kg	Cystone® 750mg/kg
AST (U/L)	103.4 ± 6.7	357.4 ± 19.1*	165.2 ± 17.0*+	132.2 ± 5.5*+&	129.4 ± 5.7+&	222.8 ± 13.0*+%	181.2 ± 23.3*+&%	163.2 ± 6.8*+&%
ALT (U/L)	25.8 ± 1.6	152.2 ± 6.8*	63.4 ± 5.0*+	40.2 ± 4.3*+&	41.4 ± 4.0*+&	92.6 ± 5.1*+%	74.4 ± 5.3*+&%	67.4 ± 5.7*+&%
ALP (U/L)	126.0 ± 6.2	511.8 ± 24.4*	237.6 ± 13.8*+	191.2 ± 9.1*+&	165.4 ± 13.0*+&	407.0 ± 18.2*+%	333.6 ± 13.1*+&%	267.2 ± 16.6*+&%
IL-10 (pg/mL)	102.0 ± 4.3	66.4 ± 2.9*	95.7 ± 2.6+	98.6 ± 4.0+	101.9 ± 5.7+	80.2 ± 3.6*+%	87.1 ± 5.0*+&%	89.1 ± 4.1*+&%
IL-1β (pg/mL)	53.8 ± 7.7	116. 8 ± 3.5*	85.3 ± 3.5*+	82.6 ± 4.2*+	74.9 ± 4.0*+	65.1 ± 5.9*+	77.5 ± 3.4*+&	78.9 ± 5.8*+&
TNF-α (pg/mL)	102.55±1.0	175.2 ± 9.7*	134.6 ± 10.4*+	120.3 ± 4.1*+&	117.5 ± 3.9*+&	146.4 ± 5.0*+	135.9 ± 4.5*+%	126.4 ± 4.6*+

Abbreviations: EG+AC, ethylene glycol + ammonium chloride (stone-induced); DC, Devil's claw; AST, Serum levels of aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; IL-10, interleukin-10; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha; U/L, Units per Liter; pg/mL, picograms per milliliter; and mg/kg, milligram per kilogram. Symbols indicate significant differences between specific groups at $p<0.05$: *: Significant difference with Control; +: Significant difference with EG+CA group; &: significant difference within prevention or treatment groups; %: significant difference between prevention and treatment groups.

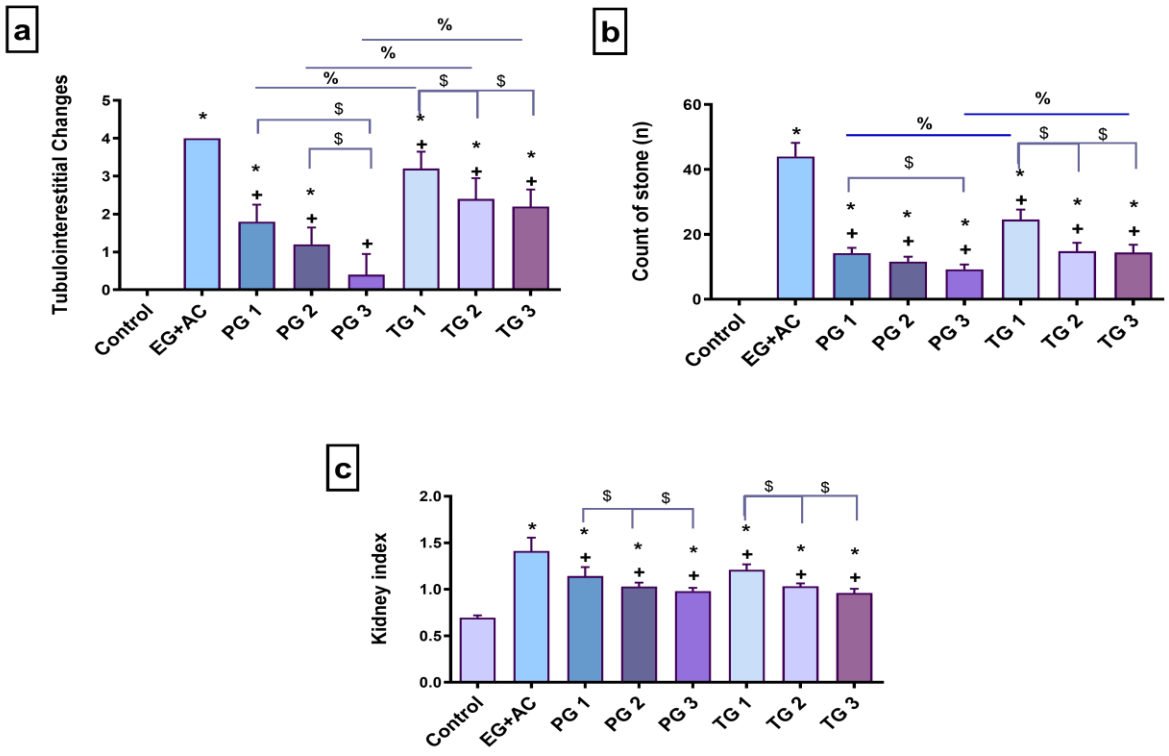

Figure 4. Histopathological and morphological kidney assessment across experimental groups, including tubulointerstitial changes (a), count of stone (b), and kidney index (c). Data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Abbreviations: EG+AC (ethylene glycol + ammonium chloride); PG1 (prevention group 1, Devil's claw 150 mg/kg); PG2 (prevention group 2, Devil's claw 300 mg/kg); PG3 (prevention group 3, Cystone® 750 mg/kg); TG1 (treatment group 1, Devil's claw 150 mg/kg); TG2 (treatment group 2, Devil's claw 300 mg/kg); TG3 (treatment group 3, Cystone® 750 mg/kg); and n, number. Symbols indicate significant differences between specific groups at $p<0.05$: *: Significant difference with Control; +: Significant difference with EG+CA group; $: significant difference within prevention or treatment groups; %: significant difference between prevention and treatment groups.

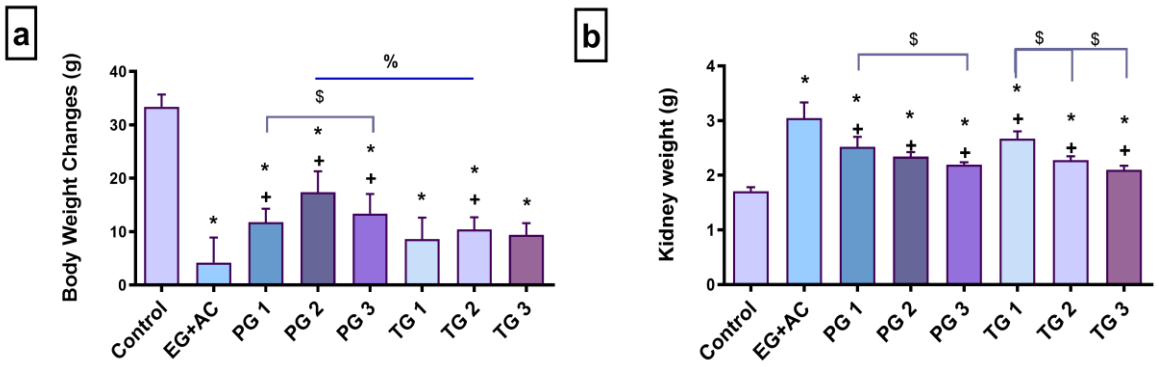

Figure 5. Body weight and renal hypertrophy dynamics in experimental urolithiasis models treated with *Harpagophytum procumbens* (Devil's Claw) extract and Cystone®. Parameters include body weight changes during the experimental period (a) and final kidney weight (b). Data are expressed as Mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Abbreviations: EG+AC (ethylene glycol + ammonium chloride); PG1 (prevention group 1, Devil's claw 150 mg/kg); PG2 (prevention group 2, Devil's claw 300 mg/kg); PG3 (prevention group 3, Cystone® 750 mg/kg); TG1 (treatment group 1, Devil's claw 150 mg/kg); TG2 (treatment group 2, Devil's claw 300 mg/kg); TG3 (treatment group 3, Cystone® 750 mg/kg); and g, gram. Symbols indicate significant differences between specific groups at $p<0.05$: *: Significant difference with Control; +: Significant difference with EG+CA group; $: significant difference within prevention or treatment groups; %: significant difference between prevention and treatment groups.

Discussion

The results of this study underscore the notable therapeutic potential of Devil's claw extract and Cystone® in improving biochemical indicators and mitigating kidney stone-related disorders. Both compounds demonstrated significant efficacy in reducing serum and urinary levels of uric acid, creatinine, calcium, protein, phosphorus, oxalate, and liver enzymes (AST, ALT, and ALP) in treatment and prevention groups. The observed reductions reflect improvements in metabolic health and renal function. Furthermore, the enhanced effectiveness of a high dose of Devil's claw extract (300 mg/kg) emphasizes the importance of optimizing administration strategies.

Devil's claw extract exhibited profound effects in reducing kidney stone formation induced by ethylene glycol and ammonium chloride, likely mediated through its robust antioxidant and anti-inflammatory properties. Active compounds such as phenolic acids, flavonoids, and essential oils contributed to alleviating oxidative stress a critical factor in calcium oxalate crystallization and renal tubular damage (Baptista et al. 2024; Sun et al. 2024). These findings align with prior studies that have highlighted the protective roles of medicinal plants in kidney stone prevention and treatment (Gyurkovska et al. 2011; McGregor et al. 2005). Similarly, Cystone® multifaceted formulation demonstrated synergistic benefits due to the combination of active constituents with complementary therapeutic effects (Azarfar et al. 2020; Erickson et al. 2011).

A key observation of this study was the reduction in liver enzyme levels across all prevention and treatment groups, which corroborates the hepatoprotective effects of Devil's claw extract and Cystone®. Previous research has established the efficacy of phytochemicals in reducing hepatic injury associated with kidney stone formation. Mechanistically, compounds such as flavonoids, terpenoids, and phenolic acids exhibit antioxidant and anti-inflammatory activities that attenuate oxidative stress and inflammation in hepatic tissues (Chinnappan et al. 2023a; Chinnappan et al. 2023b). These properties likely contributed to the observed decreases in AST and ALT levels, further affirming the systemic benefits of these herbal treatments.

Histological analyses revealed significant preservation of renal tissue integrity in the prevention and treatment groups. The protective effects of Devil's claw extract and Cystone® against kidney tissue damage were evident, highlighting their ability to reduce oxidative stress and inflammation. Improved serum protein levels and enhanced urinary calcium clearance observed in this study suggest improvements in renal function and filtration efficiency. Both Devil's claw extract and Cystone® inhibited crystallization processes and promoted the excretion of metabolic waste, contributing to reductions in serum calcium and uric acid levels. The inhibition of xanthine oxidase activity and enhanced diuretic effects were identified as potential mechanisms underlying these improvements (Allard et al. 2013; Georgiev et al. 2010). Comparative analysis suggests that Devil's claw extract at 300 mg/kg achieved effects similar to Cystone® in several parameters. This highlights its potential as a cost-effective alternative, especially in regions where Cystone® is less accessible.

Additionally, the study provided insights into the role of anti-inflammatory cytokines such as IL-10 and inflammatory markers like interleukin IL-1β in kidney stone pathology. Devil's claw extract and Cystone® demonstrated beneficial effects in modulating these cytokines, resulting in reduced inflammation and improved kidney function. These findings support the therapeutic relevance of anti-inflammatory and antioxidant mechanisms in herbal treatments for kidney disorders (Chinnappan et al. 2023b; Sun et al. 2024).

Mechanistically, the antioxidant and anti-inflammatory effects of Devil's claw may involve modulation of NF-κB and MAPK signaling pathways which were not directly measured in this study but are supported by prior literature. Future molecular assays such as immunohistochemistry or gene expression profiling are needed to confirm these mechanisms (Gyurkovska et al. 2011).

The efficacy of Devil's claw extract and Cystone® in preventing and treating kidney stone formation highlights their clinical potential. The high doses of Devil's claw extract (300 mg/kg) and Cystone® (750 mg/kg) were more effective in mitigating biochemical and histological markers of kidney dysfunction. This study reinforces the importance of understanding optimal dosing strategies for herbal compounds to maximize therapeutic outcomes.

Cystone® was used in this study as a reference herbal formulation with well-documented anti-kidney stone properties. Comparative analysis showed that Devil's claw extract produced renal protective, antioxidant, and anti-inflammatory effects that were largely comparable to those of Cystone®. Cystone® showed strong efficacy in reducing stone count and liver enzyme levels, and Devil's claw extract showed significant benefits in modulating cytokine balance. Devil's claw also improved markers of kidney function such as serum creatinine and BUN. These findings suggest that Devil's claw extract may offer potential as a complementary or alternative treatment for Cystone®.

The study further emphasized the role of Devil's claw extract and Cystone® in preventing calcium oxalate crystallization and preserving renal tubular integrity. Compounds such as phenolics, flavonoids, and terpenoids contributed to these effects by reducing oxidative stress, inhibiting inflammatory pathways, and promoting renal clearance of calcium and other ions. These mechanisms underscore the systemic benefits of these herbal remedies in supporting renal health and preventing kidney stone formation.

In conclusion, this study demonstrates that the hydroalcoholic extract of Devil's claw exerts significant nephroprotective and anti-nephrolithiasis effects in a rat model of ethylene glycol and ammonium chloride-induced nephrolithiasis. The extract improved biochemical markers, modulated inflammatory cytokines, and reduced oxidative stress. Devil's claw extract also preserved kidney tissue integrity. Compared to the reference formulation of Cystone®, Devil's claw extract showed comparable efficacy and, at high dose, had significant benefits in regulating cytokines and markers of renal function. These findings highlight its potential as a complementary or alternative herbal treatment for kidney stone management.

Future research should focus on molecular mechanisms (e.g., NF-κB and MAPK pathways), long-term safety, and clinical validation in human populations. Such studies will be essential to determine optimal dosing strategies. They can determine whether Devil's claw can be integrated into treatment protocols, either alone or in combination with established formulations such as Cyston.

Acknowledgment

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Conflicts of interest

The authors declare no conflict of interest.

Funding

This experimental laboratory research has been approved by the student research committee of the Jahrom University of Medical Sciences-Iran.

Ethical Considerations

This study was conducted under the ethical code IR.JUMS.REC.1402.002 from Jahrom University of Medical Sciences,

adhering to all ethical principles and considerations for working with laboratory animals

Code of Ethics

IR.JUMS.AEC.1402.002

Authors' Contributions

H.K.J, M.M, and H.H conceived and designed the experiments; A.S.J., H.K.J, M.M., and H.H. performed the experiments, B.E., A.M. and H.A. analyzed and interpreted the data. B.E. and H.K.J wrote the paper. All authors revised it and accepted the final version of the paper.

Abbreviations

Abbreviation Full Form
AC, Ammonium chloride
ALP, Alkaline phosphatase
ALT, Alanine aminotransferase
AST, Aspartate aminotransferase
BUN, Blood urea nitrogen
Ca, Calcium
CO_2, Carbon dioxide
COX-2, Cyclooxygenase-2
Cr, Creatinine
Cys, Cystone®
DC, Devil's Claw (Harpagophytum procumbens)
EG, Ethylene glycol
EG+AC, Ethylene glycol + ammonium chloride (stone-induced group)
ELISA, Enzyme-linked immunosorbent assay
H&E, Hematoxylin and eosin
IL-10, Interleukin-10
IL-1β, Interleukin-1 beta
IL-6, Interleukin-6
IP, Intraperitoneal
LOX, Lipoxygenase
MAPK, Mitogen-activated protein kinase
NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells
P, Phosphorus
PG, Prevention group
PG1, Prevention group 1 (Devil's claw 150 mg/kg)
PG2, Prevention group 2 (Devil's claw 300 mg/kg)
PG3, Prevention group 3 (Cystone® 750 mg/kg)
SEM, Standard error of the mean
TG, Treatment group
TG1, Treatment group 1 (Devil's claw 150 mg/kg)
TG2, Treatment group 2 (Devil's claw 300 mg/kg)
TG3, Treatment group 3 (Cystone® 750 mg/kg)
TNF-α, Tumor necrosis factor-alpha
TP, Total protein
UA, Uric acid

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