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Effect of beta-cyclodextrin encapsulated gallic acid from *Polygonum equisetiforme* L. on kidney stones produced by ethylene glycol and ammonium chloride in rats

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In this study, we examined the effects of encapsulated ellagic acid from *Polygonum equisetiforme* (PE) on kidney stones. Rats in this study randomly divided into 4 groups: Group I (control), Group II (disease group), Group III (low dose-100 mg/kg) and Group IV (high dose- 400 mg/kg). Kidney stones induced by adding 0.75% Ethylene Glycol (EG) + 2% Ammonium Chloride (AC) to drinking water and treatment groups received PE extract by gavage for 14 days. Exposure to EG/AC resulted in a significant decrease in SOD and GPx and an increase in creatinine levels. The treatment reversed the decreased GPx, SOD and the increased the creatinine levels. Histopathological findings are compatible with the ELISA results. There was no pathologic findings were detected in Group I. In Group II, the presence of calcium oxalate crystalloids in the kidney tissues as well as tubular dilatation and interstitial inflammation confirmed the formation of kidney stones. These histopathological findings in the kidneys were reversed by the PE extract. On the other hand, the histopathological results of liver tissues were different from kidney tissues. While no pathological findings were found in group I, a trace amount of damage was observed in the disease group. In addition, while a mild portal inflammation occurred in the low-dose treatment group, moderate portal inflammation and mild interface hepatitis occurred in the high-dose treatment group. This finding may restrict usability of the phenolic extract of PE in the treatment of kidney stone except of low doses of the PE extract.

Keywords: Kidney stone, Phenolic compounds, *Polygonum Equisetiforme* L, Rat

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Polygonum equisetiforme is a flowering plant belonging to the family Polygonaceae, characterized by its distribution in North Africa, North and South America, Europe and Western Asia[1,2]. This plant has been used in folk medicine due to its richness in proteins, mineral elements, polysaccharides, tannins and phenolic compounds[1,3]. Some of these compounds found in this species have exhibited various pharmacological properties such as antioxidant, anti-inflammatory, antibacterial, antiviral and antifungal activities[1] due to its polyphenol content. Polygonum species has also been used to reduce the production of kidney stones induced by ethylene glycol and chloride in rats[4].

P. equisetiforme was chosen for this study due to its long history of use in traditional medicine for urinary system problems. In addition, the plant is rich in polyphenolic compounds, which have been linked to antioxidant, anti-inflammatory, and kidney-protective effects. Previous research on various *Polygonum* species has also suggested their potential to inhibit kidney stone formation in experimental models[4,5].

Nephrolithiasis ranks as the third most frequent condition of the urinary tract, following urinary tract infections and prostate-related disorders[6]. The process of stone formation is influenced by various endogenous substances, and nearly 80-85% of urinary calculi are composed of calcium[4]. The development of calcium-based stones is generally associated with elevated urinary calcium, uric acid, and oxalate

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levels, together with a reduction in urinary citrate concentration[7].

It is also quite possible for kidney stones to recur. Considering the risk of recurrence of urinary stones, high treatment costs, and damage caused by operations, protection of kidney stones and use of some plant extracts in the treatment may be a suitable alternative[4]. Undoubtedly, the efficiency of plant-extracted biomolecules was greatly affected by the extraction processes as well as the operating conditions[8]. Indeed, the latter should be reproducible, environmentally friendly, commercially effective and preserve the stability and effectiveness of bioactive molecules[9]. In this context, various methods have been used to maintain the stability and solubility of polyphenol. The most common method used to maintain the stability of these molecules is encapsulation with β-cyclodextrin (CDs)[10,11]. CDs encapsulated systems increase the antioxidant activity of the inclusion complex, can protect polyphenols against rapid oxidation by free radicals, which can be partially explained by an increase in their bio solubility[10,11].

This plant has been widely used in traditional medicine for urinary system diseases, particularly for dissolving kidney stones and treating bladder problems. Previous studies have also reported its strong antioxidant and anti-inflammatory properties, as well as organ-protective activities such as hepatoprotection[12,13]. Furthermore, polyphenolic compounds are abundant in this species, and gallic acid has been identified as one of the major phenolic constituents, making it a promising candidate for investigating protective effects against kidney stone formation[13,14].

In a study conducted by Basant Ballabh and colleagues in 2008, the leaves of *Polygonum aviculare* Linn. were reported to alleviate kidney inflammation and pain, as well as burning sensations in the bladder and during urination[15]. Moreover, the decoction of *Polygonum bellardii* has also been traditionally used for the treatment of kidney stones[16].

Since the effects of encapsulated ellagic acid from *Polygonum equisetiforme* on kidney stones have not yet been studied. In this study, the effects of cyclodextrin-encapsulated gallic acid obtained under optimal extraction conditions of the phenolic extract of the beta-encapsulated green part of PE on kidney stones induced with ethylene glycol and ammonium chloride in male Wistar rats will be investigated. Thus, a new herbal treatment method can be added to the treatment of nephrolithiasis.

Material and Methods

Preparation of *Polygonum equisetiforme* extract as encapsulation

The complexes were prepared according to the lyophilization methods described by Pralhad and Rajendrakumar[17] and Wang[18] in the presence of GA (Gallic acid) and β-CDS (beta-cyclodextrin) with some modifications. Encapsulations were performed with 0.003 mol of each reagent (β-CDS and Polygonum extract) in 50 mL of water. The mixture was kept under continuous stirring at room temperature for about 5 h and then allowed to stand for 12 h. It was then be frozen at -80°C for 24 h[18,19], followed by lyophilization to obtain a powder consistency. The physical mixture was obtained by manual homogenization of GA with polymer and plant extract in a ratio of 1:1.

Animals

A total of 32 adult female Wistar Albino rats, each weighing 200-250 g, were included in the study. The experimental procedures were reviewed and approved by the Cukurova University Animal Care and Use Committee (SABİDAM). The experiments were conducted in line with the guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1984). Animals were maintained under controlled laboratory conditions with a 12-h light/12-h dark cycle. All experimental procedures received approval from the Cukurova University Animal Care and Use Committee (SABIDAM), under Protocol No. 22.04.2022: 3.8.

Experimental design

Animals were kept in metabolic cages at SABİDAM (Health Sciences Experimental Application and Research Center) and there was one rat in each cage. The rats randomly divided into 4 groups, each containing eight rats. The experimental protocol was 14 days. Group I (control group) was given normal drinking water, and Group II (disease group: kidney stone) was given drinking water with 0.75% Ethylene Glycol + 2% Ammonium Chloride to ensure kidney stone formation[20]. Except for the control group, all groups were given drinking water containing 0.75% Ethylene Glycol + 2% Ammonium Chloride for 14 days. Group III (low dose treatment group 100 mg /kg)[12] received phenolic compounds obtained from PE, Group IV (high dose treatment group 400 mg /kg)[21] received phenolic compounds obtained from PE by gavage for 14 days.

Histological and biochemical studies

On the 15th day, the animals were killed under sevoflurane anesthesia, and the liver and both kidneys were removed from each animal and placed in a separate tube in formaldehyde fixation solution and stored for histopathological examination. Intracardiac blood samples were taken while animals were killed to determine oxidative stress markers such as glutathione peroxidase (GPx, ng/ml) and superoxide dismutase (SOD, ng/ml) with ELİSA method. We additionally examined serum creatinine levels (ng/ml) via ELİSA method.

Statistical analysis

All experimental data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Values of $p<0.05$ were considered statistically significant. GraphPad Prism software (GraphPad; version 5.0 San Diego, CA, USA) was used to perform all analyses.

Results

ELISA results

When we compared with control group (Group 1), exposure to ammonium chloride and ethylene glycol (Group 2) resulted in a significant decrease in SOD, and GPx and caused a slightly but statistically significant increase in creatinine levels (Fig. 1 a-c). The GPx, SOD, and creatinine ELISA results expressed as mean ± standard error, are presented in Table 1.

Effects of low dose (group-3) phenolic compounds obtained from Polygonum equisetiforme in GPx, SOD, creatinine levels (ng/mL)

Low-dose PE (100 mg/kg) significantly reversed the decrease in GPx observed in the disease group, restoring it close to control levels (Fig. 1 a, +$p<0.05$ vs. Group 2). SOD levels were also increased compared to the disease group, although not reaching statistical significance (Fig. 1 b). Creatinine levels were significantly reduced relative to Group 2 (Fig. 1 c, +$p<0.05$). The GPx, SOD, and creatinine ELISA results, expressed as mean ± standard error, are presented in Table 1.

Effects of high dose (group-4) phenolic compounds obtained from *P. equisetiforme* on GPx, SOD and creatinine levels (ng/mL)

High-dose PE (400 mg/kg) improved GPx and creatinine levels similarly to the low-dose group (Fig. 1 a & Fig. 1 c, +$p<0.05$ vs. Group 2). However, SOD levels were not significantly increased compared to the disease group (Fig. 1 b). No clear dose-dependent effect was observed between the low- and high-dose groups. The GPx, SOD, and creatinine ELISA results, expressed as mean ± standard error, are presented in Table 1.

Histopathologic examinations of the kidney in all the groups

No pathological changes were observed in the kidneys of the control rats (Group 1, Fig. 2). In the disease group (Group 2), however, calcium oxalate crystal deposits, tubular dilatation, and interstitial

Table 1 — ELISA values of GPx,SOD, Creatinine (ng/ml) in rats. (Group-1 control group; Group-2 disease group (kidney stone); Group-3 low dose (100 mg/kg) treatment group; Group-4 high dose (400 mg/kg) treatment group). (*) indicates significance ($p<0.05$) according to control group (+) indicates significance ($p<0.05$) according to kidney stone group-2. values are mean ± SE, based on repeated measures ANOVA followed by Tukey post hoc test.

Group	GPx (ng/mL)	SOD (ng/mL)	Creatinine (ng/mL)
Group 1	0.4535±0.07961	2.26±0.3030	132,8±9.138
Group 2	0.1379±0.03068*	1.425±0.08874*	157,8±2.948*
Group 3	0.3873±0.03838+	2.001±0.3525	136.6±2.35+
Group 4	0.3109±0.02331	1.253±0.1447*	144.2±3.065+

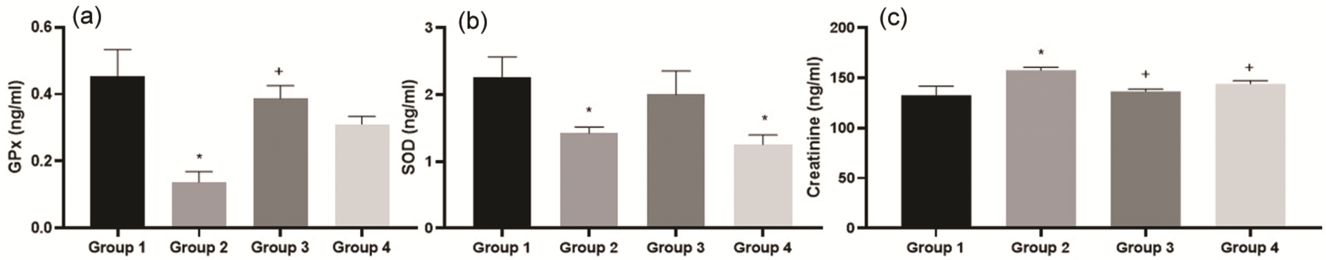

Fig. 1 — (a) ELISA values of GPx in rats, (b) ELISA values of SOD in rats, (c) ELISA values of Creatinine in rats. (Group-1 control group; Group-2 disease group (kidney stone); Group-3 low dose (100 mg/kg) treatment group; Group-4 high dose (400 mg/kg) treatment group). (*) indicates significance ($p<0.05$) according to control group (+) indicates significance ($p<0.05$) according to kidney stone group-2. (n= 8, for each groups)

inflammation were clearly present, confirming the formation of kidney stones (Fig. 3 & Fig. 4). In both treatment groups (Groups 3 and 4), these pathological findings were markedly reduced (Fig. 5 & Fig. 6).

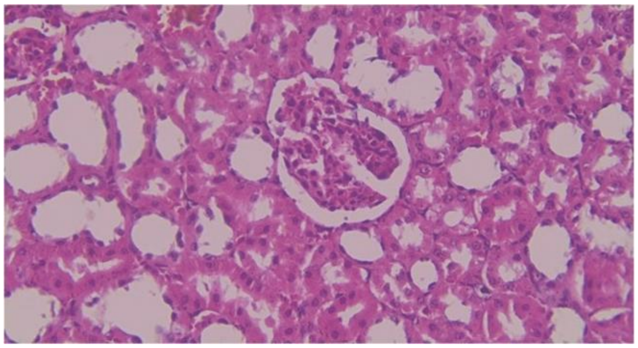

Fig. 2 — No pathologic findings were detected in the kidneys of group 1 control rats. Normal renal tubules and Bowman's capsule are observed. x400 magnification Hematoxylin & eosin (H&E stain). (n= 8)

Histopathologic examinations of the liver in all the groups

Histopathological examination of the liver revealed findings distinct from those observed in the kidney. No pathological alterations were detected in the control group (Group 1, Fig. 7 a). In the disease group (Group 2), minimal hepatic damage was noted (Fig. 7 b). In the low-dose treatment group (Group 3), mild portal inflammation was observed (Fig. 8), whereas the high-dose treatment group (Group 4) exhibited more pronounced changes, including moderate portal inflammation and mild interface hepatitis (Fig. 9). These findings indicate that while both treatment groups showed hepatic alterations, the extent of changes was greater in Group 4 compared to Group 3.

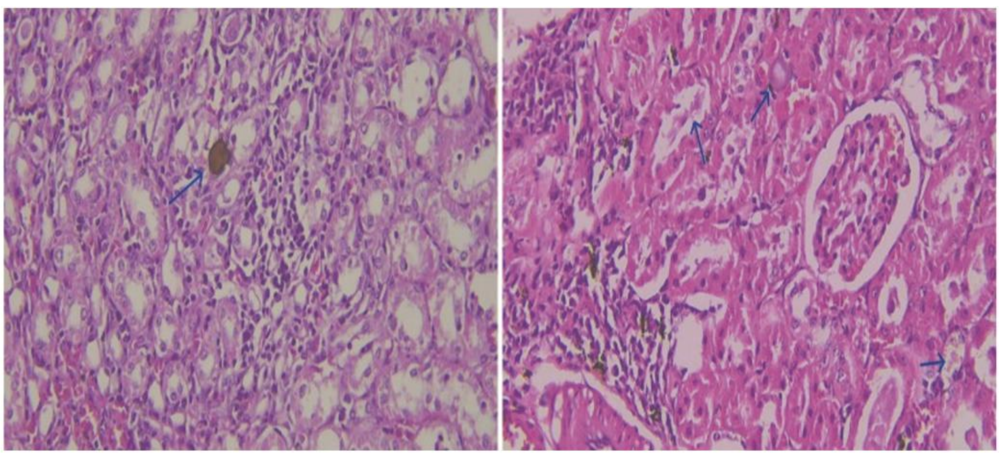

Fig. 3 — Kidney stones in group 2 rats are indicated by the blue arrow. x400 magnification Hematoxylin & eosin (H&E stain). (n= 8)

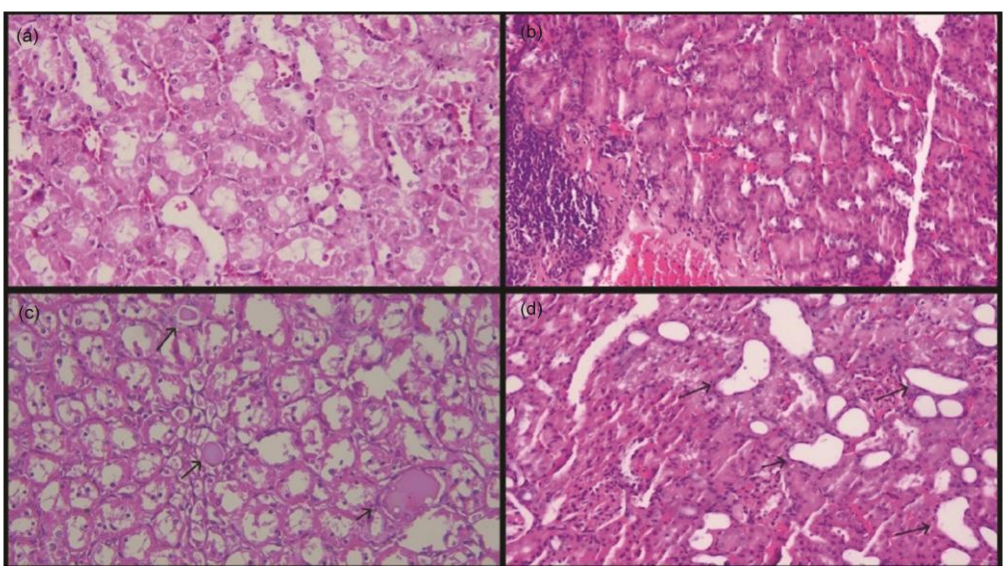

Fig. 4 — Findings showing the tubulointestinal damage formation most prominently observed in Group 2; (a) Tubular necrosis area, (b) Interstitial inflammation, (c) Hyaline cast formation, (d) tubular dilatation. x400 magnification Hematoxylin & eosin (H&E stain). (n= 8)

Discussion

In this study, administration of ammonium chloride and ethylene glycol led to decreased SOD and GPx activities and increased creatinine levels compared with controls. Treatment with the phenolic extract of *Polygonum equisetiforme* partially reversed these alterations, suggesting a protective role against experimentally induced kidney stones in rats.

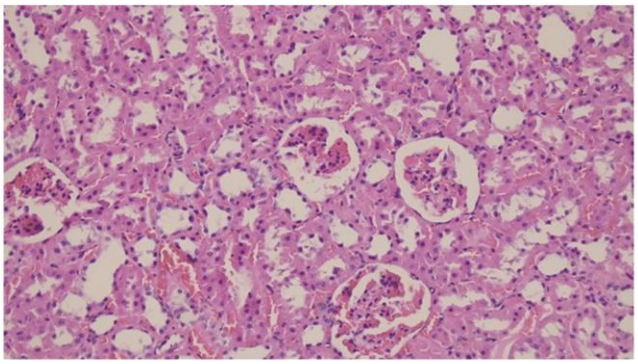

Fig. 5 — In group 3 (low dose (100 mg/kg) treatment group) registered specimen has normal kidney tissue morphology. H&E stain x400. (n= 8)

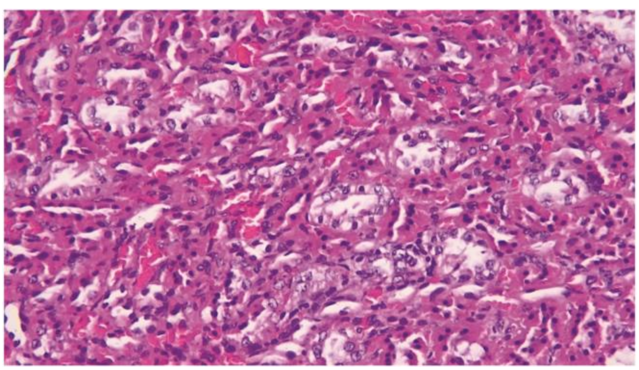

Fig. 6 — In group 4 (high dose (400 mg/kg) treatment group) registered specimen shows evidence of regeneration of tubule epithelium and congested vascular structures. H&E stain x400. (n= 8)

Previous studies indicate that exposure to ammonium chloride and ethylene glycol disrupts the antioxidant defense system and promotes oxidative stress, leading to reduced activity of radical scavenging enzymes such as SOD and GPx[10]. The pronounced protective effect observed with the encapsulated extract may stem from its improved bioavailability. Nano-encapsulation techniques are reported to enhance both cellular uptake and overall bioavailability of active compounds[10,11,22]. Moreover, since SOD needs copper and zinc to require its activity[23] and ammonium chloride and ethylene glycol induce copper deficiency, the activity of SOD decrease proportionally. However, the improvement observed with our extract could be related to its copper composition[14]. According to certain reports, the increased levels of urea and serum creatinine observed in ammonium chloride and ethylene glycol-exposed rats might result from the degradation of liver proteins into amino acids, which are then metabolized to urea and creatinine[23]. Renal failure in ammonium chloride and ethylene glycol-treated rats can be interpreted by increases urea and creatinine concentrations relative to controls. In our study, increasing values of creatinine and decreasing values of SOD and GPx in kidney stone group, reversed with the treatment of *P. equisetiforme.* Although creatinine levels decreased and antioxidant enzyme activities improved after treatment, no clear dose-dependent effect was observed between low and high extract doses. This may reflect limitations of the selected dose range.

The present study demonstrated that both biochemical and histopathological data confirmed renal damage in the stone-forming group, whereas treatment with the extract alleviated these alterations. The therapeutic effect observed with *P. equisetiforme*

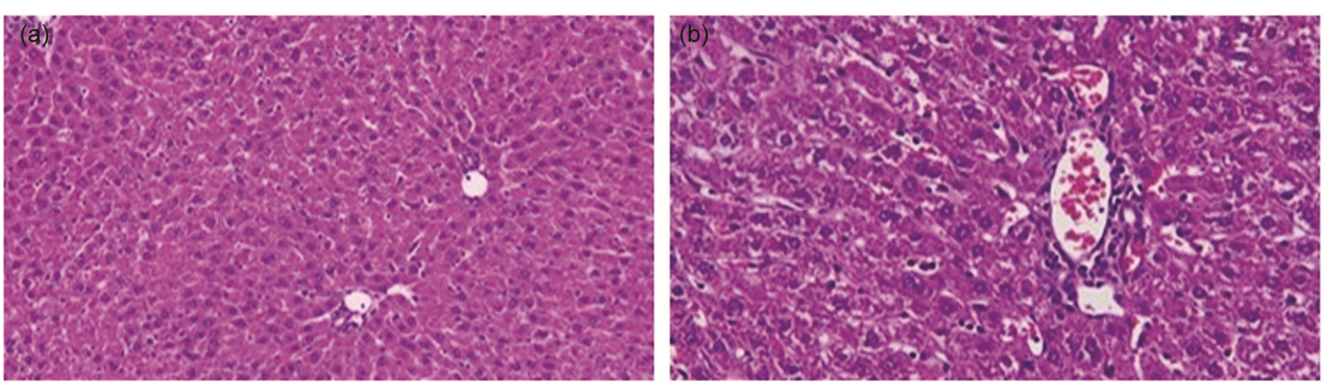

Fig. 7 — (a) Group 1 has normal liver parenchyma and portal areas. H&E stain x200, (b) Group 2 shows only minimal grade 1 portal inflammation with no interface hepatitis and focal lytic necrosis. H&E stain x200. (n= 8)

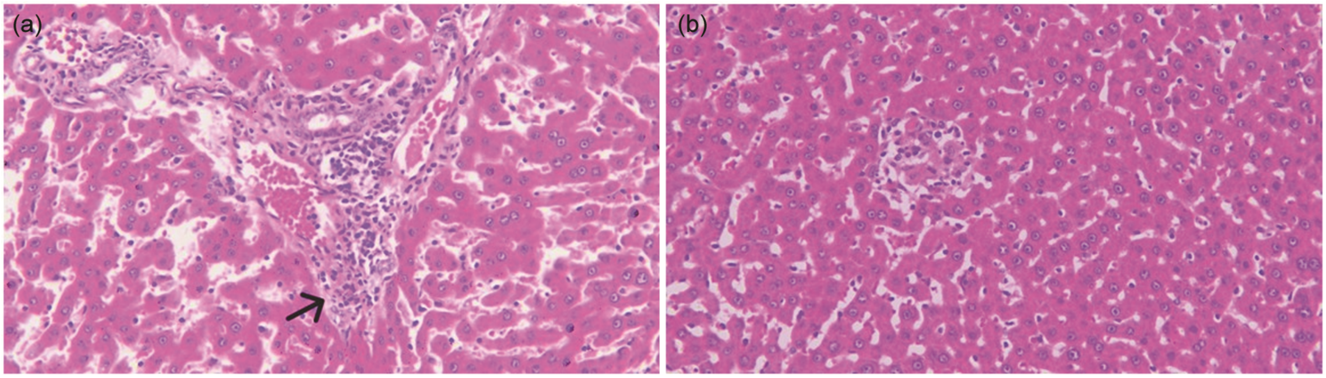

Fig. 8 — (a) There is an area of grade 2 portal inflammation in group 3 liver tissue. and (b) Focal lytic necrosis area is observed. H&E stain x200. (n= 8)

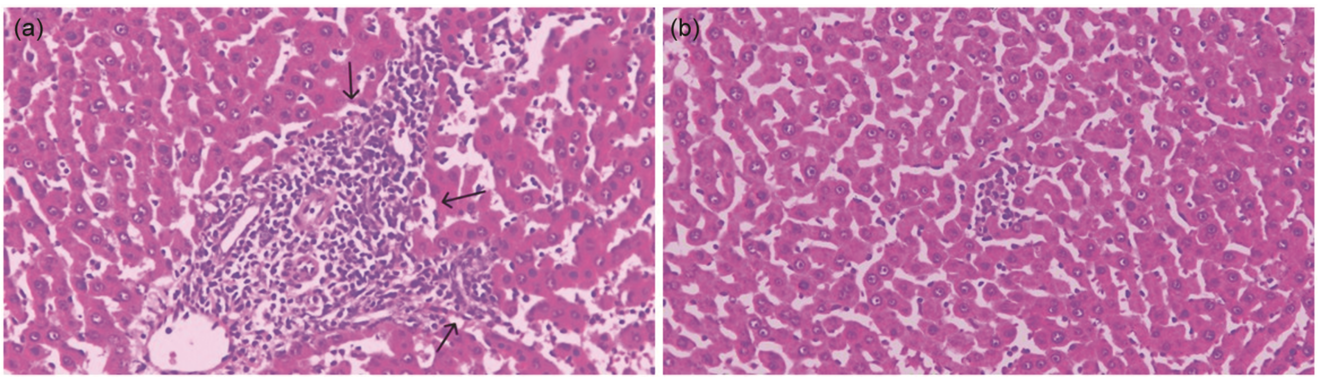

Fig. 9 — (a) Areas of portal inflammation and interface hepatitis in liver tissue are shown in group 4. In this area, portal inflammation is grade 3 and interface hepatitis is grade 2, (b) Focal lytic necrosis is observed in the same group. H&E stain x200. (n= 8)

is consistent with previous ethnopharmacological reports describing the traditional use of other *Polygonum* species in the management of urinary tract and kidney disorders[15,16]. Taken together, our findings provide experimental support for these traditional uses and highlight the potential of *P. equisetiforme* as a complementary therapeutic option.

Histopathological assessment supported the biochemical data: calcium oxalate deposits, tubular dilatation, and interstitial inflammation were prominent in the stone group but were markedly reduced in treated groups. Although *P. equisetiforme* extract clearly protected the kidneys, the liver responded differently. Low doses caused only mild portal inflammation, while higher doses resulted in moderate portal inflammation and some interface hepatitis. Supporting this, El-Toumy *et al*. demonstrated that administration of the methanolic extract at 100 and 200 mg/kg increased antioxidant enzyme levels and exerted a significant hepatoprotective effect, primarily attributed to its flavonoid content based on histopathological evaluations. Together, these findings suggest that moderate doses of the extract may protect the liver, whereas excessive amounts could cause hepatic alterations, emphasizing the importance of careful dose selection in accordance with traditional medicinal practices[12,13]. Consistent with our findings, previous studies suggest that herbal products are not always risk-free. Several reviews have documented liver injury following prolonged or high-dose use of herbal remedies[24,25]. In addition, studies on other *Polygonum* species, particularly *P. multiflorum*, have reported dose-dependent and immune-mediated mechanisms of hepatotoxicity[26,27]. Altogether, these results reinforce that the therapeutic and adverse effects of herbal remedies depend on dose, duration, and method of preparation, supporting the traditional concept that the proper dose is essential for safety and efficacy.

Herbal therapy has been widely practiced in many regions around the world for thousands of years. While herbal remedies have become increasingly popular as effective and affordable treatments for disease, hepatotoxicity is one of the most common side effects of herbal therapy.

Overall, the study supports the potential of *P. equisetiforme* as a protective agent against kidney stone formation, while highlighting the need for careful dose selection due to possible hepatic effects. In addition, some previous studies[12,19,28-30] have suggested that certain *Polygonum* species possess hepatoprotective potential, emphasizing the need for more comprehensive future investigations. The low-dose outcomes observed in our study are in line with these reports and underscore the importance of evaluating *P. equisetiforme* within a dose-dependent framework when considering its possible therapeutic use.

Conclusion

Histopathological findings are compatible with the ELISA results of control and kidney stone groups. Histopathologic examinations showed that the presence of significant calcium oxalate crystalloids in the kidney tissues in group-2 compared to the control, the presence of tubular dilatation and interstitial inflammation confirms the formation of kidney stones in this group. On the other hand, increasing values of creatinine and decreasing values of SOD and GPx in kidney stone group, reversed with the treatment of *P. equisetiforme*. These findings may indicate that the phenolic extract of *P. equisetiforme* may be partially effective in the treatment of experimentally induced kidney stones in rats. Kidney stone formation is a multifactorial process, and antioxidant protection alone may not be sufficient to completely prevent stone formation.

Otherwise, our histopathologic examinations of liver tissue obtained from the rats showed that portal inflammation, hepatitis or necrosis in treatment groups (group 3 and 4) significant increased compared to control (group 1) and disease group (group 2). However, the pathological findings in the liver at the low dose of PE extract were significantly less than those seen at the high dose, and appeared almost similar to the control group.

This finding may restrict usability of the phenolic extract of *P. equisetiforme* in the treatment of kidney stone except of low doses of the *P. equisetiforme* extract.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

HSB: designed and performed the experiments, analyzed the results, FE: designed experiments, methodology, PU: performed the experiments and drafted the manuscript. ŞE and NEK: performed the experiments. PUE: read and approved the final version manuscript. CG: designed and performed the experiments, analyzed the results, and drafted the manuscript. All authors read and approved the final version of the manuscript.

Ethics Approval

All animal experiments were conducted with the approval of the Animal Care Committee of Cukurova University (Protocol No.22.04.2022: 3.8) and were performed in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals (publication No. 86-23, revised 1984).

Data Availability

Data will be made available on request.

References

1 Mahmoudi M, Boughalleb F, Bouhamda T, Abdellaoui R & Nasri N, Unexploited *polygonum* equisetiforme seeds: potential source of useful natural bioactive products, *Ind Crop Prod*, 122 (2018) 349-357. doi:10.1016/j.indcrop.2018.06.017

2 Vechiu E, Dincă L & Bratu I, The characteristics of *polygonum* plants present in the alexandru beldie herbarium, *Res J Agric Sci*, 50 (4) (2018) 378-385.

3 Altay V, Ozyigit I I, Keskin M, Demir G & Yalcin I E, An ecological study of endemic plant *Polygonum istanbulicum* Keskin and its environs, *Pak J Bot*, 45 (1) (2013) 455-459.

4 Saremi J, Jahromi H K & Pourahmadi M, Effect of *Polygonum aviculare* L. on nephrolithiasis induced by ethylene glycol and ammonium chloride in rats, *Urol J*, 15 (3) (2018) 79-82. doi: 10.22037/uj.v0i0.3815

5 Benrahou K, Driouech M, El-Guourrami O, Mrabti H N, Cherrah Y, *et al.*, Medicinal uses, phytochemistry, pharmacology, and taxonomy of *Poygonum aviculare* L.: A comprehensive review, *Med Chem Res*, 32 (2023) 409-423. doi:10.1007/s00044-023-03021-1

6 Wigner P, Bijak M & Soluk-Bijak J, Probiotics in the prevention of the calcium oxalate urolithiasis, *Cells*, 11 (2) (2022) 284. doi: 10.3390/cells11020284

7 Park S & Pearle M S, Pathophysiology and management of calcium stones, *Urol Clin North Am*, 34 (3) (2007) 323-334. doi: 10.1016/j.ucl.2007.04.009

8 Azwanida N N, A review on the extraction methods use in medicinal plants, principle, strength and limitation, *Med Aromat Plant*, 4 (3) (2015) 196. doi:10.4172/2167-0412.1000196

9 Panahirad S, Dadpour M, Peighambardoust S H, Soltanzadeh M, Gullón B, *et al.*, Applications of carboxymethyl cellulose- and pectin-based active edible coatings in preservation of fruits and vegetables: A review, *Trends Food Sci Technol,* 110 (2021) 663-673. doi:10.1016/j.tifs.2021.02.025

10 Mercader-Ros M T, Lucas-Abellán C, Gabaldón J A, Fortea M I, Martínez-Cachá A, *et al*., Kaempferol complexation in cyclodextrins at basic pH, *J Agric Food Chem,* 58 (8) (2010) 4675-4680. doi: 10.1021/jf904218j

11 Liu D, Mao Y, Ding L & Zeng X-A, Dihydromyricetin: A review on identification and quantification methods, biological activities, chemical stability, metabolism and approaches to enhance its bioavailability, *Trends Food Sci Technol,* 91 (2019) 586-597. doi: 10.1016/j.tifs.2019.07.038

12 El-Toumy S A H, Salib J Y, Shafik N H, Elkarim A S A, Salama A, *et al.,* Evaluation of hepatoprotective activity of *Polygonum equisetiforme* methanolic extract, *J Appl Pharm Sci*, 9 (11) (2019) 54-59. doi: 10.7324/JAPS.2019.91107

13 Idoudi S, Tourrette A, Bouajila J, Romdhane M & Elfalleh W, The genus *Polygonum*: An updated comprehensive review of its ethnomedicinal, phytochemical, pharmacological activities, toxicology, and phytopharmaceutical formulation, *Heliyon,* 10 (8) (2024) e28947. doi: 10.1016/j.heliyon.2024.e28947

14 Mahmoudia M, Boughalleb F, Maaloul S, Bouhamd T, Nasri N, *et al.,* Organ-dependent variability in mineral composition, phytochemicals and antioxidant potentials in *Polygonum equisetiforme* parts, *Int J Plant Based Pharm,* 4 (1) (2024) 40-46. doi10.62313/ijpbp.2024.177

15 Ballabh B, Chaurasia O P, Ahmed Z & Singh S B, Traditional medicinal plants of cold desert Ladakh-used against kidney and urinary disorders, *J Ethnopharmacol,* 118 (2) (2008) 331-339. doi: 10.1016/j.jep.2008.04.022

16 Gurdal B & Kultur S, An ethnobotanical study of medicinal plants in Marmaris (Mugla, Turkey), *J Ethnopharmacol,* 146 (2013) 113-126. doi: 10.1016/j.jep.2012.12.012

17 Pralhad T & Rajendrakumar K, Study of freeze-dried quercetin-cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis, *J Pharm Biomed Anal,* 34 (2) (2004) 333-339. doi: 10.1016/S0731-7085(03)00529-6

18 Wang J, Cao Y, Sun B & Wang C, Characterization of inclusion complex of trans-ferulic acid and hydroxypropyl-β-cyclodextrin, *Food Chem,* 124 (3) (2011) 1069-1075. doi:10.1016/j.foodchem.2010.07.080

19 da Rosa C G, Borges C D, Zambiazi R C, Nunes M R, Benvenutti E V, *et al.,* Microencapsulation of gallic acid in chitosan, β-cyclodextrin and xanthan, *Ind Crop Prod,* 46 (2013) 138-146. doi:10.1016/j.indcrop.2012.12.053

20 Stiani S N, Syahidah F M, Fikriani H, Subarnas A & Rusdiana T, Anticalculi activity of Apigenin and Celery (*Apium graveolens* L.) extract in rats induced by Ethylene Glycol-Ammonium Chloride, *J Pharm Bioallied Sci*, 11 (4) (2019) S556-S561. doi: 10.4103/jpbs.JPBS_202_19

21 Mazid M A, Datta B K, Nahar L, Bashar S A M K, Bachar S C, *et al.*, Antinociceptive, anti-inflammatory and diuretic properties of *Polygonum barbatum* (L.) Hara var. Barbata, *Rev Bras Farmacogn*, 19 (3) (2009) 749-754. doi:10.1590/S0102-695X2009000500017

22 Aggarwal D, Kaushal R, Kaur T, Bijarnia R K, Puri S, *et al.,* The most potent antilithiatic agent ameliorating renal dysfunction and oxidative stress from *Bergenia ligulata* rhizome, *J Ethnopharmacol*, 158 (2014) 85-93. doi: 10.1016/j.jep.2014.10.013

23 Chen J, Jiang Y, Shi H, Peng Y, Fan X, *et al.*, The molecular mechanisms of copper metabolism and its roles in human diseases, *Pflügers Arch,* 472 (10) (2020) 1415-1429. doi: 10.1007/s00424-020-02412-2

24 Teschke R, Frenzel C, Glass X, Schulze J & Eickhoff A, Herbal hepatotoxicity: a critical review, *Br J Clin Pharmacol*, 75 (3) (2013) 630-636. doi: 10.1111/j.1365-2125.2012.04395.x.

25 Ballotin V R, Bigarella L G, de Mello Brandão A B, Balbinot R A, Balbinot S S, *et al.*, Herb-induced liver injury: systematic review and meta-analysis, *World J Clin Cases*, 9 (20) (2021) 5490-5513. doi: 10.12998/wjcc.v9.i20.5490

26 Gong L, Shen X, Huang N, Wu K, Li R, *et al.*, Research progress on hepatotoxicity mechanism of *Polygonum* multiflorum and its main components, *Toxicon*, 248 (2024) 108040. doi: 10.1016/j.toxicon.2024.108040

27 Rao T, Liu Y-T, Zeng X-C, Li C-P & Ou-Yang D-S, The hepatotoxicity of *Polygonum multiflorum*: The emerging role of the immune-mediated liver injury, *Acta Pharmacol Sin*, 42 (2021) 27-35. doi: 10.1038/s41401-020-0360-3

28 Chiu Y-J, Chou S-C, Chiu C-S, Kao C-P, Wu K-C, *et al.,* Hepatoprotective effect of the ethanol extract of *Polygonum orientale* on carbon tetrachloride-induced acute liver injury in mice, *J Food Drug Anal,* 26 (2018) 369-379. doi: 10.1016/j.jfda.2017.04.007

29 Mahmoudia M, Boughalleb F, Mabrouk M, Tlili N, Potter D, *et al.,* Chemical analysis of the antioxidants from the aerial parts of wild *Polygonum equisetiforme* from Tunisia, *Food Biosci,* 29 (2019) 24-29. doi:10.1016/j.fbio.2019.03.004

30 Esfanjani A F & Jafari S M, Biopolymer nano-particles and natural nano-carriers for nano-encapsulation of phenolic compounds, *Colloids Surf B Biointerfaces,* 146 (2016) 532-543. doi: 10.1016/j.colsurfb.2016.06.053