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The potential therapeutic role of viburnum opulus juice in the management of kidney stones

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ABSTRACT: This study examined the therapeutic potential of Viburnum opulus (Gilaburu) juice in an experimental nephrolithiasis model induced by ethylene glycol (EG) and ammonium chloride (AC). Gilaburu juice administration began on Day 8, and outcomes were evaluated through biochemical and histopathological parameters. Urinary analysis revealed that the positive control group had markedly elevated oxalate and reduced citrate levels—key risk factors for kidney stone formation. In contrast, Gilaburu-treated rats showed lower oxalate excretion and higher citrate levels, indicating its capacity to reduce oxalate accumulation and promote crystallization inhibitors. The treatment group also exhibited partial recovery in urinary magnesium levels and increased urine output, suggesting a diuretic effect. Serum biochemical findings demonstrated reduced creatinine and uric acid levels in the treatment group compared to the positive control, implying renal protective effects of Gilaburu. Histopathological examination (H&E staining) showed that while the positive control group displayed severe tubular injury, including epithelial necrosis and inflammation, the treatment group exhibited milder alterations and better preservation of tubular architecture. Pizzolato staining confirmed a significant reduction in both the number and size of calcium oxalate crystals in Gilaburu-treated rats ($p < 0.05$). In conclusion, *V. opulus* juice exhibited oxalate-lowering, citrate-enhancing, diuretic, and nephroprotective effects in an experimental kidney stone model. These findings support its potential as a phytotherapeutic agent for preventing and managing nephrolithiasis, although further preclinical and clinical investigations are necessary to confirm these results.

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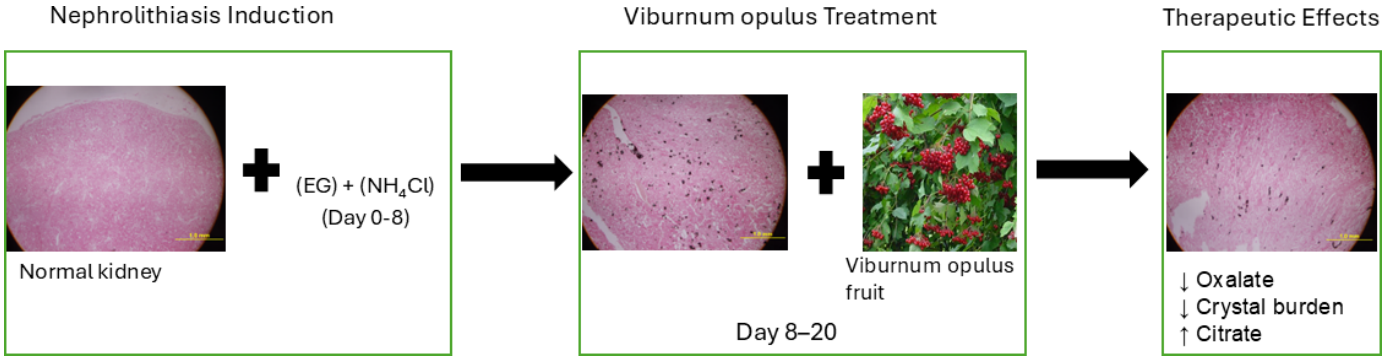
GRAPHICAL ABSTRACT



1. INTRODUCTION

Recent studies report a significant rise in urinary stone disease in Turkey. Globally, prevalence ranges from 5% to 12%, involving multiple interacting pathophysiological mechanisms (Curhan, 2007). Treatment depends on stone size, location, resources, surgeon expertise, and patient preference. Recurrence rates remain high, up to 50% after treatment (Koursh et al., 2004). Calcium oxalate (CaOx) stones are the most common type (Curhan, 2007), and their pathogenesis is not fully understood (Khan, 2013). The disease is more frequent in males than females (Khan, 1997). Animal models play a crucial role in the development of treatment strategies. In particular, rats are commonly used in experimental CaOx stone models induced by ethylene glycol (EG) and ammonium chloride (AC) (Khan, 2013). Preventive strategies in kidney stone management include increased fluid intake, consumption of herbal teas and products with diuretic effects, and avoidance of a sedentary lifestyle (Hoopes, 2003). As interest in complementary and alternative medicine resurges, the potential therapeutic effects of natural, plant-based products are being increasingly investigated. One such traditional remedy is Gilaburu (*Viburnum opulus L.*), which has long been used in Anatolia for its reputed stone-dissolving effects. Due to its high content of vitamin C, phenolic compounds, and organic acids, it is hypothesized that Gilaburu may prevent CaOx crystal formation by lowering urinary pH. This traditional use is particularly prevalent in Central Anatolia, where the fruit has been consumed for generations as a supportive treatment for kidney stones (Baytop, 1984; Herrara, 1987). Gilaburu belongs to the class *Magnoliopsida* and the family *Caprifoliaceae*, with a wide natural distribution spanning from Europe to Asia (Dinç et al., 2012). In addition to its effects on kidney stones, Gilaburu juice is also traditionally used in the treatment of gallbladder and certain liver disorders in Anatolia due to its perceived litholytic properties (Herrara, C.M., 1987; Soylak et al., 2002; Yildiz, 2019). *Viburnum opulus* (Gilaburu) fruits and extracts are rich in various bioactive compounds relevant to kidney stone prevention. They contain iridoids, such as opuloside, which exhibit anti-inflammatory and antioxidant effects. The fruits are also a source of phenolic compounds and flavonoids, including chlorogenic acid, caffeic acid, quercetin, and rutin, which help reduce oxidative stress and protect renal epithelial cells from crystal-induced damage. In addition, *V. opulus* contains organic acids, such as citric, malic, and tartaric acids, that can form soluble complexes with calcium (Ca), thereby inhibiting stone formation. Saponins are present as well, which may decrease crystal aggregation through their surface-active properties. Moreover, the fruits provide essential minerals such as potassium, Ca, and magnesium (Mg), which can further influence urinary chemistry. Collectively, these compounds suggest that *V. opulus* possesses a multifaceted biochemical profile that may contribute to its traditional use in urinary tract health and kidney stone prevention (Effatparvar et al., 2025; İlhan et al., 2014; Kajszczak et al., 2020)

Several studies have reported that CaOx accumulation in the kidney leads to an increase in urinary pH, which may serve as an indicator of stone formation (Sezik et al., 2001; Takawale et al., 2012). It has also been documented that compounds found in Gilaburu fruit—such as ascorbic acid, phenolic acids, and organic acids—may inhibit stone formation by lowering urinary pH (İlhan et al., 2014).

The aim of this study is to investigate the potential therapeutic effect of Gilaburu juice on experimentally induced kidney stones in a rat model.

2. MATERIALS AND METHODS

2.1. Ethical approval

This study was supported by the Scientific Research Projects Unit of Erciyes University under the project code

TDK-2015-5949. All experimental procedures involving animals were approved by the Local Ethics Committee for Animal Experiments of Erciyes University (Decision no: 12/89, dated 12.08.2012) and conducted at the Hakan Çetinsaya Experimental Research and Application Center (DEKAM), in accordance with ethical guidelines for animal welfare.

2.2. Collection and extraction of Gilaburu

Gilaburu fruits (*Viburnum opulus L.*) were separated from their leaves and stems and allowed to ferment in water for approximately 2 months using traditional methods. The matured fruits were then pressed to obtain the juice. This juice was concentrated under vacuum at 37–38°C, lyophilized, and stored at –20°C. Approximately, 58 g of powdered extract was obtained from 1 kg of fresh fruit.

2.3. Dose determination

In traditional use, 200 mL of undiluted Gilaburu juice per day is recommended for humans. When adapting this dose for rats, a body weight–based calculation was performed. If 200 mL is appropriate for an average 70-kg adult, the equivalent dose for a 350-g rat is approximately 1 mL. Since the Gilaburu juice was lyophilized, this amount corresponds to approximately 1–1.5 g of dry extract. For oral administration by gavage, 1–1.5 g of Gilaburu extract was dissolved in 2.5 mL of water and administered accordingly.

2.4. Experimental animals

A total of 20 male Sprague-Dawley rats, weighing between 190 and 320 g, were used in the study. Prior to the experiment, animals were acclimated in metabolic cages for 3 days. Throughout the study, they were fed standard rat chow and water ad libitum under optimal environmental conditions. Water intake was recorded daily, and health status was monitored twice a day.

2.5. Experimental groups

The animals were randomly assigned into three experimental groups:

Negative Control Group (n=6): Fed a standard diet with no stone induction.

Positive Control Group (n=7): Received 0.75% EG and 1% AC (NH_4Cl) in drinking water for the first 8 days to induce stone formation, followed by 0.75% EG only.

Treatment Group (n=7): Underwent the same stone induction protocol as the positive control group and additionally received a daily oral gavage of Gilaburu extract (dose adjusted to body weight) for 12 consecutive days.

The daily dose of Gilaburu was calculated as half of 10% of the animal's body weight (mL), multiplied by 0.09 to determine the required amount of extract. The extract was dissolved in 2.5 mL of water, vortexed, and administered using a 5 mL syringe at the same time each day.

2.6. Urinary biochemical analysis

Daily water consumption (mL) and 24-hour urine volume (mL) were recorded throughout the study. Urine samples were collected daily in 100 mL containers, and total urine volume per animal was measured. On days 0, 4, 8, 12, 16, and 20, 2 mL of each urine sample was stored in Eppendorf tubes and sent to the Central Biochemistry Laboratory at Erciyes University for the measurement of Ca, uric acid, Mg, and creatinine (Cr). The remaining urine samples were stored at –20 °C for later determination of oxalate and citrate levels. Oxalate (REF OX8850) and citrate (REF C18820) kits from B.E.N s.r.l. were used for manual analysis.

2.7. Blood sampling and biochemical analysis

At the end of the experiment, animals were anesthetized via intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). Following disinfection of the abdominal area with alcohol, a V-shaped incision was made at the pubic level to expose the abdominal cavity. The organs were gently moved aside, and blood was drawn from the aortic bifurcation using a syringe. Blood samples were sent to the Central Biochemistry Laboratory at Erciyes University for measurement of urea, Cr, Ca, and Mg levels.

2.8. Histopathological procedure

Kidney tissues from each animal were fixed in 10% buffered formalin for histopathological examination. After frontal trimming, tissues underwent standard histological processing, including washing in tap water, dehydration through graded alcohols (50%, 70%, 80%, 90%, and 100%), clearing in xylene (two steps), and embedding in paraffin (two steps).

Paraffin blocks were formed using paraffin that melted at 56–58°C and stored at –10 °C until sectioning.

Sections of 5 µm thickness were obtained from paraffin blocks using a microtome. Due to detachment issues observed in standard slides during trial staining, sections were mounted on pre-coated lysine slides. The slides were stained using Hematoxylin–Eosin (HE) and Pizzolato's (PZ) method and examined under a light microscope.

2.9. Statistical analysis

All data were analyzed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA). Individual rat data were entered into the software, including daily water intake, urine volume, and urinary levels of Ca, Cr, Mg, uric acid, oxalate, and citrate on days 0, 4, 8, 12, 16, 18, and 20. Blood levels of Mg, Cr, uric acid, and Ca were also statistically compared. Descriptive statistics (mean ± standard deviation) were first applied. Normality of distribution was tested using the Shapiro–Wilk test, and nonnormal distribution ($p<0.05$) was confirmed. Therefore, nonparametric tests were employed: the Mann–Whitney U test for pairwise comparisons and the Kruskal–Wallis test for comparisons among three groups. A p-value <0.05 was considered statistically significant. For biochemical comparisons in serum, one-way ANOVA and Tukey's HSD post hoc test were applied.

3. RESULTS

3.1. Changes in body weight

Body weights of the animals in each experimental group were measured at the beginning (Day 0) and at the end (Day 20) of the study. An average weight gain of 49.3 g was observed in the negative control group, while the positive control group showed a gain of only 9.1 g, and the treatment group exhibited an average increase of 33.6 g (Figure 1).

The administration of EG and AC in the drinking water to induce kidney stone formation in the positive control group resulted in a notably smaller weight gain compared to the negative control group. In contrast, animals in the treatment group receiving Gilaburu extract demonstrated a more pronounced weight gain.

Statistical analysis revealed a significant decrease in weight gain in both the positive control group ($p<0.01$) and the treatment group ($p<0.05$) when compared to the negative control group. Moreover, comparison between the positive control and treatment groups showed a statistically significant increase in body weight in the treatment group ($p<0.05$).

3.2. Daily water intake and 24-hour urine output findings

Daily water intake and 24-hour urine output were measured at the same time each day for all experimental animals. In the negative control group, the average water intake ranged between 25 and 30 mL. In contrast, the positive control and treatment groups showed an initial increase in water consumption, followed by a decrease between Days 4 and 8.

After discontinuation of AC on Day 8, a sharp rise in water intake was observed, reaching approximately 50–60 mL by Day 14. Toward Day 20, water intake declined again to around 40–50 mL Compared to the negative control group, both the positive control and treatment groups demonstrated a statistically significant increase in water intake on Days 4, 16, and 20 ($p<0.01$). No significant difference in water consumption was found between the positive control and treatment groups.

When examining daily urine output, rats in the negative control group exhibited a consistent range of 7–10 mL/day throughout the study period. In both the positive control and treatment groups, urine volume increased proportionally with water intake until Day 4, followed by a decline until Day 8. After the cessation of AC on Day 8, a notable increase in urine output was observed, rising to an average of 20–25 mL, in parallel with the increase in water consumption (Figure 3).

When urine volumes were statistically compared across groups, a significant increase was detected in the positive

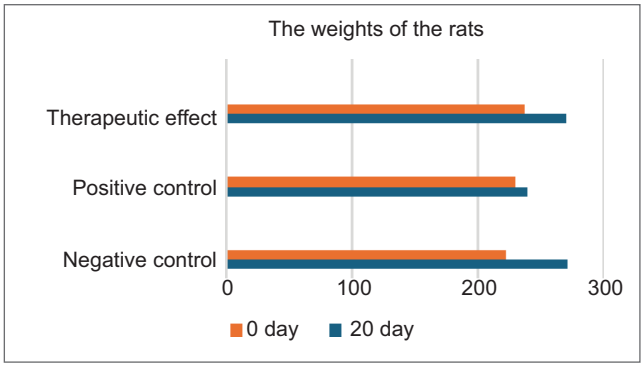


Figure 1. Body weight changes between Day 0 and Day 20 in the experimental groups.

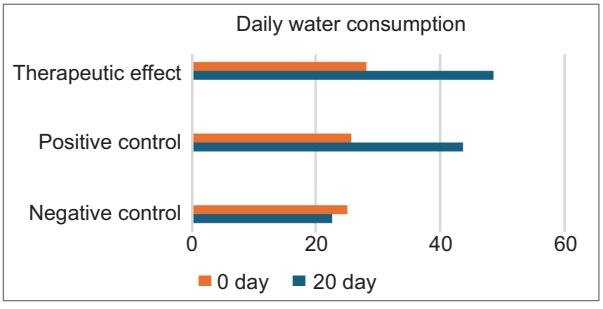


Figure 2. Water consumption trends from Day 0 to Day 20.

control and treatment groups on Days 4, 8, and 12 (**p<0.05**) and on Days 16 and 20 (**p<0.01**) compared to the negative control group. The treatment group had the highest average urine output, correlating with the elevated water consumption compared to other groups.

3.3. Biochemical analysis findings

Urinary biochemical parameters were measured in all experimental groups on Days 0, 4, 8, 12, 16, and 20. The results are presented below in both graphical and tabular formats.

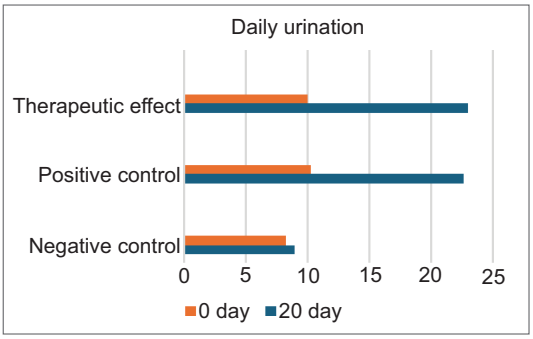


Figure 3. Daily urine volume from Day 0 to Day 20 in all experimental groups.

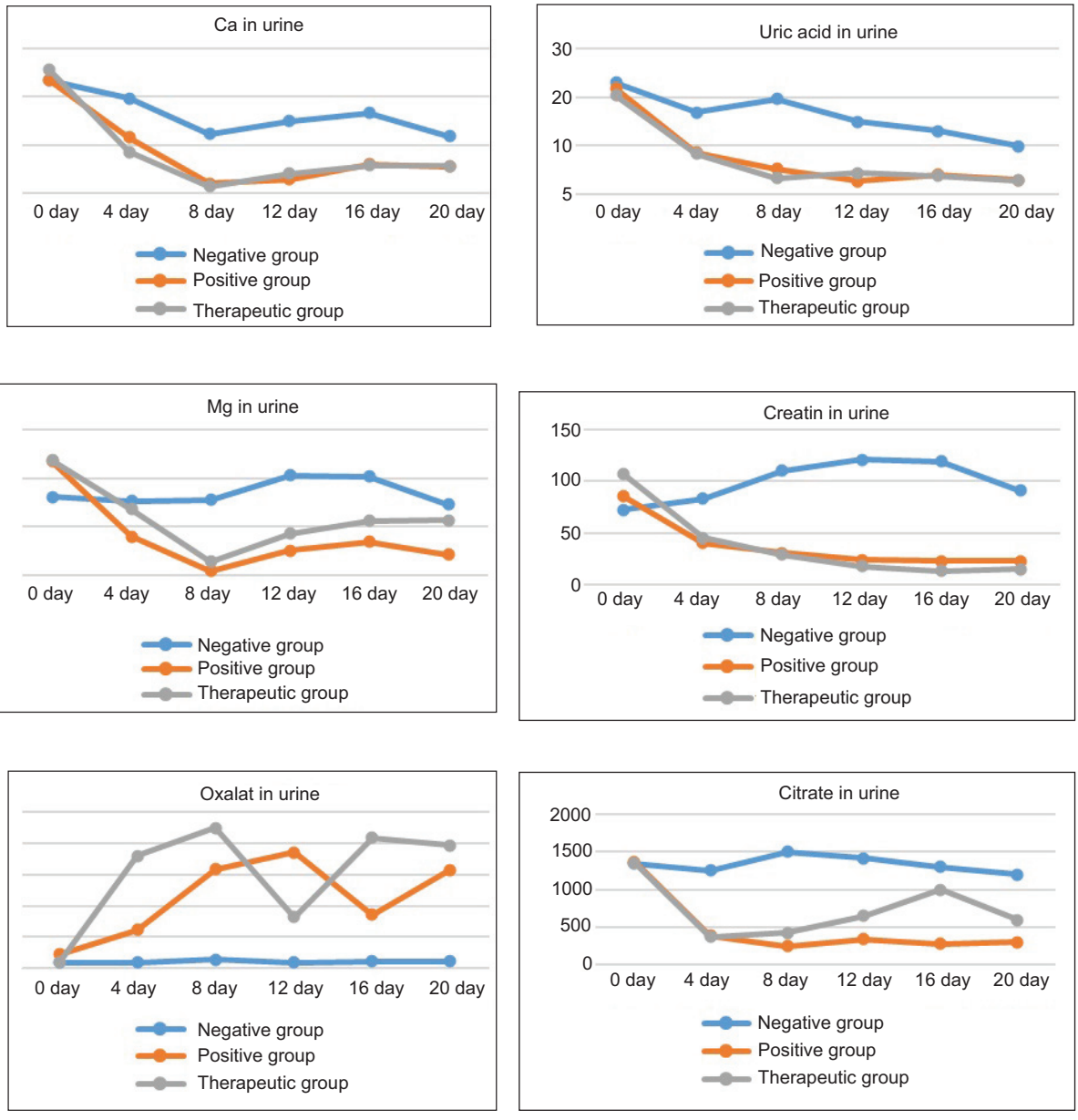


Figure 4. Urinary biochemical analysis results.

3.4. Urinary Ca levels

In the positive control group, urinary Ca levels showed a marked decline beginning on Day 8, which is considered a typical indicator of kidney stone formation. A similar initial decrease was observed in the treatment group; however, starting from Day 12, Ca levels demonstrated recovery and stabilization. This suggests a potential regulatory effect of *Gilaburu* on Ca metabolism.

3.5. Urinary uric acid levels

A general decrease in urinary uric acid levels was observed over time in all groups, with no statistically significant differences between them. These findings show that Gilaburu extract does not exert a prominent effect on uric acid levels.

3.6. Urinary Mg levels

The positive control group showed a decline in urinary Mg excretion, whereas this reduction was less pronounced in the treatment group. Moreover, a gradual recovery in Mg levels was noted over time in treated animals. These results suggest that Gilaburu may help support Mg levels, a known inhibitor of stone formation.

3.7. Urinary Cr levels

Cr levels remained high and stable in the negative control group. In contrast, both the positive control and treatment groups exhibited a significant decline in urinary Cr, reflecting possible impairment of renal function due to stone formation. The limited recovery of Cr levels in the treatment group suggests that Gilaburu may have a minimal or indirect effect on this parameter.

3.8. Urinary oxalate levels

A substantial increase in urinary oxalate levels was detected in the positive control group, consistent with active stone formation. In the treatment group, oxalate levels were comparatively lower and showed a fluctuating but overall suppressed pattern. This supports the hypothesis that Gilaburu may exert an inhibitory effect on oxalate accumulation.

3.9. Urinary citrate levels

Citrate levels, a key inhibitor of stone formation, significantly decreased in the positive control group, which is associated with increased risk of stone development. In contrast, the treatment group exhibited a notable increase in urinary citrate levels, suggesting a protective role of Gilaburu extract by enhancing the excretion of stone-inhibitory substances.

3.10. Serum biochemical findings

In this study, serum biochemical parameter including Cr, uric acid, Ca, and Mg were measured and analyzed among negative control, positive control, and treatment groups using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test.

3.11. Cr

ANOVA revealed a statistically significant difference in serum Cr levels among groups ($p < 0.05$). Tukey's test indicated that the positive control group had significantly higher Cr levels compared to the negative control ($p = 0.0316$). Although Cr levels in the treatment group were lower than those in the positive control and approached values observed in the negative control group, this difference was not statistically significant ($p = 0.0739$). These findings suggest that *Gilaburu* may exert a regulatory and protective effect on renal function.

3.12. Uric acid

ANOVA shows a significant difference in uric acid levels between groups ($p < 0.05$). According to Tukey's post hoc analysis, the positive control group had significantly elevated uric acid levels compared to the negative control group ($p = 0.0224$). Although uric acid levels were reduced in the treatment group, this decrease did not reach statistical significance ($p = 0.1088$), implying a potentially mild corrective effect of *Gilaburu* on uric acid metabolism.

3.13. Ca

No statistically significant difference was found among groups for serum Ca levels in the ANOVA test ($p > 0.05$). Similarly, no significant differences were observed in Tukey's

multiple comparison analysis. This suggests that systemic Ca levels remained relatively unaffected by the experimental model or treatment.

3.14. Mg

ANOVA showed a statistically significant difference in serum Mg levels between groups ($p < 0.05$). Tukey's analysis confirmed that the positive control group had significantly higher Mg levels compared to the negative control group. Although the treatment group showed a moderate elevation, the difference between the treatment and positive control groups was not statistically significant. These results suggest that *Gilaburu* may contribute to maintaining Mg balance, which is important due to Mg's role as a known inhibitor of kidney stone formation.

3.15. Histopathological findings

Histological sections obtained from the kidney tissues of animals in the negative control, positive control, and treatment groups were examined. H&E staining revealed clear differences in tissue architecture among the groups (Figures 5 and 6). Crystal formation was assessed using PZ staining method (Figure 7A, 7B, 7C).

3.16. H-E staining findings

In the positive control group, severe damage was observed in the proximal tubules by Day 20, including extensive structural disruption and a high number of pyknotic nuclei. Tubular epithelial cells appeared flattened. The distal tubules showed marked degeneration with prominent vacuolization and necrosis of epithelial cells. In addition, there was significant dilatation in both the collecting ducts and distal tubules.

In the treatment group, which received *Gilaburu* extract in addition to EG after Day 8, histological appearance was relatively better preserved compared to the positive control group. Fewer structural irregularities were noted in both distal and proximal tubules. While some proximal tubular damage was still present, the distal tubules appeared relatively intact.

Tubular structures in the renal medulla exhibited more severe damage compared to those in the cortex. Histopathological alterations in the medulla were primarily characterized by dilatation of the collecting ducts and accumulation of sloughed epithelial cells within the tubular lumen (Figures 5 and 6).

3.17. Crystal quantification and PZ staining findings

When compared to the negative control group, sections stained using the PZ method from the positive control group exhibited both larger and more numerous crystal structures

Table 1.
Serum biochemical values in experimental groups.

Group	Creatinine (mg/dL)	Uric Acid (mg/dL)	Calcium (mg/dL)	Magnesium (mg/dL)
Biochemical Findings in Blood				
Negative Control	0.22 ± 0.05	1.37 ± 0.05	10.70 ± 0.17	0.88 ± 0.09
Positive Control	0.50 ± 0.28	3.20 ± 0.85	10.76 ± 0.60	1.46 ± 0.56
Treatment	0.46 ± 0.12	2.19 ± 0.61	10.83 ± 0.40	1.05 ± 0.15

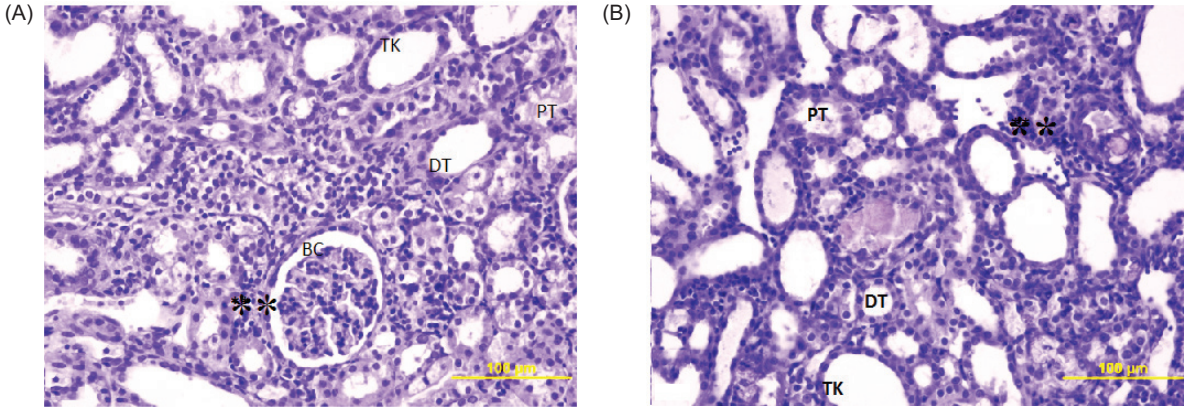


Figure 5. Hematoxylin and eosin (H&E) staining in the positive control group at ×40 magnification. A: Renal cortex; B: Renal medulla.TK: Collecting duct, DT: Distal tubule, BC: Bowman's capsule, PT: Proximal tubule, **: Necrotic structure.

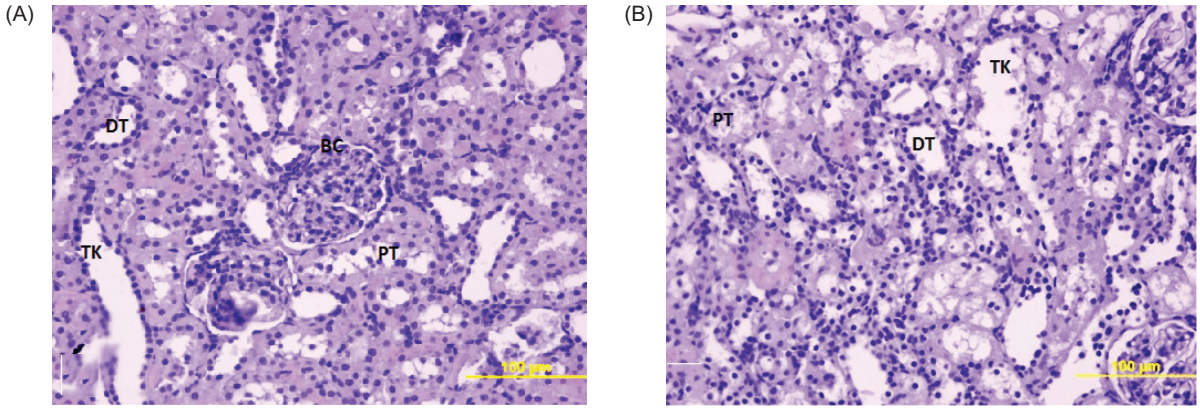


Figure 6. Hematoxylin and eosin (H&E) staining in the treatment group at ×40 magnification. A: Renal cortex; B: Renal medulla. TK: Collecting duct, DT: Distal tubule, BC: Bowman's capsule, PT: Proximal tubule.

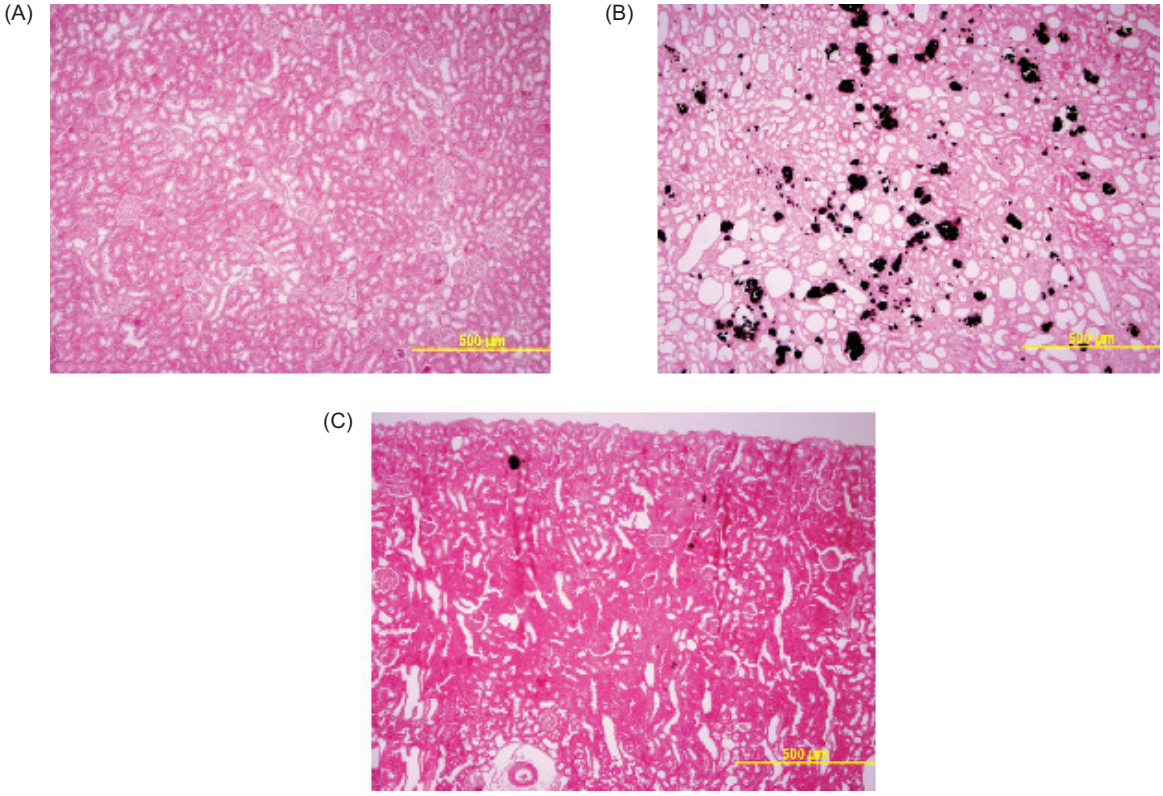


Figure 7. Pizzolato's (PZ) staining at ×10 magnification. A: Negative control group, B: Positive control group, C: Treatment group.

(Figure 7B). In contrast, the treatment group showed markedly fewer crystals, and those observed were smaller in size compared to the positive control group (Figure 7C).

Crystal counts were performed on kidney sections from the positive control and treatment groups using PZ staining. In the positive control group, the number of crystals per sample ranged from 1 to 147 (Table 2), with individual means ranging between 2 and 36. In the treatment group, crystal counts varied from 0 to 49 per sample, with individual means ranging from 1 to 12 (Table 2).

Since the treatment protocol aimed to intervene after crystal formation had already begun (post-Day 8), the observed reduction in crystal number in the treatment group suggests a potential therapeutic effect of Gilaburu juice.

Statistical analysis of the mean number of crystals between groups revealed that the positive control group had a

Table 2.
Crystal count results in the positive control and treatment groups. Crystal numbers were quantified based on Pizzolato-stained kidney sections by three independent observers. Mean values reflect individual and group-level assessments.

N	Researcher 1					Researcher 2					Researcher 3					Overall Mean
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Positive Control																
1	1	3	2	6	3	1	2	5	6	3.5	2	5	7	0	3.5	3.3
2	3	2	4	3	3	2	6	0	0	2	5	4	3	2	3.5	2.8
3	37	40	31	35	35.75	34	18	38	23	28.25	40	32	47	28	36.75	33.6
4	6	8	16	5	8.75	3	14	7	3	6.75	5	16	11	15	11.75	9.1
5	31	26	34	28	29.75	34	30	22	34	30	34	27	22	26	27.25	29
6	24	28	32	26	27.5	27	29	21	18	23.75	26	29	19	24	24.5	25.3
7	9	19	7	7	10.5	35	15	12	11	18.25	6	14	14	13	11.75	13.5
Overall Mean																**16.6**
Treatment Group																
1	21	10	9	9	12.25	21	7	3	9	10	13	13	12	6	11	11.1
2	5	5	2	3	3.75	4	2	5	6	4.25	7	8	2	5	5.5	4.5
3	7	7	9	12	8.75	12	8	9	9	9.5	12	13	7	7	9.75	9.3
4	8	5	4	3	5	11	3	7	5	6.5	9	7	9	7	8	6.5
5	0	3	1	1	1.25	0	1	1	1	0.75	1	1	0	1	0.75	0.9
6	3	1	2	2	2	1	1	1	1	1	2	0	1	2	1.25	1.4
7	1	3	2	0	1.5	1	2	0	0	0.75	2	5	1	0	2	1.4
Overall Mean																5.0

significantly higher crystal count than the treatment group, and this reduction was found to be statistically significant ($p < 0.05$).

To assess inter-rater reliability, we calculated the Intraclass Correlation Coefficient (ICC) using a two-way random effects model with absolute agreement and average measures (ICC[2,k]). For the Positive Control group, the ICC was 0.9793, indicating excellent agreement among the three researchers. For the Treatment group, the ICC was 0.9809, also reflecting excellent consistency across raters.

According to established thresholds, ICC values greater than 0.90 denote *excellent reliability*. Thus, both groups demonstrated very high levels of consistency in scoring, confirming that the evaluation method produced stable and reproducible results across different raters.

4. DISCUSSION

Plant-derived therapies have long been investigated for their antiurolithic potential. Lemon juice has been shown to prevent stone formation in rats by increasing urinary citrate while reducing crystal deposition and oxidative stress (Touhami et al., 2007). The methanolic extract of *Glochidion velutinum* leaves decreased urinary Ca and oxalate levels and protected renal tissue in an EG-induced nephrolithiasis model (Thatikonda et al., 2013). *Herniaria hirsuta* demonstrated preventive effects against CaOx stone formation through its diuretic and crystal-inhibitory properties (Atmani and Silimani, 2003), whereas *Berberis vulgaris* bark extract reduced hyperoxaluria-associated crystal deposition and exhibited antioxidant activity (Yıldız, 2019). In addition, *Taraxacum officinale* inhibited CaOx monohydrate crystal growth in vitro, suggesting that phytochemicals may directly modulate crystallization processes (Akyol, 2016). Collectively, these studies support the concept that plant-based compounds can modulate urinary biochemistry, oxidative stress, and tubular epithelial injury, thereby preventing stone formation.

V. opulus (VO; Gilaburu) has recently attracted attention for its antiurolithic properties. Most earlier studies have used preventive rather than therapeutic models. For example, Çapkın (2014) and İlhan et al. (2014) reported reductions in urinary oxalate and Ca levels, along with prevention of crystal deposition, in rats treated with VO prior to stone induction. Erdem et al. (2016) demonstrated that VO, alone or combined with *Persea americana*, attenuated renal crystal

deposition in an EG-induced nephrolithiasis model. More recent studies have confirmed the ability of VO to prevent CaOx accumulation and tubular injury (Ömerli et al., 2023; Şam et al., 2023). Clinically, VO has also been reported to facilitate the passage of distal ureteral stones (Kızılay et al., 2019; Tuğlu et al., 2014).

CaOx nephrolithiasis is a multifactorial condition involving hyperoxaluria, hypocitraturia, alterations in urinary mineral composition, oxidative stress, and tubular epithelial injury (Coe et al., 2005; Goldberg et al., 1989; Kaufman et al., 2008; Mitchell et al., 2019). Rat models induced with EG and AC provide a reliable system for evaluating both preventive and therapeutic interventions, allowing the assessment of renal histopathology, urinary biochemistry, and crystal formation dynamics.

The present study makes a meaningful contribution to the literature by evaluating *therapeutic* administration of VO juice after stone induction. VO treatment began on Day 8, when crystal formation had already commenced, providing a model more reflective of clinical conditions. VO significantly improved urinary biochemical parameters by increasing citrate and Mg levels while decreasing oxalate, Cr, and uric acid. These changes are consistent with the high polyphenolic content and antioxidant/anti-inflammatory activity of VO (31). Notably, the increase in citrate—a key endogenous inhibitor of crystal aggregation—is particularly important for suppressing stone development (Goldberg et al. 1989).

Histopathological analyses revealed marked reductions in tubular degeneration and crystal deposition in VO-treated rats. Quantitative assessment using H&E and PZ staining, scored blindly by independent observers, confirmed a significant decrease in crystal burden compared with positive controls. These findings suggest that VO may facilitate crystal clearance and attenuate tubular epithelial injury, effects also reported with lemon juice, *Berberis*, and *Glochidion* extracts (Bashir et al. 2010;Thatikonda et al., 2013; Touhami et al., 2007). Importantly, the present study is among the few directly comparing VO fruit juice and aqueous extract, demonstrating that extraction techniques may influence therapeutic efficacy.

Mechanistically, VO polyphenols likely exert their protective effects through antioxidant, anti-inflammatory, and diuretic actions, thereby reducing tubular injury and CaOx crystal retention (Hong and Qin, 2023). These mechanisms align with findings from studies using *Taraxacum officinale*, *Berberis vulgaris*, and *Herniaria hirsuta* (Akyol, 2016; Atmani and Silimani, 2003; Bashir, 2010). Our study offers several novel contributions to the existing literature. First, the therapeutic administration of VO following stone induction addresses an important gap left by earlier studies that focused exclusively on preventive models. Second, the longitudinal assessment of urinary promoters and inhibitors over a 20-day period enables a dynamic evaluation of metabolic alterations associated with stone formation. Third, the use of blinded observers for quantitative histopathological scoring of crystal deposition enhances methodological robustness and minimizes bias. Finally, the direct comparison between VO fruit juice and its aqueous extract provides a valuable insight into how different extraction methods influence therapeutic efficacy.

The observed reductions in CaOx–related parameters and histopathological crystal burden are consistent with the known antioxidant and anti-inflammatory profile of *V. opulus*. Bioactive constituents such as chlorogenic and caffeic acids, quercetin, and rutin can scavenge reactive oxygen species, mitigate lipid peroxidation, and preserve renal epithelial integrity, thereby reducing sites for crystal adhesion and retention. Iridoids (e.g., opuloside) and saponins may further dampen inflammatory signaling and decrease crystal aggregation via surface-active effects. In addition, organic acids (citric, malic, and tartaric) can chelate Ca and modulate urinary chemistry, potentially lowering supersaturation and nucleation. Together, these mechanisms provide a biologically plausible explanation for the improvements observed in urinary biomarkers and renal histology, aligning with prior reports of *V. opulus* exerting renoprotective effects through oxidative stress reduction, anti-inflammatory actions, and modulation of crystal dynamics (Effatparvar, 2025; İlhan et al., 2014; Kajszczak et al., 2020; Şam et al., 2023).

5. LIMITATIONS

This study has several limitations. The relatively small sample size and short treatment duration may restrict the generalizability of the findings. Only a single dose of Gilaburu extract was tested, and dose–response relationships remain to be clarified. Furthermore, while the EG/AC rat model is widely used, it cannot fully replicate the complex pathophysiology of human CaOx stone disease. Finally, mechanistic insights into the molecular pathways underlying the observed effects were not explored. Future studies with larger cohorts, longer treatment periods, multiple dosing regimens, and mechanistic analyses are warranted to validate and extend these findings.

6. CONCLUSION

These findings indicate that *V. opulus* juice may exert antiurolithiatic effects in an EG-induced rat model through antioxidant, anti-inflammatory, and urinary chemistry–modulating pathways. However, the results are preclinical and

derived from a single-species, short-duration study with a single dosing regimen. Rigorous validation in humans—through controlled dose-finding studies, standardized extract characterization, and clinical trials assessing efficacy, safety, and reproducibility—is required before any therapeutic claims or clinical recommendations can be made.

AUTHOR CONTRIBUTIONS

Ayşe Ömerli did research concept and design, collection and/or assembly of data, writing the article, and final approval of the article; Harun Ulger did research concept and design and critical revision of the article; Mehtap Nisari did collection and/or assembly of data and critical revision of the article; Hatice Susar, Arzu Hanım, and Ozge Al did data analysis and interpretation; Gökçe Şeker Karatoprak did collection and/or assembly of data and data analysis and interpretation.

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MANDATORY DISCLOSURE ON USE OF ARTIFICIAL INTELLIGENCE

During the preparation of this work, the author(s) used artificial intelligence tools to check the accuracy and quality of the English translation. After using this tool, the author(s) reviewed and edited the content as necessary and take full responsibility for the content of the publication.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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