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Research Article

Exploring the Antiurolithiatic and Diuretic Potential of Nausadar and Plant Extracts in Ethylene Glycol-Induced Urolithiasis in Wistar Rats

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Abstract

The rising demand for natural products has spurred interest in bioactive compounds from plants like *Piper nigrum* (black pepper), *Morus alba* Linn (white mulberry), and *Vitis vinifera* (grapevine). These plants are known for their rich content of alkaloids, flavonoids, and polyphenols, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties. In this study, we evaluated the anti-urolithiasis and diuretic effects of ethnomedicine using extracts from *P. nigrum*, *M. alba* Linn, and *V. vinifera*, focusing on the active compounds piperine, quercetin and lupeol, respectively. Urolithiasis was induced in wistar rats (150-200g body weight) through the administration of 0.75% ethylene glycol and 1% ammonium chloride solution (oral in drinking water) over 14 days. Following this, curative treatment was initiated using plant extracts at a dose of 300 mg/kg body weight and a combination of extracts with nausadar and ethnomedicine (*black pepper* and *nausadar* with water). The findings indicating an LD₅₀ >2000 mg/kg for the extracts. However, a marked therapeutic response was observed. Key biochemical parameters including serum (calcium, magnesium, uric acid, BUN, creatinine), kidney (weight, calcium, urea, phosphate, uric acid, catalase, oxalate), and urine (volume, pH, crystal urea, calcium, magnesium, uric acid, creatinine, phosphate, citrate, oxalate) demonstrated significant improvement following ethnomedicine treatment. Overall, the ethnomedicine demonstrated promising results in reducing kidney stone formation and improving overall kidney function, showing comparable efficacy to the standard marketed formulation, Cystone. Future research should focus on refining the dosages and mechanisms of action of these plant-based compounds and exploring their long-term therapeutic efficacy in treating urolithiasis in larger models.

Key words: Urolithiasis, Kidney stone, *P. nigrum*, *M. alba* Linn, and *V. vinifera*, Nausadar

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1. Introduction

Kidney stones, or urolithiasis, are among the most prevalent disorders of the urinary tract, affecting millions of individuals worldwide, this condition occurs when supersaturated urine leads to the crystallization and aggregation of minerals, most commonly calcium oxalate, resulting in the formation of solid stones in the kidneys or urinary system(Allam, 2024; Tsujihata,

2008). The recurrence rate for kidney stones is high, making it a significant health issue(Wang et al., 2022). Conventional treatments for urolithiasis include hydration, pharmacological agents to dissolve stones or inhibit their formation, and, in severe cases, surgical removal(Abou Chakra et al., 2020). However, these treatments may have limitations such as high costs, side effects, and limited accessibility in remote areas(Morton

et al., 2002; Noorulla et al., 2024). As a result, there has been growing interest in alternative treatments, particularly those rooted in traditional medicine systems, including ethno-medicine(Thanh Ha, 2021).

Ethno-medicines, which are grounded in the collective knowledge and practices of Indigenous and local communities, offer an important resource for addressing kidney stones naturally (Das, 2021). These practices involve the use of local plants, herbs, and other natural substances passed down through generations(Kumar et al., 2021). In many communities worldwide, particularly in rural or underserved areas, ethno-medicine remains a primary form of healthcare(Wanzala & Minyoso, 2024). This is especially true in Assam, a northeastern region of India, where diverse Indigenous and tribal groups rely on their traditional ecological knowledge to treat various ailments(Debnath & Das, 2019).

Despite the growing availability of modern medical treatments, these ethno-medicinal practices persist due to their cultural significance, accessibility, and perceived effectiveness(Rahman et al., 2023). Such traditional systems of healing underscore the importance of ethno-medicine not only as a therapeutic resource but also as a vital aspect of cultural heritage(Reddy et al., 2023). Plant-based remedies for kidney stones, particularly those used in traditional medicine, often exhibit a range of beneficial pharmacological properties(Oswal et al., 2023). Diuretic, anti-inflammatory, and antioxidant effects are commonly associated with the plants used in ethno-medicine(Prasad et al., 2021). Diuretics help increase urine output, thereby flushing out toxins and reducing the concentration of solutes that form stones (Kumar and Sindhu., 2024; Disher, 2021). Antioxidants reduce oxidative stress in the kidneys, which is linked to stone formation, while anti-inflammatory compounds alleviate the pain and discomfort associated with passing stones(Oswal et al., 2023). These effects, combined with their accessibility and cultural integration, make ethno-medicines valuable alternatives for managing urolithiasis, particularly in regions where modern treatments are not readily available(Sumarni et al., 2022).

However, despite their extensive use, the effectiveness and safety of many ethnomedicinal treatments for urolithiasis have yet to be scientifically validated(Beldar et al., 2022). Given the complexity of the condition, it is essential to rigorously assess the therapeutic potential of these traditional remedies in preclinical and clinical studies(Raj et al., 2024; Wang et al., 2021). Ethylene glycol, an industrial compound widely used as an antifreeze and coolant, is employed in research as a key substance for inducing urolithiasis in experimental models(Intasian et al., 2021). By simulating calcium oxalate stone formation in animals, ethylene glycol provides researchers with a controlled environment to test the antiurolithiatic efficacy of various treatments(Yousefi Ghale-Salimi et al., 2018). This preclinical evaluation is crucial for determining the ability of traditional plants to inhibit stone formation, reduce stone size, or promote stone expulsion(Sansores-España et al., 2022).

The present study focuses on the preclinical evaluation of ethnomedicine used for their antiurolithiatic and diuretic effects. Ethylene glycol-induced urolithiasis in animal models will serve as the basis for assessing the therapeutic potential of these plants. By analyzing the pharmacological properties of the selected plants, this research aims to provide scientific validation for their traditional use, thereby bridging the gap between ethno-medicine and modern healthcare. Furthermore, the study emphasizes the importance of sustainability, as the promotion and validation of these ethnomedicinal practices also highlight the need for the conservation of local plant species and traditional knowledge systems. As global interest in plant-based remedies continues to rise, this research seeks to contribute to the development of alternative treatments for kidney stones. In doing so, the integration of traditional ethnomedicinal knowledge with modern scientific methodologies holds the potential to offer new, effective, and sustainable treatments for urolithiasis, advancing both healthcare and cultural preservation.

2. Materials and Methods

2.1. Chemicals, Reagents, Instrumentation and Plant Materials

Ethylene glycol, used to induce urolithiasis in rats, was purchased from Merck Ltd., Mumbai, India. All other chemicals and reagents, including ammonium chloride and analytical-grade solvents, were sourced from certified suppliers.

The biochemical parameters, serum analysis, and kidney homogenate metrics were assessed using specific analytical instruments. The haematological parameters were measured with the Siemens ADVIA 2120i haematology analyzer. For biochemistry analysis, the Agappe Mispa Clinia was utilized, while electrolyte levels were determined using the Nirmal Medical Nulyte Smart analyzer. Each of these devices is designed to provide precise and reliable results in their respective domains, ensuring comprehensive evaluation of the samples.

Commercially available dried crude herbs, specifically *Vitis vinifera* fruits, *Piper nigrum* fruits, and *Morus alba* fruits, were procured from the market for examination. Upon visual inspection, the raw herbs were found to be satisfactory, showing no signs of foreign particles. To ensure their authenticity, identification and approval were conducted by an ISO-certified Vedanta Testing and Research Laboratory, Madhya Pradesh, India. Following this, a comprehensive organoleptic characterization of all three herbs was completed. This assessment involved observing both the fresh and dried forms of the herbs for various attributes, including color, odor, taste, size, shape, touch, and fracture.

2.2. Experimental Design and Induction of Urolithiasis

The present study received IAEC approval for the ethical use of animals, specifically under Protocol approval no. UH/IAEC/MS/21/03/2024/04. The study involved the use of healthy *Wistar rats*, each weighing between 150-200 grams, which were divided into 10 groups with 6

rats assigned to each group. The animals were housed in a controlled environment, maintained at a temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of 45–55%, under a 12-hour light-dark cycle. They were provided with food (Chakan Oil Mills, Pune, India) and water ad libitum. Prior to the experimentation, an acclimatization period of 7 days was observed to ensure the animals adapted to their new environment.

An acute toxicity study following the OECD 423 Test Guidelines, where the LD50 (lethal dose for 50% of the population) was determined (Panda et al., 2024). Subsequently, the induction of urolithiasis was conducted over a period of 14 days using ethylene glycol (EG) administered orally at a concentration of 0.75%, combined with 1% ammonium chloride. Following this induction phase, a further 14-day treatment was carried out using various plants extracts and ethnomedicine to evaluate their effects on urolithiasis in the experimental groups.

The group details are below:

Group 1 (G1): Normal control (distilled water)

Group 2 (G2): Standard control diuretic (Furosemide 10 mg/kg)

Group 3 (G3): Disease control 0.75% with 1% Ammonium chloride in drinking water

Group 4 (G4): Standard-EG + Cystone 500 mg/kg

Group 5 (G5): Ethnomedicine (*Piper nigrum* with Nausadar and water (1:2:4) 600 mg/kg

Group 6 (G6): Extracts of *P. nigrum*, *M. alba* and *V. vinifera* 300 mg/kg

Group 7 (G7): *M. alba* Extract with Nausadar 300 mg/kg

Group 8 (G8): *V. vinifera* Extract with Nausadar 300 mg/kg

Group 9 (G9): *P. Nigrum* Extract with Nausadar 300 mg/kg

Group 10 (G10): Extracts of *P. nigrum*, *M. alba* and *V. vinifera* with Nausadar 300 mg/kg

2.3. Collection and Analysis of Urine, Serum and Kidney Samples

Initially, the body weight of the animals was measured at key intervals throughout the study specifically on days 7, 14, 21, and 28. During these assessments, any clinical signs or physical abnormalities were recorded, and other biochemical and enzymatic parameters also measured with slight modification in reported protocols (Mammate et al., 2024). Throughout the experimentation, each animal was housed in individual metabolic cages for a 24-hour period to facilitate urine collection. Urine samples were gathered on days 0, 14, and 28 for comprehensive analysis. The parameters measured included urine volume, pH, crystal urea, calcium, magnesium, uric acid, urea, phosphate, citrate, phosphorus, and oxalate.

Following the urine collection, blood samples were drawn from the retro-orbital plexus under light anaesthesia. The serum was then separated via

centrifugation and analyzed for key parameters such as calcium, magnesium, uric acid, blood urea nitrogen (BUN), and creatinine. After blood collection, both kidneys were excised, cleaned, and rinsed with ice-cold physiological saline. A 10% homogenate of the kidney was prepared in Tris-HCl buffer (0.02 mol/L, pH 7.4) for further analysis of oxalate, catalase, calcium, uric acid, phosphate, and urea.

Additionally, to evaluate diuretic effects, urine volume, pH, sodium, potassium, and chloride ions were measured six hours post-treatment on day 15. All aforementioned markers were assayed using automatic analysers as detailed in Section 2.1.

2.4. Statistical Analysis

Data were expressed as Mean $\pm$ SEM. Statistical comparisons were performed using one-way ANOVA, followed by Dunnett's post hoc test. A p-value of less than 0.05 was considered statistically significant.

3.0. Results and Discussion

Urolithiasis is the most common form of urinary stone disease, characterized by the pathological processes of crystal nucleation, growth, and aggregation (Mosquera et al., 2020; Sodimbaku et al., 2016). These events lead to the formation of kidney stones, which occur when crystals in supersaturated urine coalesce into larger particles. Understanding these mechanisms is crucial for developing effective treatments and preventive strategies against kidney stone formation. Urinary calculi rank as the third most common urinary tract disorder, with approximately 80% composed of calcium oxalate. Treatment options for urolithiasis are limited, primarily focusing on dietary management and pain relief rather than specific medications for stone dissolution. In contrast, this study employed a controlled design with appropriate groups, ensuring that the observed effects are attributable to the treatments rather than confounding factors. The use of disease control groups provides a clear baseline for comparison, enhancing the reliability of the results. The consistent dosage of 300 mg/kg body weight for the herbal combinations and 500 mg/kg for Cystone allows for a direct comparison of efficacy. This controlled approach ensures that the observed differences in response can be confidently attributed to the treatments rather than variations in dosage. The extensive range of biochemical parameters measured (including urine volume, pH, and various metabolites) provides a holistic view of the treatment effects, supporting the conclusions drawn about the efficacy of the different regimens (Mosquera et al., 2020).

The current study presents compelling evidence regarding the efficacy of various treatment regimens on urinary, serum parameters and kidney homogenate in the context of kidney stones. During the entire duration of 28 days exposure no significant difference in bodyweight was observed as indicated in figure 1.

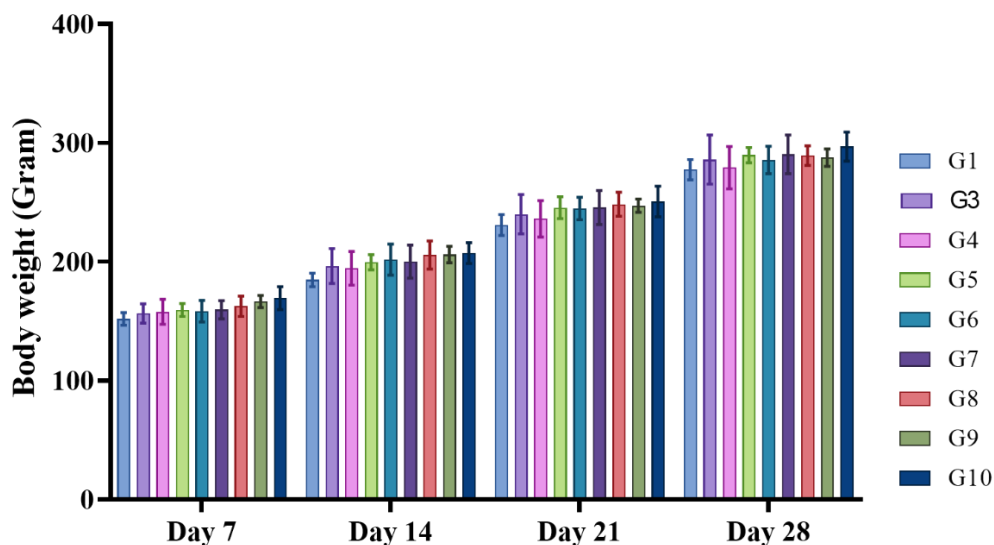


Figure 1 No significant impact was observed on the weekly body weight of rats between day 7 to day 28 of exposure duration.

Our analysis revealed that disease control groups (G3) exhibited highly significant alterations in biochemical markers compared to the vehicle control group (G1), indicating a detrimental effect on renal health. Specifically, there was a notable biphasic response in

pH, urine volume, citrate, and magnesium levels, alongside a significant increase in calcium, uric acid, blood urea nitrogen (BUN), creatinine, crystal urea, urea, phosphate, and phosphorus levels ($p < 0.0001$) indicated in figure 2-4.

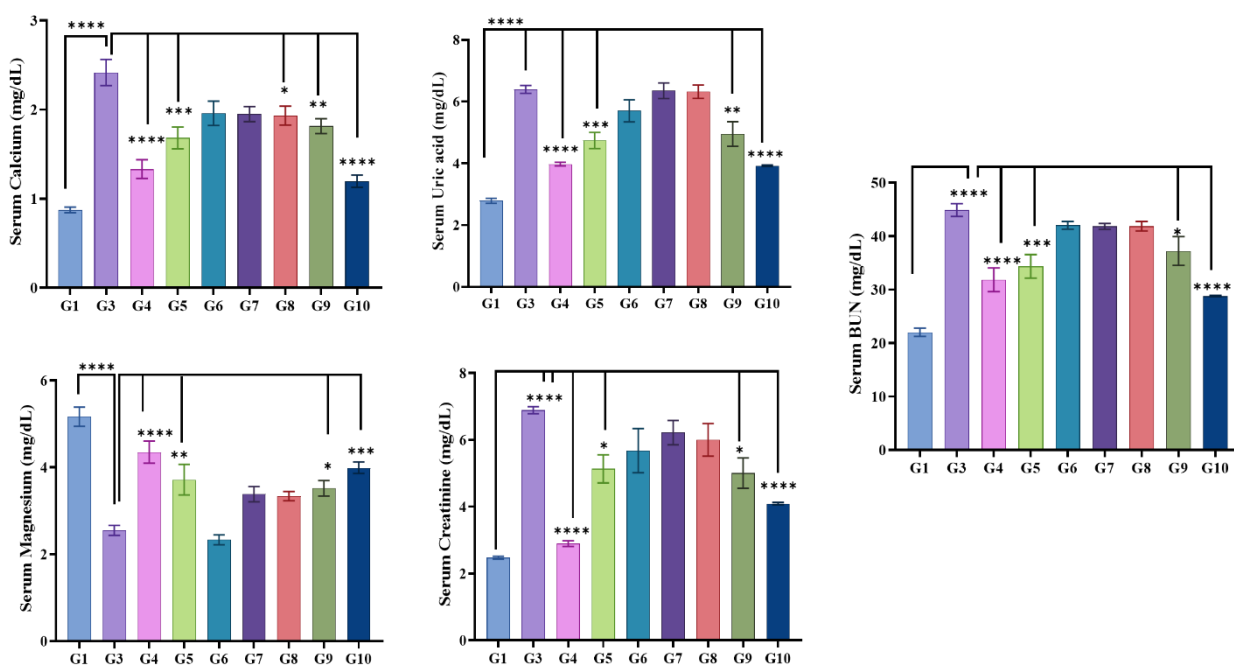


Figure 2: Significant changes were observed on the various serum biochemical parameters of wistar rats

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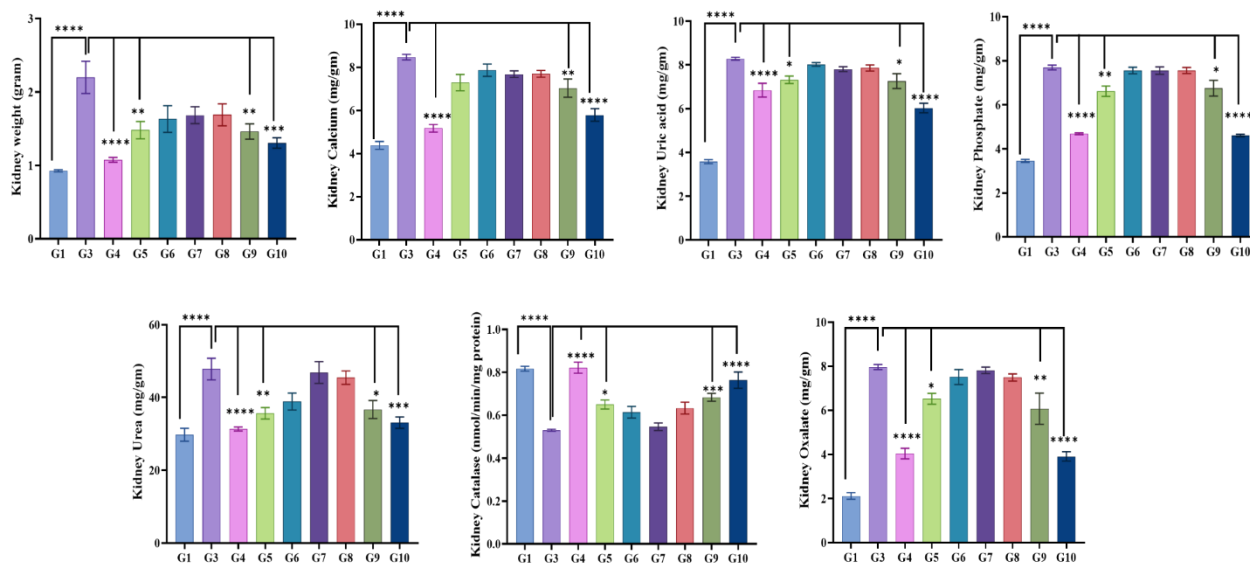


Figure 3: Significant changes were observed on the urine volume and various urine biochemical parameters of rats

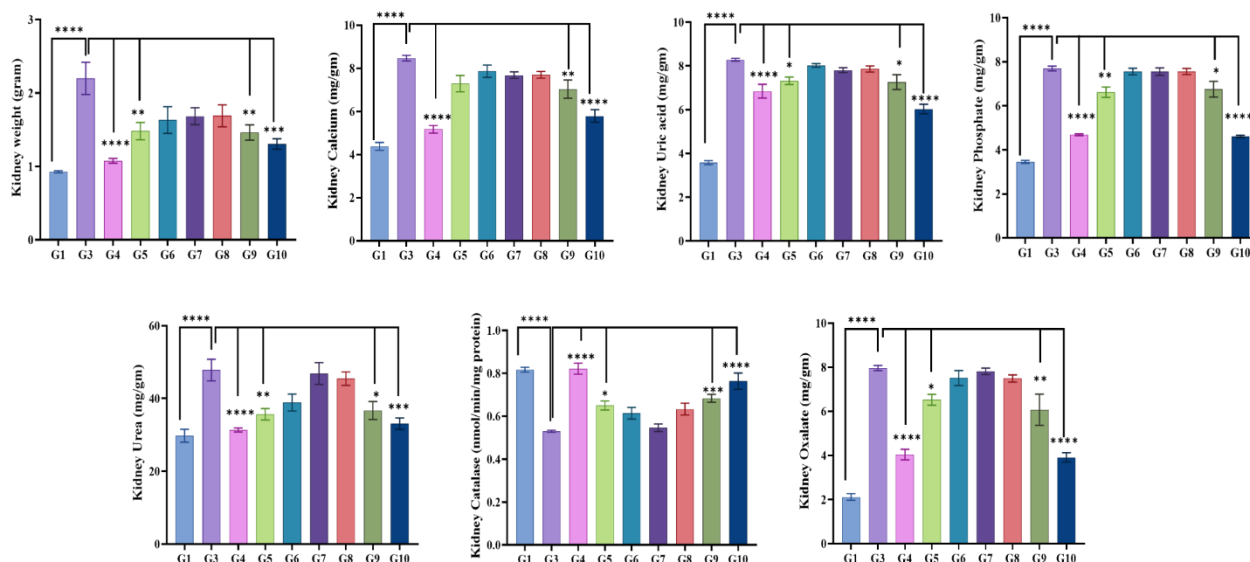


Figure 4: Significant changes were observed on the kidney weight and kidney homogenate biochemical parameters of rats

Furthermore, treatment with the standard drug Cystone (G4) at a dosage of 500 mg/kg body weight demonstrated a remarkable improvement in these parameters, reinforcing the drug's established role in managing urolithiasis. Importantly, the combination of the extracts from *P. nigrum*, *M. alba*, and *V. vinifera* with Nausadar (G10) yielded results that were not only statistically significant but also comparable to those achieved with Cystone, underscoring the potential of these herbal combinations in therapeutic applications. Similarly, it has been reported that extracts from *Plectranthus mollis*, *Didymocarpus pedicellata*, *Taraxacum officinale*, and *Dendrophthoe elastic* were administered at two doses over 28 days, results showed significant increases in calcium oxalate deposition and urinary levels of oxalate and calcium in control rats compared to normal, indicating the potential therapeutic

effects of these plants against urolithiasis (Baheti & Kadam, 2013).

In brief, the observed decrease in pH, citrate, and magnesium levels, along with the increase in calcium and uric acid, aligns with the pathophysiology of urolithiasis. The reduction in citrate, a known inhibitor of stone formation, suggests an unfavorable condition for stone dissolution, while elevated calcium and uric acid levels may contribute to stone formation. These findings reflect a typical biochemical profile, highlighting the impact of the disease on renal function and metabolic balance. Meanwhile, the significant improvement observed with Cystone treatment reinforces its established role in clinical practice. The drug's ability to modulate serum and urine parameters effectively may be attributed to its multifactorial mechanism of action, which includes promoting diuresis and inhibiting crystallization. This finding suggests that

pharmacological interventions remain critical in managing urolithiasis, particularly in severe cases. Administration of fruit extracts alone and in mixture did not show any significant changes when compared to standard control treatment, however some parameters show good response but the trend was not uniform with respect to all tested parameters. For example, *Benincasahispida* extract at doses of 250 and 500 mg/kg body weight orally for 35 days significantly reduced urinary excretion and kidney retention of oxalate, protein, and calcium. This suggests a potential nephroprotective effect of the extract in managing kidney stone formation. Additionally, elevated serum levels of sodium, creatinine, calcium, and phosphorus were notably decreased following treatment with the extract (Ali Esmail Al-Snafi, 2023). Although the combination without Nausadar (G6) showed a positive response, the lack of statistical significance implies that Nausadar plays a crucial role in enhancing the effects of the herbal extracts. The results from the ethnomedicine groups (G5) indicate that while the combination of black pepper and Nausadar yielded significant results, it was less effective than the three-herb combination with

nausadar. This suggests that the inclusion of additional herbal components may offer a more robust therapeutic benefit. Beyond their antiunitary activity, the biochemical parameters such as sodium, potassium, chloride, urine volume, and urine pH exhibited a clear pattern indicative of diuretic activity, favoring the removal of kidney stones in the individual treatment groups in a biphasic manner as indicated in figure 5. In contrast, the combination of *P. nigrum*, *M. alba*, and *V. vinifera* with Nausadar (G10) demonstrated remarkable efficacy, underscoring the potential for synergistic effects among these herbal constituents. Similar to present investigation, a study investigated the effects of a 0.75% ethylene glycol solution on kidney stone formation in rats over 28 days, which resulted in significantly elevated levels of urinary calcium, phosphate, oxalate, and protein, while magnesium levels decreased. Treatment with *Adonis aestivalis* at doses of 60 mg/kg daily effectively reduced these elevated parameters and inhibited stone formation. This suggests that *Adonis aestivalis* may have potential as a therapeutic agent for managing kidney stones (Ali Esmail Al-Snafi, 2023)

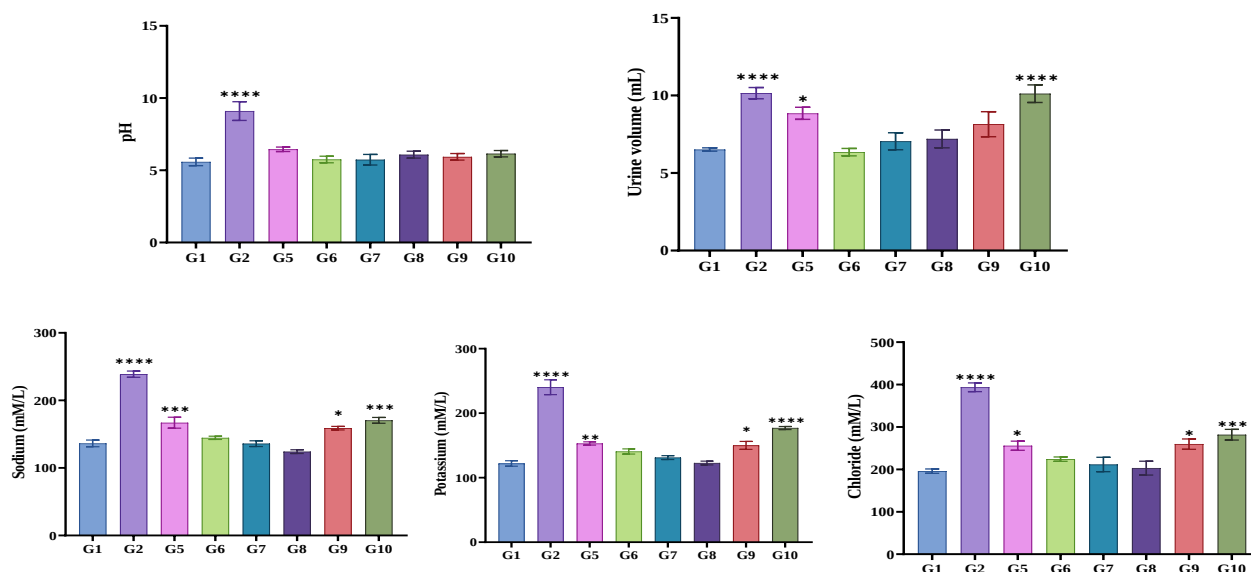


Figure 5: Significant changes were observed on the various urine parameters showing diuretic activity in rats 6 hour post treatment exposure

The histopathological examination revealed significant and severe histological changes in the renal tissues of the disease control groups. The presence of tubular dilatation and initial cystic changes indicates significant structural alterations within the renal tubules. These changes often result from sustained damage or obstruction, potentially linked to the accumulation of calcium oxalate crystals. The cystic formations suggest

that the renal architecture is compromised, impairing normal kidney function. This may lead to further complications such as impaired filtration and increased susceptibility to renal failure. The observed tubular atrophy signifies a reduction in the size and function of the renal tubules. Atrophy typically results from prolonged injury or stress on the renal tissues, indicating chronic damage. This finding correlates with the

dysfunction observed in the biochemical parameters of the disease control groups, where the kidneys are unable to maintain homeostasis effectively. The deposition of calcium oxalate crystals is a hallmark of nephrolithiasis. In the disease control groups, the abundance of these crystals suggests a pathological state conducive to stone formation. The presence of these crystals can lead to further injury, causing inflammation and contributing to the observed interstitial mononuclear cell infiltration. This infiltration is indicative of an inflammatory response, further compounding the damage to renal

tissues. In contrast, the standard treatment and other therapeutic groups demonstrated a reduction in these histological abnormalities. The amelioration of tubular dilatation, atrophy, and calcium oxalate deposits supports the efficacy of the treatments administered. This suggests that the treatments not only alleviate symptoms but also promote renal tissue recovery, restoring structural integrity and function as indicated in figure 6.

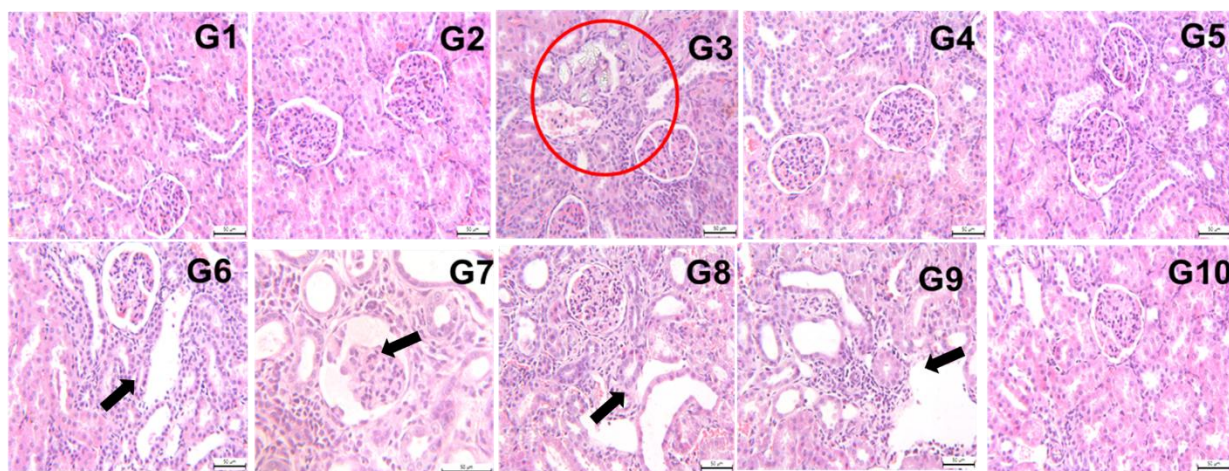


Figure 6: Representative images showing histopathology findings of kidney tissue of (G1) vehicle control, (G2) FSM standard treated, (G3) EG induced calculi group, showing severe histological changes including tubular dilatation and initial cystic, tubular atrophy, calcium oxalate crystals deposits and interstitial mononuclear cell infiltration; crystal of calcium oxalate shown in the circle (G4) Cystone, standard drug treated, (G5, G6, G7, G8, and G9) curative treatment with alone and in combination of all extracts showing improvement histological changes respectively; G10 showed no crystal deposition and no major histological changes.

Magnesium and citrate are recognized for their roles as crystal inhibitors in the urinary tract. The significant decrease in these parameters in the disease control groups highlights an unfavourable environment for preventing stone formation. Low citrate levels, in particular, are associated with reduced inhibition of calcium crystal precipitation, promoting stone formation. Conversely, the standard and other effective treatment groups showed significant increases in magnesium and citrate levels. This restoration of crystal-inhibiting compounds likely contributes to the reduced crystal formation and subsequent histological improvements observed in the treated groups. The pH and urine volume were also significantly decreased in the disease control groups. A lower urine volume can lead to concentrated urine, fostering an environment conducive to stone formation. The standard and other effective treatments, however, resulted in increased urine volume and pH, which can help dilute urinary constituents and reduce the likelihood of crystal formation. The decrease in catalase activity in the disease control groups suggests increased oxidative stress within the renal tissues. Oxidative stress can contribute to cellular damage and inflammation, exacerbating the pathological changes observed. In contrast, the standard and other effective treatment groups exhibited increased catalase levels, indicating

enhanced antioxidant defence mechanisms that may protect against renal injury and promote healing.

The significance of these findings suggests that the combined phytochemical properties may enhance the therapeutic outcomes beyond what is achieved with individual herbs alone. This synergism could be attributed to the complementary mechanisms of action, including antioxidant, anti-inflammatory, and diuretic effects, which collectively support renal health.

The promising results of the combination treatments warrant further investigation into other herbal combinations and their potential synergistic effects on renal health. Future studies could also explore optimal dosages and treatment durations. Understanding the underlying mechanisms through which these herbal extracts exert their effects will be crucial. Future research could employ *in vitro* and *in vivo* models to elucidate the specific pathways involved in the observed biochemical changes. Given the favorable outcomes observed in this preclinical study, clinical trials assessing the efficacy and safety of these herbal combinations in human populations are essential. Such studies could pave the way for integrating these natural remedies into standard therapeutic protocols for managing urolithiasis.

Conclusion

In summary, the findings from both biochemical and histopathological assessments underscore the severity of renal damage in the disease control groups and the protective effects of the treatments administered. The reduction of tubular injuries, inflammation, and calcium oxalate crystal deposits in treated groups highlights the potential therapeutic benefits of the interventions used. The findings demonstrate that the combination of three extracts with Nausadar possesses significant antiurolithiatic properties in *wistar rats* with ethylene glycol induced urolithiasis and effectively improved urine parameters, reduced serum markers of kidney dysfunction, and may offer a protective effect against renal damage associated with urolithiasis. The improvements in critical biochemical parameters further reinforce the efficacy of the treatments, suggesting a multifaceted approach to managing renal health and preventing stone formation. The findings not only reinforce the efficacy of conventional treatments like Cystone but also highlight the potential of herbal medicine in enhancing renal health. The observed synergistic effects of the herbal extracts suggest a promising avenue for further exploration, with implications for both future research and clinical practice.

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