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Science Journal of University of Zakho

Vol. 13, No.3 pp357–366July-September,2025

p-ISSN: 2663-628X
e-ISSN: 2663-6298

PROTECTIVE ROLES OF *Lactuca serriola* AND *Glycyrrhiza glabra* ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN MALE ALBINO RATS (*Rattus norvegicus*)

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Received: 26 Feb 2025 Accepted: 16 Mar 2025 Published: 20 Aug 2025 https://doi.org/10.25271/sjuoz.2025.13.3.1494

ABSTRACT:

This study aimed to evaluate the protective effects of *Lactuca serriola* (*L. serriola*) and *Glycyrrhiza glabra* (*G. glabra*) against ethylene glycol (EG)-induced urolithiasis in male albino rats. Twenty-eight rats were divided into four groups: control, EG-treated, and two groups co-administered with *L. serriola* or *G. glabra* alongside EG. Body weight, food and water intake, kidney and liver function, serum electrolytes, and lipid profiles were assessed. Histological examination and urine crystal analysis were also performed. Co-administration of *L. serriola* and *G. glabra* protected against EG-induced increases in serum creatinine, urea, and glutamic oxaloacetic transaminase (GOT) levels. *Lactuca serriola* also prevented triglyceride (TG) elevation, while *G. glabra* effectively maintained food intake. Both herbs significantly reduced the kidney weight increase, crystal formation, and tissue damage. Histological analysis showed reduced calcium oxalate (CaOx) crystal deposition and tissue injury with herb supplementation. In conclusion, *L. serriola* and *G. glabra* demonstrated protective roles against urolithiasis and may serve as potential natural anti-urolithiasis agents.

KEYWORDS: Ethylene Glycol, *Glycyrrhiza glabra*, Histology of Kidneys, *Lactuca serriola*, Renal Calculi

1. INTRODUCTION

Medicinal plants are referred to as herbs that are used to treat various diseases and medical problems due to the presence of numerous bioactive compounds (Al-Chalabi & Hajani, 2023 ; Mahmud, 2021). Since ancient times, they have been used as remedies for various human diseases via their therapeutic constituents (Mahmud *et al*., 2016; Mahmud, 2017a; Mahmud, 2017b). The plants' medicinal value is related to the presence of a wide variety of secondary metabolites (Sajadi *et al*., 2019), including phenolics, terpenoids, alkaloids, tannins, flavonoids, saponins, phytosterols, polyphenols, and others (Mohammed, & Omer 2023). Recently, it has been found that the WHO recorded that about 80% of the world's population uses traditional drugs, mainly herbal medicine (Khdhr & Mahmud, 2023).

Lactuca serriola, also commonly known as prickly lettuce, wild lettuce, or milk thistle, is a member of the Asteraceae family and is recorded as a medicinal plant used in traditional medicine for the treatment of various diseases (Abdul-Jalil, 2020). *Lactuca serriola* is rich in lactucarium and a wide amount of mineral nutrients, vitamins, natural antioxidants, flavonoids, phenolics (El-Esawi *et al*., 2017), alkaloids, glycosides, volatile oil, organic acids, carotene, saponins**,** phytosterols, phenolic compounds, tannins, triterpenoids and sesquiterpene esters (Abdul-Jalil, 2020). Concerning its chemical constituents, *L. serriola* can be used as an anti-inflammatory, anticarcinogenic, antioxidant potential (El-Esawi *et al*., 2017), sedative, diuretic, antispasmodic (Aziz *et al*., 2016; Abdul-Jalil, 2020), antibacterial, hypnotic, expectorant, cough suppressant, antiseptic, vasorelaxant, manage gastrointestinal, and various other ailments (Abdul-Jalil, 2020).

Glycyrrhzia glabra (liquorice) is a small perennial herb belonging to the Fabaceae family (Shah *et al*., 2018). It is native to the Mediterranean region, central to Southern Russia and Asia, and is now widely cultivated throughout Europe and the Middle East (Shah *et al*., 2018). Similar to other medicinal herbs, the *G. glabra* contains several chemical compounds such as alkaloids, glycosides, carbohydrates, starches, lipids, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, tannins, sterols, steroids, and glycyrrhizin. *Glycyrrhzia glabra* was applied orally to treat numerous health disorders, including cystitis, kidney stones, diabetes, stomachache, tuberculosis, and Addison's disease, as well as a mild laxative, contraceptive, and to improve sexual function (Al-Snafi, 2018). Several studies showed that *G. glabra* can be employed as a memory enhancer, hypolipidemic, antimicrobial, anticancer, antidiabetic, antidepressant (Al-Snafi, 2018)*,* antiviral, immune modulator, hepatoprotective (Gaur *et al*., 2016)*,* antioxidant, and anti-ulcer effects (Mutaillifu *et al*., 2020).

Kidney diseases are the main reason for kidney failure and cardiovascular diseases (Evans *et al*., 2022). Urolithiasis is classified as one of the most painful and most common problems in the urinary system. Urolithiasis or nephrolithiasis or kidney stones is selected as a global disorder since ancient times which affecting human health (Wang *et al*., 2021), its incidence about 12% in the common population (Al-Bajari *et al*., 2019), but the rate of occurrence is strongly higher in men (12 in 100) as compared to women (6 in 100) (Wang *et al*., 2020). There are different forms of kidney stones: calcium stones (CaOx and calcium phosphate), magnesium ammonium phosphate stones, uric acid stones, cysteine stones, and drug-induced stones. Calcium stone is the most common type of kidney stone, which accounts for more than 80% of all stones (Shastri *et al*., 2023).

The current study was designed to find the protective roles of *L. serriola* and *G. glabra* in EG-induced urolithiasis in male albino rats.

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2. MATERIALS AND METHODS

Plant Materials:

The aerial part of *L. serriola* and the root of *G. glabra* were collected from Soran-Erbil (GPS position 36.673689 N, 44.548769 E), Kurdistan Region-Iraq. The plant parts were washed using tap water and then shade-dried at room temperature for 20-25 days. They were then crushed into small pieces and grinded using an electrical mill (Clartonic AT 3644 blenders, Germany). The produced powders were stored in cloth bags at 5ºC until use (Khdhr & Mahmud, 2023).

Animal housing and breeding:

Twenty-eight male albino rats, about 250-320 g body weight (B.W.), were used during the present study. *Rattus norvegicus* were bred in the animal house, Department of Biology/Faculty of Science/ Soran University–Soran, Iraq. They were maintained in cages bedded with wooden chips and kept under standard laboratory conditions, with a photoperiod of 12 hrs light and 12 hours darkness and at 22 ± 2ºC. Rats were fed on normal rat chow (66.6% wheat, 25.6% soya, 4.4% oil, 1.5% lime stone, 0.63% salt, 0.158% methionine, 0.062% choline chloride, and 0.05% trace elements) and tap water *ad libitum* (Abdulla *et al*., 2017).

Experimental design:

This study was conducted to evaluate the protective effects of *L. serriola* and *G. glabra* against EG-induced urolithiasis. The rats were randomly divided into four experimental groups, with treatments administered for 10 days:

Group A: Control group (n=8)

Rats were given normal water *ad libitum* and a diet.

Group B: EG and AC group (n=6)

Rats received drinking water containing 1% EG (10 days) and 1% ammonium chloride (AC) (4 days) for inducing urolithiasis and a normal diet.

Group C: EG+*L. serriola* (n=7)

Rats were supplied with a normal diet containing 5% *L. serriola* and water containing 5% L. serriola, 1% EG (10 days), and 1% AC (4 days).

Group D: EG+*G. glabra* (N=7)

Rats were supplied with a normal diet containing 5% of *G. glabra* and water containing 5% of *G. glabra*, 1% EG (10 days), and 1% AC (4 days).

Collection of blood samples:

At the end of the experiment, rats were anesthetized using Ketamine hydrochloride (50 mg/kg) and Xylazine (10 mg/Kg) administered intraperitoneally. Blood samples were collected via cardiac puncture into chilled, ethylenediaminetetraacetic acid (EDTA)-free gel tubes. The samples were centrifuged at 3000 rpm for 15 minutes (Khdhr & Mahmud, 2016), and the obtained serum was stored at -20°C for biochemical analysis.

Physiological and Biochemical Assessments, Body Weight, Kidney Weight, Food, and Water Intake:

Body weight gain/loss (body weight gain/loss = final weight - initial weight) and the kidney weight-to-body weight ratio were measured using an electronic balance (Das *et al*., 2025).

Food and water intake were recorded for each experimental group.

Kidney and liver function tests:

Serum levels of creatinine, urea, and uric acid were measured as markers of kidney function.

Liver function was assessed by measuring glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels.

Lipid profile and glucose analysis:

Serum cholesterol, TG, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were analyzed. Blood glucose levels were measured.

Electrolyte analysis:

Serum potassium (K), sodium (Na), and calcium (Ca) concentrations were determined.

All biochemical parameters were analyzed using a Cobas c411 biochemical analyzer and a SMART LYTE electrolyte analyzer.

Microscopic examination of urinary crystals:

Urine samples were collected from all groups at the end of the experiment and centrifuged at 2000 rpm for 5 minutes. The sediment was examined under a light microscope for the presence of CaOx crystals.

Statistical analysis:

All data were expressed as means ± standard error (S.E.), and statistical analysis was carried out using the GraphPad Prism 8 program (Version 8) (GraphPad Software, USA).

The comparisons among groups were done using one-way ANOVA. P-values less than 0.05 ($P<0.05$) were considered statistically significant. In all figures, the symbols (*, **, and ****) represent that mean differences are significant at the 0.05, 0.01, 0.001, and 0.0001 levels, respectively.

3. RESULTS

Body weight gain/loss:

Ethylene glycol treatment significantly ($P<0.0001$) decreased body weight compared to the control group. Co-administration of *L. serriola* with EG reduced ($P<0.05$) this weight loss, while *G. glabra* root with EG showed a stronger protective effect ($P<0.001$) by increasing body weight compared to EG-treated rats (Fig. 1).

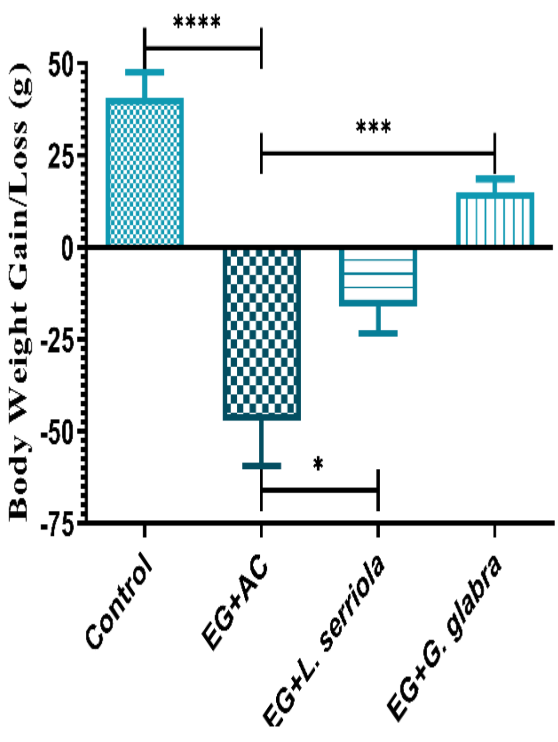


Figure 1: The effect of *L. serriola* and *G. glabra* root on body weight gain/ loss in EG-induced urolithiatic rats.

Kidney weight/ body weight:

Ethylene glycol treatment significantly increased the kidney weight/ body weight ratio ($P<0.01$) compared to the control group. However, co-administration of *L. serriola* and *G. glabra* root with EG significantly reduced this increase ($P<0.05$) compared to EG alone (Fig. 2).

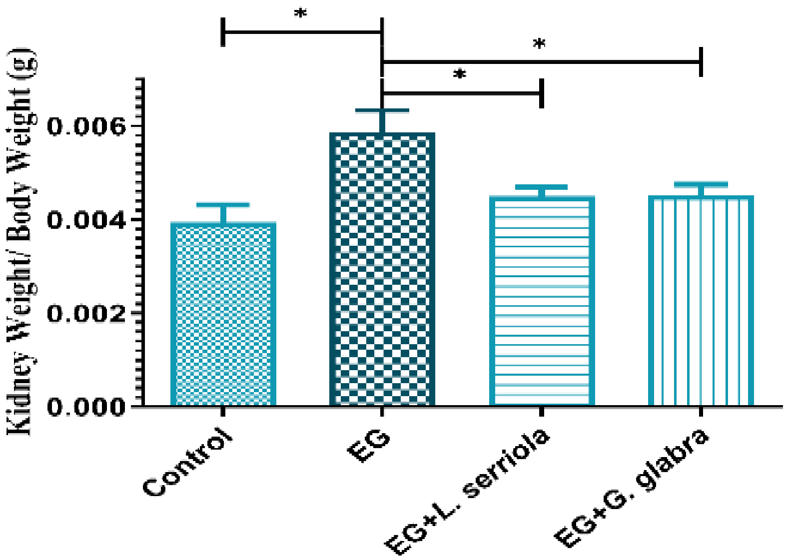


Figure 2: The effect of *L. serriola* and *G. glabra* root on kidney weight/ body weight in EG-induced urolithiatic rats.

Food and water intake:

Statistical data analysis revealed that in EG-treated rats, the rate of food intake effectively (P<0.0001) declined as compared to control animals. *Lactuca serriola* failed to counteract this effect, but administration of *G. glabra* root successfully (P<0.0001) restored food intake to levels closer to control values (Fig. 3).

Similarly, EG treatment significantly **(**P<0.0001**)** reduced water intake compared to the control group. Co-administration of *L. serriola* and *G. glabra* root with EG significantly (P<0.0001) restored water intake levels to values comparable to the control group (Fig. 3).

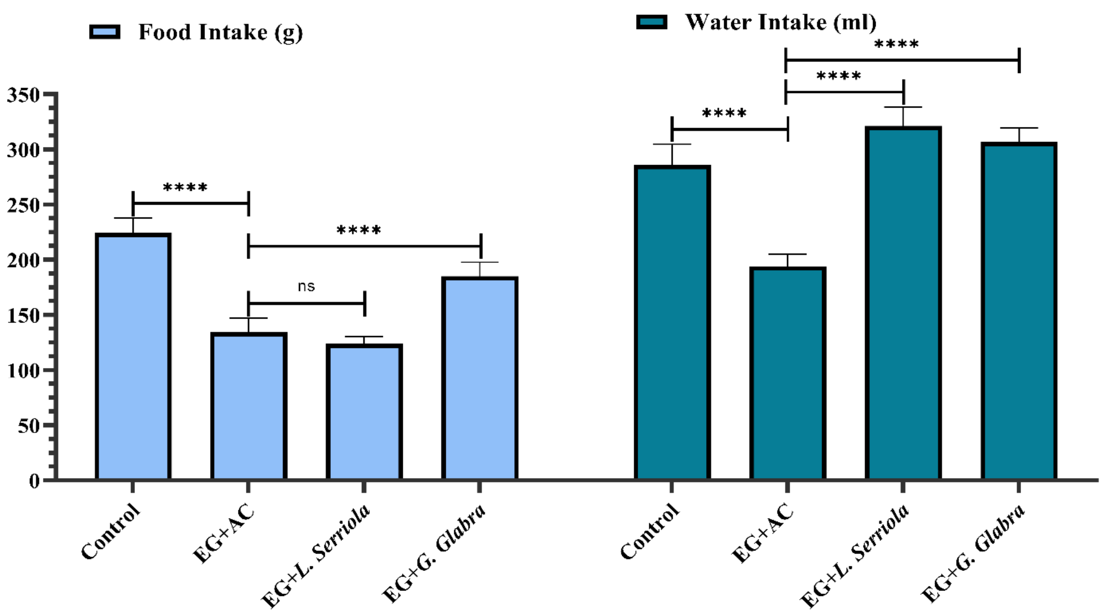


Figure 3: The effect of *L. serriola* and *G. glabra* root on food and water intakes in EG-induced urolithiatic rats.

Serum uric acid, creatinine, and urea:

There were no significant differences in serum uric acid levels among the control, EG-treated, and *L. serriola*-treated groups. However, *G. glabra* root administration with EG resulted in a non-significant reduction in uric acid levels compared to EG-treated rats (Fig. 4).

Statistical analysis showed that serum creatinine concentration significantly (P<0.01) increased in the EG-treated group as compared with the control group. Co-administration of *L. serriola* and *G. glabra* root significantly (P<0.05 and P<0.01, respectively) prevented this elevation compared with rats only treated with EG (Fig. 4).

Ethylene glycol treatment significantly (P<0.0001) increased serum urea levels compared to control rats. Co-administration of *L. serriola* and *G. glabra* root with EG significantly **(**P<0.0001**)** prevented this increase and restored urea levels close to control values (Fig. 4).

Serum K, Na, and Ca:

Serum K, Na, and Ca levels among the experimental groups did not significantly change (Fig. 5).

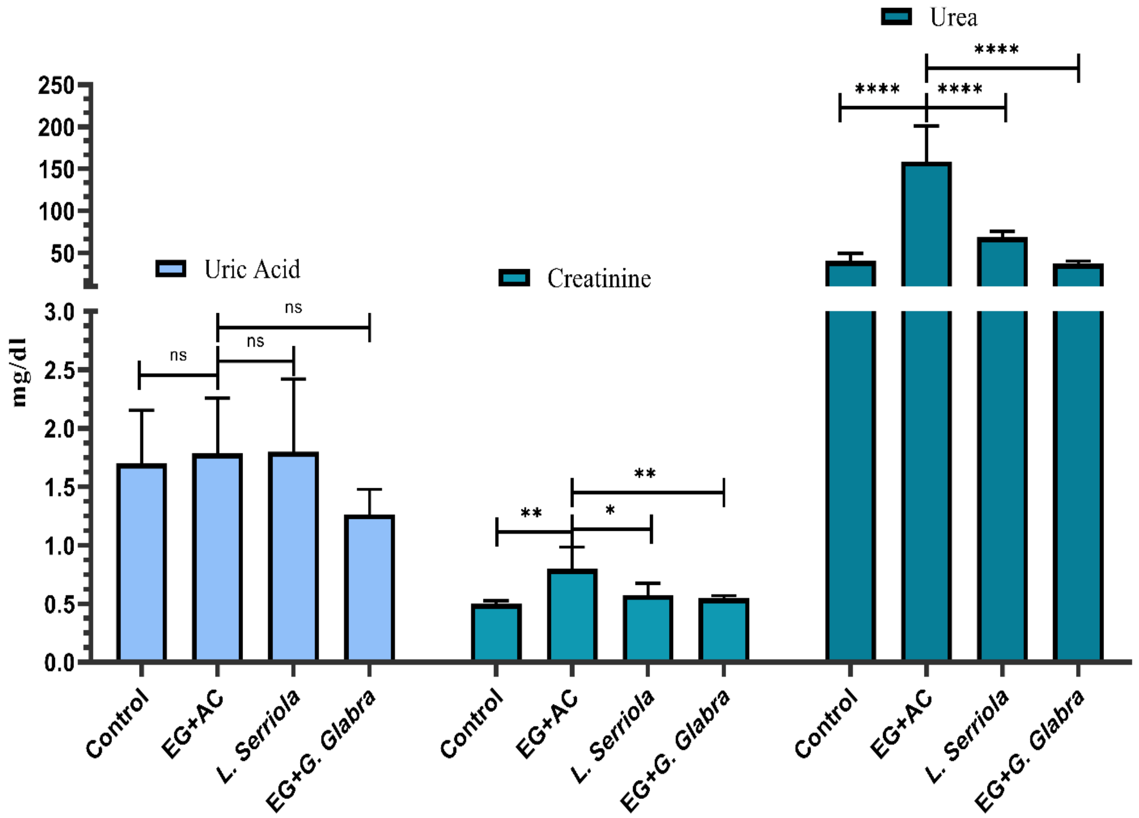


Figure 4: The effect of *L. serriola* and *G. glabra* root on serum uric acid, creatinine, and urea in EG-induced urolithiatic rats.

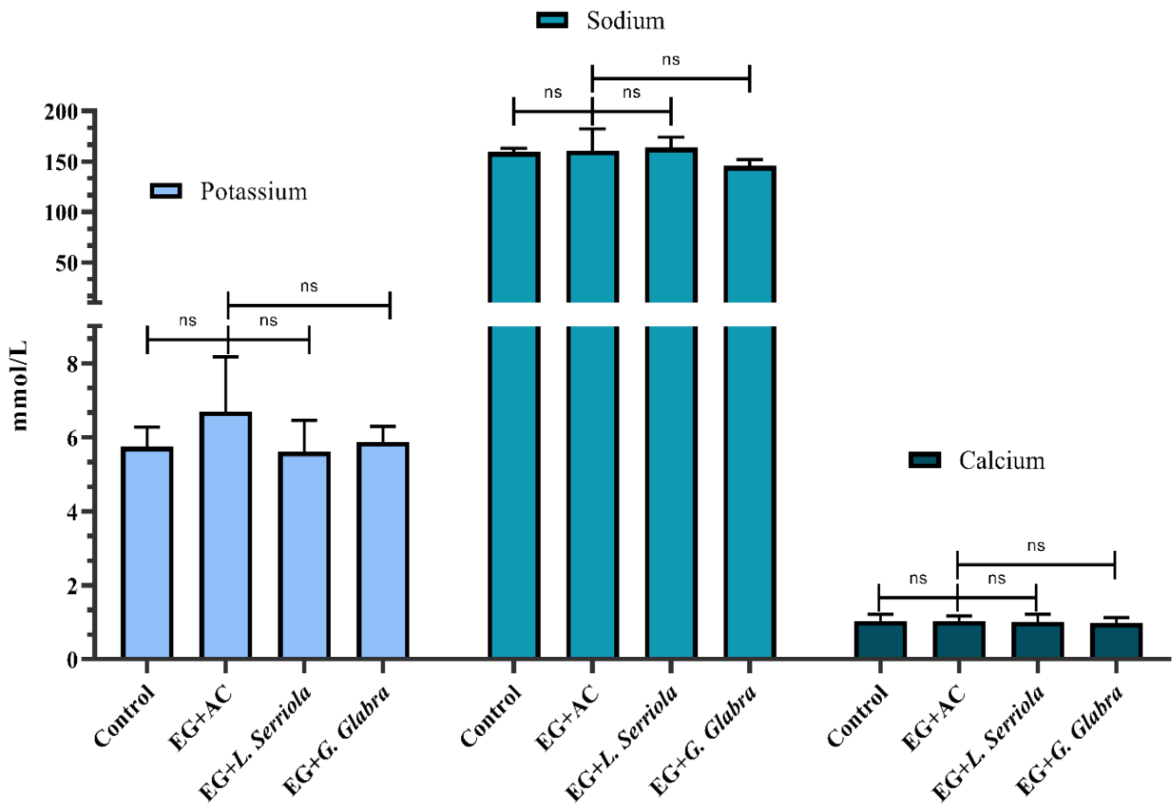


Figure 5: The effect of *L. serriola* and *G. glabra* root on K, Na, and Ca ions in EG-induced urolithiatic rats.

Serum GOT or AST and GPT or ALT:

Administration of EG significantly **(**P<0.01**)** increased serum GOT levels compared to the control group. However, administration of *L. serriola* and *G. glabra* root with EG significantly **(**P<0.01 and P<0.001**, r**espectively) prevented this increase. No significant changes were observed in serum GPT levels among the groups (Fig. 6).

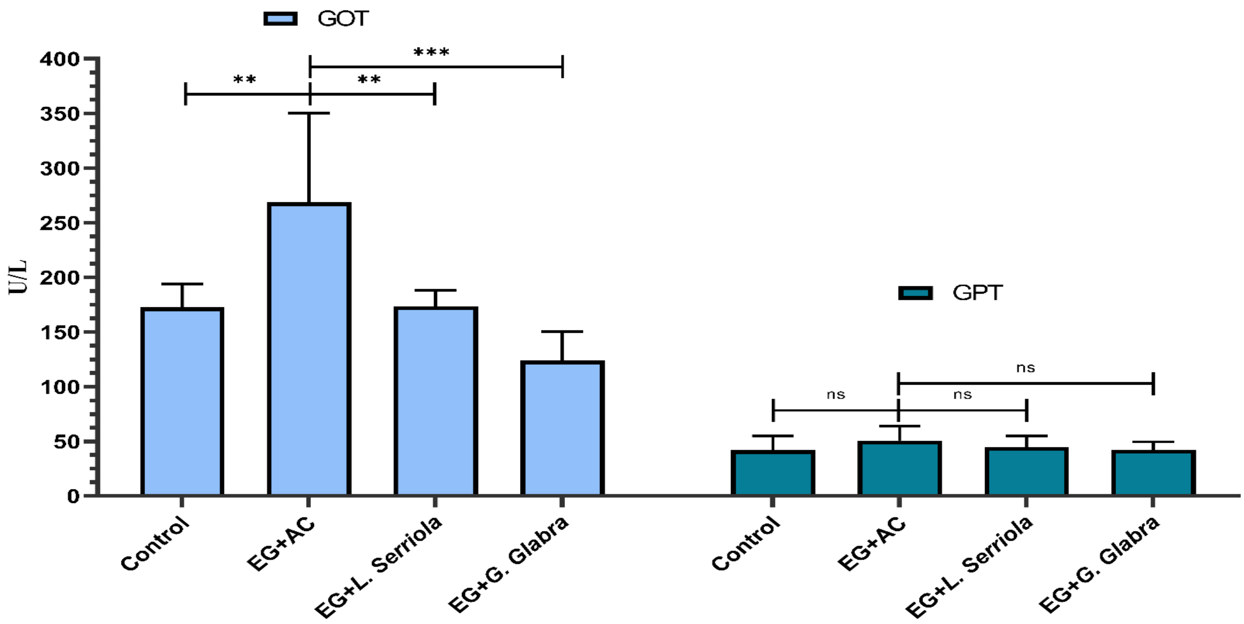


Figure 6: The effect of *L. serriola* and *G. glabra* root on GOT and GPT in EG-induced urolithiatic rats.

Serum cholesterol, non-HDL cholesterol, LDL, TG, VLDL, HDL, and LDL/HDL ratio:

Serum cholesterol, non-HDL cholesterol, LDL, VLDL, HDL, and LDL/HDL ratio did not show significant changes among the groups (Fig. 7). However, serum T.G. levels significantly (P<0.05) increased in EG-treated rats compared to control rats. Co-administration of *L. serriola* with EG significantly (P<0.05) prevented this increase, but *G. glabra* root failed to prevent it (Fig. 7).

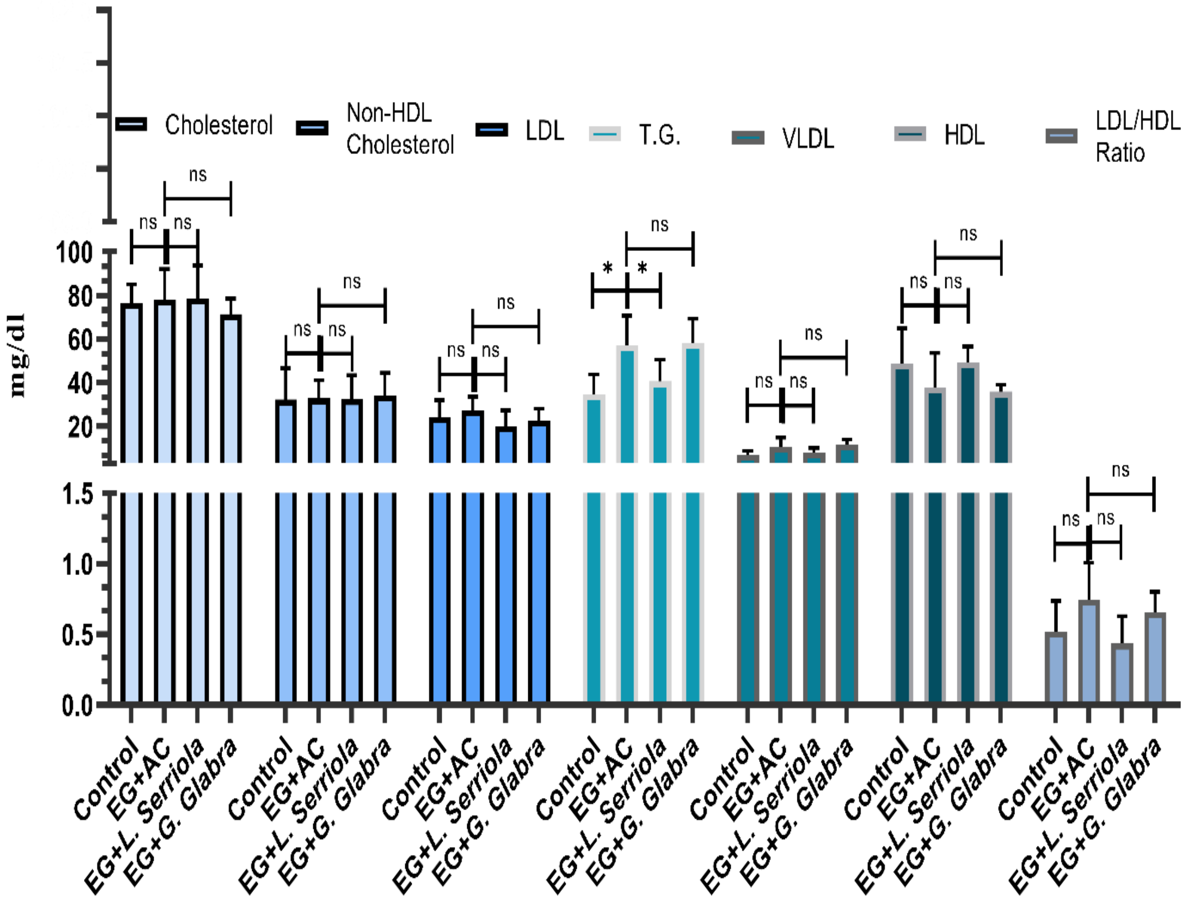


Figure 7: The effect of *L. serriola* and *G. glabra* root on serum cholesterol, non-HDL cholesterol, LDL, T.G., VLDL, HDL, and LDL/ HDL ratio in EG-induced urolithiatic rats.

Serum glucose:

Ethylene glycol treatment caused a non-significant increase in serum glucose levels compared to controls.

Co-administration of *L. serriola* and *G. glabra* root with EG maintained glucose levels close to control values (Fig. 8).

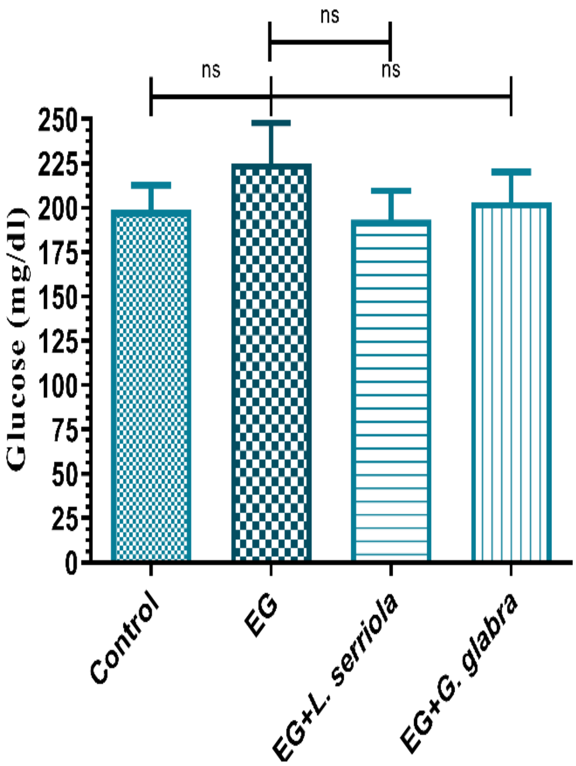
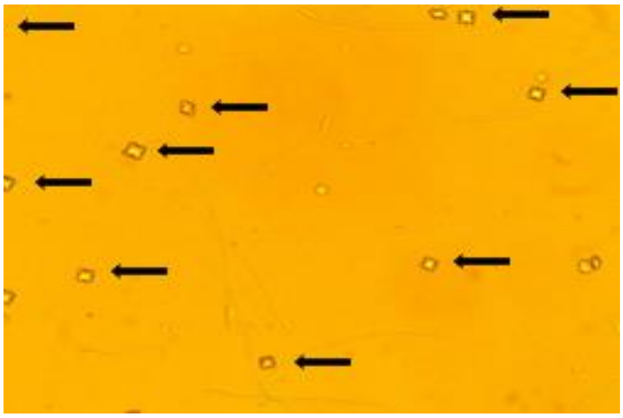


Figure 8: The effect of *L. serriola* and *G. glabra* root on serum glucose in EG-induced urolithiatic rats.

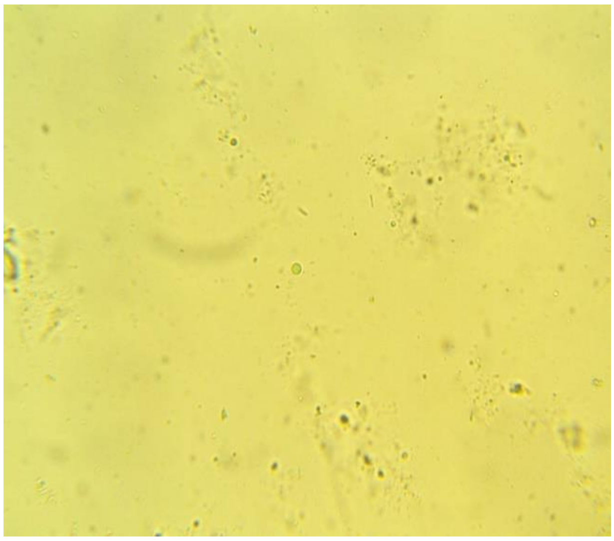
Microscopic urine analysis:

Microscopic examination of urine revealed numerous CaOX crystals in the EG-treated group compared to the control group (Fig. 9A & B). Co-administration of *L. serriola* and *G. glabra* root with EG significantly reduced CaOX crystal deposition, showing a protective effect against crystal accumulation (Fig. 9C & D).

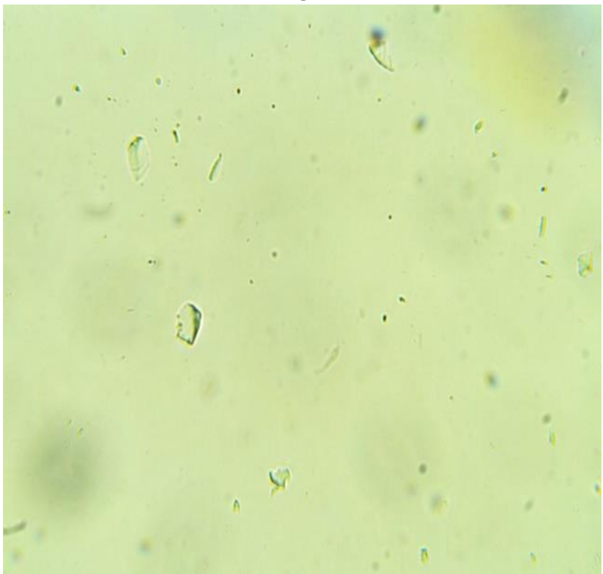
A



B



C



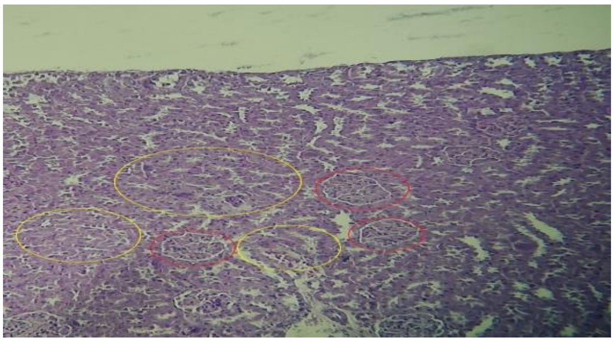
D

Figure 9: The CaOX crystals viewed by light microscope (40X) from urine, **(A)** control rats revealing normal appearance, **(B)** EG treated rats showing numerous CaOX crystals accumulation, **(C)** EG + *L. serriola* supplemented animals revealing prevented CaOX crystals deposition, **(D)** EG + *G. glabra* root supplemented animals revealing prevented CaOX crystals deposition.

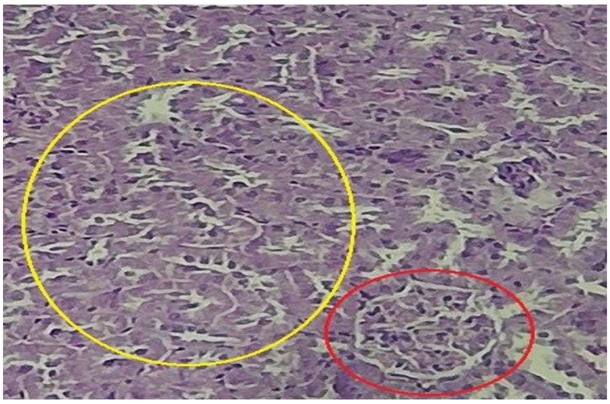
Kidney's histological examination:

Histological analysis showed normal kidney structures in control rats, including intact glomeruli, proximal and distal convoluted tubules, and collecting tubules (Fig. 10A1 & A2). In contrast, EG-treated rats exhibited CaOX crystal deposition in the kidney tubules, along with hyperplasia, necrosis, hypertrophy of tubular tissue, and acidic deposits in the tubular lumen (Fig. 10B1 & B2). Pyknotic nuclei in tubular and glomerular cells and infiltration of inflammatory cells in interstitial tissues were also observed.

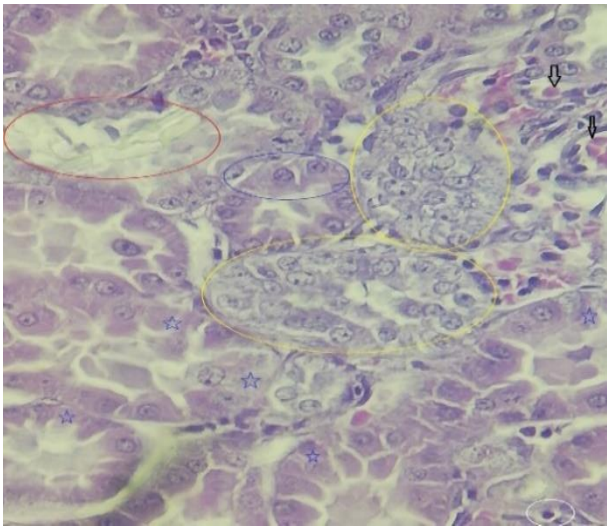
Co-administration of *L. serriola* with EG showed a protective effect, with mild congestion, reduced inflammatory cell infiltration, and fewer renal stones in the tubular cavities (Fig. 10C1 & C2). Similarly, *G. glabra* root with EG resulted in mild stone crystal formation, moderate inflammatory cell infiltration, formation of multinucleated giant cells around crystals, and mild renal tissue congestion (Fig. 10D1 & D2).



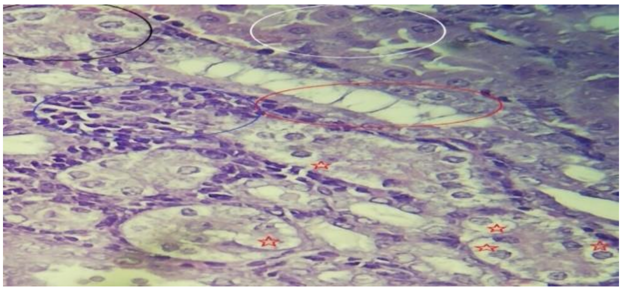
A1



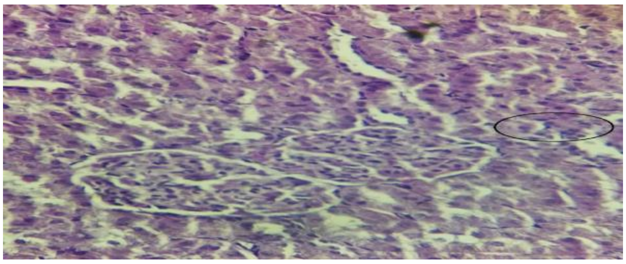
A2



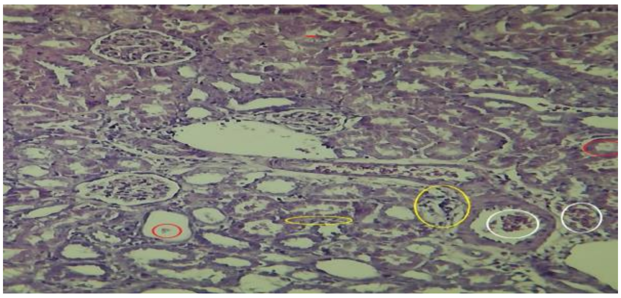
B1



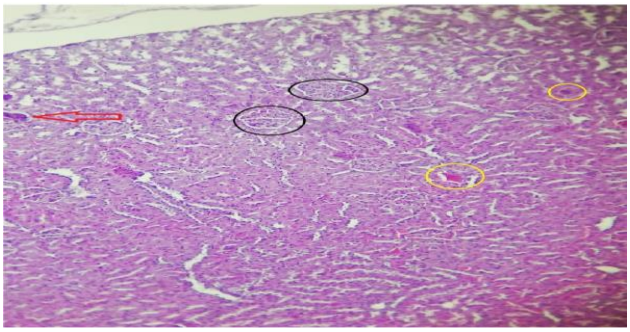
B2



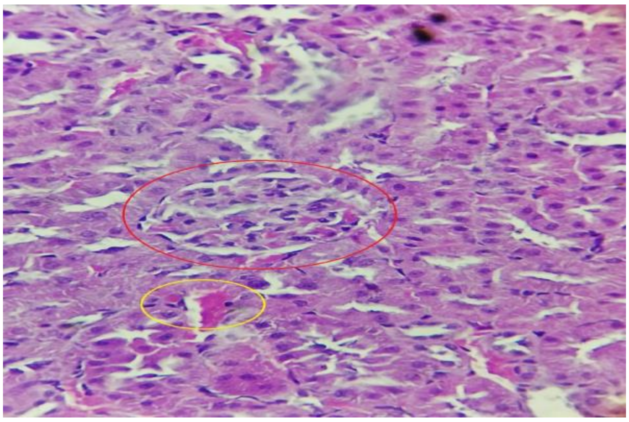
C1



C2



D1



D2

Figure 10: Control rats showed the normal structure of glomeruli (red circle), and renal tubules (yellow circle) A1 and A2. The urolithiatic rats showed tubular hypertrophy (blue and white circles), necrosis (blue star and black circle), hyperplasia (yellow circle), CaOx crystals (red circles) deposition in the lumen of renal tubules, acidic deposition in the tubular lumen, pyknotic cell noted in glomerular and tubular cells, and inflammatory cell infiltration B1 and B2. However, in group C, administration of *L. serriola* parallel to EG revealed mild congestion (white circle), inflammatory cell infiltration (yellow and black circles), and small particles of stones (red circle), C1 and C2. Also, as rats received *G. glabra* with EG agent showed mild urolithiasis, acidic molds (yellow circle), multinucleated giant macrophages surrounding molds (red arrow), and normal glomeruli (red and black circle) D1 and D2. (A1, D1 100X; C1 200X; A2, B1, B2, C2, D2 400X; H&E stain).

4. DISCUSSION

Effect of EG and medicinal plants on body weight, food intake, and water intake:

This study demonstrated that body weight significantly decreased in EG-treated animals compared to the control group. Administration of *L. serriola* and *G. glabra* root effectively mitigated the body weight reduction caused by EG. Moreover, EG treatment reduced water and food intake rates. Co-administration of *L. serriola* and *G. glabra* reversed the reduction in water intake, while G. glabra alone improved food intake levels.

These findings align with previous studies. Aslan & Aksoy (2015) reported that increased water intake reduces stone formation by increasing urine output and accelerating the urinary system cycle. Aziz *et al*. (2016) demonstrated that *L. serriola* increases urine output due to its diuretic properties. Similarly, Abhirama and ShanmugaSundaram (2018) found that renal calculi negatively impact food and water intake, leading to body weight reduction.

The body weight decline observed in EG-treated rats may be linked to metabolic disruption and reduced appetite, consistent with findings by Kumar *et al*. (2016) and Sikarwar *et al*. (2017). Gonzales and Membreve (2019) also reported that pain from stone formation reduces food intake and body weight. The protective effect of *L. serriola* and *G. glabra* could be attributed to their phytochemicals (polyphenols, flavonoids, saponins, and tannins), which are known to have antiurolithiatic (Anand *et al*., 2021) and appetite-stimulating effects (Abdulla, 2020).

Effect of EG and medicinal plants on kidney weight relative to body weight:

The present study showed that kidney weight relative to body weight significantly increased in EG-treated rats, indicating kidney swelling and inflammation. However, co-administration of *L. serriola* and *G. glabra* prevented this increase.

Mahmud *et al*. (2021) reported that EG administration induces inflammation and swelling in kidney tubules, which increases kidney weight. Aziz & Hassan (2020) confirmed that EG-induced crystal formation contributes to increased kidney weight. The protective effect of *L. serriola* and *G. glabra* may be attributed to their flavonoid content, which exhibits anti-inflammatory (Naqvi *et al*., 2025) and nephroprotective (Abdul-Jalil, 2020) properties. In addition, El-Esawi *et al. reported that flavonoid is the main phytochemical in L. serriola, and L. serriola may act* against the EG action through its flavonoid content (El-Esawi *et al*., 2017).

Effect of EG and medicinal plants on serum creatinine and urea levels:

Serum creatinine and urea levels were elevated in EG-treated rats, indicating impaired renal function. However, co-administration of *L. serriola* and *G. glabra* normalized these values. Increased creatinine and urea levels are associated with reduced glomerular filtration due to lithiasic obstruction (Pawar & Vyawahare, 2017). Previous studies have shown that medicinal plants containing polyphenols, flavonoids, and saponins can prevent crystal formation and protect renal function (Cheraft-bahloul *et al*., 2017; Kayalvizhi *et al*., 2015). The diuretic action of *L. serriola* and the nephroprotective action of *G. glabra* likely contributed to the restoration of renal function (AbulAzeez & Hussein, 2016Chauhan *et al*., 2020;). Furthermore, Nirumand *et al*. demonstrated that EG treatment leads to an increase in kidney function measurements, but medicinal plants can normalize the kidney biochemical parameters (Nirumand *et al*., 2018).

In *G. glabra* the phytochemicals particularly phenolic compounds, flavonoids, saponins and tannins (Al-Snafi, 2018), play an essential role in anticrystallization and antioxidant activities (Namburu *et al*., 2017) and a huge number of medicinal plants with saponin constituent have antiurolithiatic activity (Patel *et al*., 2016) beacuse saponins are possessing antiurolithiatic action via its diuretic and disaggregating the suspension of mucoproteins (Gürocak & Küpeli, 2006). The major bio-active constituent of *G. glabra* root is a saponin triterpenoid (Varsha *et al*., 2013). The mechanism by which the triterpenoids render protection against oxalate-induced toxic manifestations and free radical production may involve the inhibition of CaOx crystal aggregation and enhancement of the body defense systems (Korah *et al*., 2020).

Histological changes in the kidney :

Medicinal plants have vast potential for managing and remedying many diseases like nephrotoxicity (Zangeneh *et al*., 2018). The kidneys are exposed to toxic substances that show some degree of inflammation observed in the cortex, pulp, and kidney subcomponents due to the excessive production of free radicals that are expressed as a response to cytokines (Mishra *et al*., 2014). These toxic substances cause cellular harm that frees up the cytoplasmic ingredients into circulation, thereby increasing the level of creatinine in, destroying the structural integrity of the kidneys. The use of EG resulted in kidney inflammation due to stone formation and cellular harm of necrosis and tissue response represented by hyperplasia and leakage of inflammatory cells led to kidney hyperplasia, these results were consistent with what when using experimentally induced carbon tetrachloride (CCL4) for nephrotoxicity, where necrosis, hyperplasia, kidney hyperplasia, and high serum creatinine were observed due to cellular harm (Mishra *et al*., 2014; Zangeneh *et al*., 2018).

It has been reported that some medicinal plants containing compounds such as saponin possess diuretic and subtracting effects of mucous proteins in the kidneys on acidic molds to the tubular lumen (Gürocak & Küpeli, 2006). The reason for the lack of congestion and the infiltration of inflammatory cells in the interstitial tissue as well as the erosion of kidney stones in the renal tubules and the restoration of normal integration of the kidney tissue is that the *G. glabra* plant contains some phytochemical compounds such as glycyrrhizin (saponin) which has anti-inflammatory, neovascularization action and rearrange collagen fiber and glycyrrhetinic acid, which has a direct effect on the receptors of mineralocorticoids producing effects similar to inflammation (Shah *et al*., 2018). Maybe one of the reasons for lowering one of the saponin effects. This is what has been noted in the results of the current study in terms of decreased congestion, blood vessels, and at the same time, the infiltration of inflammatory cells in the interstitial tissue.

Almost similar results were obtained when using the *L. serriola* plant, where low congestion, infiltration of inflammatory cells, and significant reduction of CaOx stones to small and very small stones were observed. The air parts extract of *L. serriola* plant is anti-snake and scorpion poison effects for each of the liver, kidney, and lung tissue, where protects and prolonged survival against the LD50 dose of *Buthus atlantis* snake poison, where *L. serriola* plant protects the rat by 66.66% when using methanol extract when concentrating 2g/kg B.W. (Bouimeja *et al*., 2019) as both plants were also shown to contain phytochemical compounds, especially flavinoids and saponins, which represent powerful antioxidants and antiurolithiatic agents and maintain the normal integrity of tissues (Patel *et al*., 2016). This is explained by the results of the current research when using *L. serriola* and *G. glabra,* where the return of kidney tissue to normal structural integration and its normal size is observed in comparison with both the EG and control groups.

CONCLUSION

The findings suggest that *L. serriola* and *G. glabra* root have protective roles in EG-induced urolithiasis. They improved body weight, food and water intake, and kidney function while reducing kidney damage. These effects are likely mediated by the phytochemical constituents of the plants, including flavonoids and saponins, which possess diuretic, antioxidant, and antiurolithiatic properties. Future studies should focus on identifying the specific mechanisms involved and potential clinical applications.

Acknowledgment :

The researchers would like to extend great thanks to the Department of Biology/ Soran University to support the work partly.

Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Salahaddin, College of Science.

Authors' Contribution Statement:

Sarbast Ahmad Mahmud and **Aveen Rustam Khdhr** had a principal role in study design, collection of plant samples, performing experiments and collection of samples of blood, urine and kidneys, then determination of measurements and urine analysis and also data statistical analysis. **Imad Taher Abdulla** had a principal role in histological preparation and studying. **Khalid Qader Gardi** participated in study design sections and manuscript writing with the other authors.

Ethical approval: All animal experiments had conducted following the approval by the **Animal research ethics committee** of the College of Science at the University of Salahaddin, reference no. 45/90, issue date 5-12-2023. All animal experiments were complied with the ARRIVE guidelines and carried out following the National Research Council's Guide for the Care and Use of Laboratory Animals.

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