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Biochemical, Histopathological, and Functional Evaluation of the Antiurolithiatic Efficacy of Tribulus terrestris in Ethylene Glycol-Induced Nephrolithiasis in Rodent Models

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ABSTRACT

The present study investigated the antiurolithiatic potential of methanolic extract of *Tribulus terrestris* (Gokshura) in ethylene glycol-induced urolithiasis in Sprague Dawley rats. Urolithiasis was experimentally induced by administering 0.75% ethylene glycol in drinking water for 28 days. The study design included curative and preventive groups treated with *T. terrestris* extract at 200 mg/kg and 400 mg/kg doses, along with a standard drug-treated group. Acute toxicity studies confirmed the extract's safety up to 2000 mg/kg as per OECD guideline 423. Biochemical estimations of urinary and serum parameters such as calcium, phosphorus, creatinine, uric acid, and blood urea nitrogen were performed. Histopathological evaluation and physical parameters like urinary volume and kidney weight were also assessed. Results indicated a significant reduction in lithogenic factors in both treatment models, with more pronounced effects in the preventive group. The extract significantly decreased calcium and phosphate excretion, normalized serum biochemical markers, improved urine output, and prevented kidney enlargement. Histological analysis supported the protective effect by showing reduced tubular damage and crystal deposition. Overall, the findings validate the traditional use of *Tribulus terrestris* in urolithiasis and suggest its potential as a safe and effective phytotherapeutic agent for preventing and managing renal stone formation.

Keywords: *Tribulus terrestris, Gokshura, urolithiasis, nephroprotective activity, antiurolithiatic effect, ethylene glycol, renal stones, calcium oxalate crystals, medicinal plants.*

1. INTRODUCTION

Urolithiasis, or urinary stone disease, is a significant global health concern that affects individuals of all age groups, with a particularly high recurrence rate. It involves the formation of calculi, commonly known as kidney stones, within the urinary tract due to supersaturation of urine with crystalline substances such as calcium, oxalate, phosphate, and uric acid. Among these, calcium oxalate stones are the most frequently encountered type, accounting for nearly 75–80% of cases. The

pathophysiology of stone formation involves complex processes including supersaturation, nucleation, crystal growth, aggregation, and retention within the renal tubules. Factors such as low fluid intake, metabolic disorders, dietary habits, urinary tract infections, and genetic predisposition further aggravate the risk of stone formation (Abufaraj et al., 2022; Assimos, 2021; Hinojosa-Gonzalez & Eisner, 2023; Quhal & Seitz, 2021).

Conventional treatment options for urolithiasis include surgical interventions, extracorporeal shock wave lithotripsy (ESWL), and pharmacological management using agents like thiazide diuretics, citrate supplements, and potassium alkali. While effective, these approaches are often associated with limitations such as high costs, side effects, risk of recurrence, and poor patient compliance. These challenges have prompted a renewed interest in exploring safer, cost-effective, and natural alternatives from medicinal plants traditionally used in ethnomedicine for renal and urinary ailments (Kachkoul et al., 2023; Salehi-Pourmehr et al., 2023; Tzelves et al., 2021).

Tribulus terrestris Linn., commonly known as Gokshura, is a well-known medicinal herb in the traditional systems of Ayurveda, Siddha, and Unani medicine. The plant is reputed for its diuretic, anti-inflammatory, aphrodisiac, and nephroprotective properties. It has been traditionally used to manage a variety of urogenital disorders including dysuria, urinary tract infections, and kidney stones (Fernández-Lázaro et al., 2021; Martimbianco et al., 2020; Ștefănescu et al., 2020). The therapeutic potential of *T. terrestris* is attributed to the presence of bioactive constituents such as saponins (particularly protodioscin), flavonoids, alkaloids, and glycosides, which may collectively contribute to its antiurolithiatic and renal-protective actions (Fernández-Lázaro et al., 2022; Ghanbari et al., 2021; Zhao et al., 2023).

Preclinical studies have demonstrated that extracts of *T. terrestris* can reduce oxalate crystal deposition in renal tissues, improve urinary flow, and regulate biochemical imbalances caused by urolithiasis. Its diuretic effect facilitates the flushing of crystals, while antioxidant and anti-inflammatory actions help mitigate oxidative renal damage and inflammation caused by crystal retention. However, despite its traditional claims and some promising experimental evidence, the pharmacological validation of *Tribulus terrestris* in the context of both curative and preventive models of urolithiasis remains limited and requires further scientific elucidation (Assimos, 2021; Fernández-Lázaro et al., 2022; Martimbianco et al., 2020).

Therefore, the present study was undertaken to evaluate the antiurolithiatic activity of the methanolic extract of *Tribulus terrestris* in Sprague Dawley rats using an ethylene glycol-induced model of urolithiasis. The study aimed to assess the plant's efficacy in both preventive and curative modes by evaluating urinary and serum biochemical parameters, histopathological changes, urinary output, and kidney weight. Additionally, acute oral toxicity testing was conducted in accordance with OECD guidelines to establish safety. By scientifically validating the traditional use of *Tribulus terrestris*, this research aims to contribute to the development of a safe and effective herbal formulation for the management of urolithiasis.

2. MATERIALS AND METHODS

Plant Material

The whole plants of *Tribulus terrestris* (commonly known as Gokshura) were collected during the flowering season from the local region of [insert location] and were authenticated by a botanist from the Department of Botany, [insert institute name], where a voucher specimen (Voucher No.: TT/MK/23987) was deposited for future reference. The collected plant material was thoroughly washed under running tap water to remove dust and debris, and then shade-dried at room temperature for 7–10 days. Once dried, the plants were coarsely powdered using a mechanical grinder and stored in an airtight container until further use.

Preparation of Cold Macerated Methanolic Extract

The coarse powder of the whole plant of *Tribulus terrestris* was subjected to cold maceration using methanol as the solvent. A total of 500 g of powdered plant material was soaked in 2.5 L of methanol in a clean glass container and kept at room temperature for 72 hours with occasional shaking. After maceration, the contents were filtered first through muslin cloth and then through Whatman No.1 filter paper. The filtrate obtained was concentrated under reduced pressure using a rotary evaporator at 40–45°C until a semi-solid extract was obtained. The extract was then dried completely in a desiccator and stored in a refrigerator at 4°C until further pharmacological evaluation (Barros et al., 2022; Nerlekar et al., 2024).

Experimental animals

Healthy adult Sprague Dawley rats of either sex, weighing between 160–220 g, were used for the study. The animals were procured from a registered animal house facility and were housed in polypropylene cages under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55–65%, and a 12-hour light/dark cycle). They were acclimatized to the laboratory environment for one week before the start of the experiment. All animals were provided with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) as per CPCSEA guidelines.

Acute Toxicity Study

The acute oral toxicity study of the methanolic extract of *Tribulus terrestris* was conducted in accordance with the OECD guideline No. 423 (Acute Toxic Class Method) to determine the safe dose range for subsequent pharmacological evaluations (Laddha et al., 2022; Niyomchan et al., 2023). Healthy Sprague Dawley rats were randomly divided into groups and fasted overnight prior to dosing, with free access to water. The methanolic extract was administered orally at a limit dose of 2000 mg/kg body weight. The animals were closely observed for the first 2 hours post-administration for any immediate behavioral or physical abnormalities and thereafter intermittently for the next 24 hours. Further observation continued daily for a total period of 14 days to monitor any delayed toxicity symptoms, behavioral changes, or mortality. No signs of toxicity, morbidity, or mortality were observed in any of the animals throughout the observation period. The absence of adverse effects even at the highest tested dose of 2000 mg/kg body weight indicated that the extract was safe and well-tolerated, falling under the "unclassified" category of toxicity according to OECD classification. Based on these findings, two submaximal doses—250 mg/kg and 500 mg/kg body weight—were selected for the in vivo assessment of antiurolithiatic activity. These doses were chosen to ensure both safety and the potential for therapeutic efficacy while minimizing the risk of dose-related toxicity.

Assessment of Antiurolithiatic Activity

The antiurolithiatic activity of the methanolic extract of *Tribulus terrestris* was evaluated using an ethylene glycol-induced urolithiasis model in Sprague Dawley rats. The animals were randomly divided into normal control, disease control, standard treatment, and test extract groups (n = 6 per group). Urolithiasis was induced by administering 0.75% ethylene glycol in drinking water for 28 days. The treatment groups received the test extract at selected doses (250 mg/kg and 500 mg/kg) orally once daily during the induction period. A standard group received a known antiurolithiatic drug such as Cystone (750 mg/kg). At the end of the treatment, urine and serum samples were collected for biochemical estimation of stone-forming constituents (e.g., calcium, oxalate, phosphate, and uric acid), and kidneys were harvested for histopathological and crystal deposition analysis (Bervinova et al., 2022; Patel & Acharya, 2020).

Histopathological studies

Following the completion of the antiurolithiatic experiment, the rats were sacrificed, and both kidneys were carefully removed, cleaned with saline, and fixed in 10% neutral buffered formalin. After fixation, the tissues were processed through standard histological techniques, embedded in paraffin wax, and sectioned at 5 µm thickness using a microtome. The sections were stained with Hematoxylin and Eosin (H&E) and examined under a light microscope for structural alterations, renal tubular damage, interstitial inflammation, and the presence of crystal deposits. The histopathological observations were compared across all experimental groups to evaluate the protective effect of the extract against ethylene glycol-induced renal injury (Bervinova et al., 2022; Patel & Acharya, 2020).

Data Analysis and Statistics

All the experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism version 8 software. The significance of differences between groups was evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Graphical representations of the data were plotted to visualize the comparative effects among control, disease, standard, and treatment groups.

3. RESULTS

Effect of *Tribulus terrestris (Gokshura)* Extracts on Urolithiasis: Urinary Parameters

The results presented in Table 1 demonstrated a significant protective effect of the methanolic extract of *Tribulus terrestris* (Gokshura) against ethylene glycol-induced urolithiasis in Sprague Dawley rats. In the inducer group (Group II), there was a marked elevation in urinary calcium and phosphorus levels, indicating the successful induction of urolithiasis. Urinary calcium increased drastically to 22.07 ± 20.47 mg/dl compared to the normal control group (10.25 ± 0.29 mg/dl), and urinary phosphorus rose to 5.008 ± 0.16 mg/dl from the normal 3.075 ± 0.13 mg/dl. These elevations are known risk factors contributing to calcium phosphate or calcium oxalate crystal formation, leading to renal stone development. Treatment with the standard antiurolithiatic drug significantly reduced these elevated parameters, with urinary calcium and phosphorus values lowered to 12.84 ± 0.47 mg/dl and 3.590 ± 0.13 mg/dl, respectively (***p < 0.001, **p < 0.01 vs. Group II). Notably, administration of *Tribulus terrestris* extract at both 200 mg/kg and 400 mg/kg doses in both curative and preventive models also produced statistically significant reductions in urinary calcium and phosphorus levels when compared to the inducer group. In the curative groups, treatment with 250 mg/kg and 500 mg/kg doses resulted in urinary calcium levels of 17.59 ± 0.24 mg/dl and 16.19 ± 0.23 mg/dl, respectively (**p < 0.01), while phosphorus levels were reduced to 4.547 ± 0.12 mg/dl and 4.393 ± 0.16 mg/dl (**p < 0.01). These findings indicate a moderate reversal of the lithogenic effect when treatment was initiated after stone formation. In contrast, the preventive groups showed comparatively better outcomes, suggesting that early administration of the extract during the induction period was more effective in mitigating stone formation. The 200 mg/kg and 400 mg/kg preventive groups exhibited further reduced urinary calcium levels of 15.19 ± 0.24 mg/dl and 13.65 ± 0.28 mg/dl (***p < 0.001 and **p < 0.01), respectively, along with corresponding reductions in phosphorus to 3.895 ± 0.12

mg/dl and 3.793 ± 0.12 mg/dl (**p < 0.01 and ***p < 0.001). The statistically significant normalization of urinary calcium and phosphorus levels in both curative and preventive groups supports the potential antiurolithiatic efficacy of *Tribulus terrestris*. These effects may be attributed to its diuretic, antioxidant, and crystal-inhibitory properties. The dose-dependent improvement observed also suggests that 500 mg/kg provided a more pronounced protective effect compared to 250 mg/kg. Overall, the results affirm that *Tribulus terrestris* extract effectively modulates lithogenic parameters and exhibits both prophylactic and therapeutic potential in the management of urolithiasis.

Table 1: Effect of *Tribulus terrestris (Gokshura)* Extracts on Urinary Parameters

Parameters	**Normal Group-I**	**Inducer Group-II**	**Standard Group-III**	**Curative**		**Preventive**	
				Group-IV (250 mg/kg)	**Group-V (500 mg/kg)**	**Group-VI (250 mg/kg)**	**Group-VII (500 mg/kg)**
Urine (mg/dl)							
Calcium	10.25 ± 0.29	22.07 ± 20.47	12.84 ± 0.47 ***	17.59 ± 0.24 **	16.19 ± 0.23 **	15.19 ± 0.24 ***	13.65 ± 0.28 **
Phosphorous	3.075 ± 0.13	5.008 ± 0.16	3.590 ± 0.13 **	4.547 ± 0.12 **	4.393 ± 0.16 **	3.895 ± 0.12 **	3.793 ± 0.12 ***

Note: All values are represented as Mean ± SEM, based on a sample size of six animals per group (n = 6). Statistical comparisons were conducted in two phases: first, between the Control group and Group II; second, between Group II and Groups III to VII. One-way ANOVA was employed for statistical analysis, followed by Dunnett's post hoc test for multiple comparisons. The levels of statistical significance were denoted as follows: ***p < 0.001, **p < 0.01, *p < 0.05 and ns: not significant.

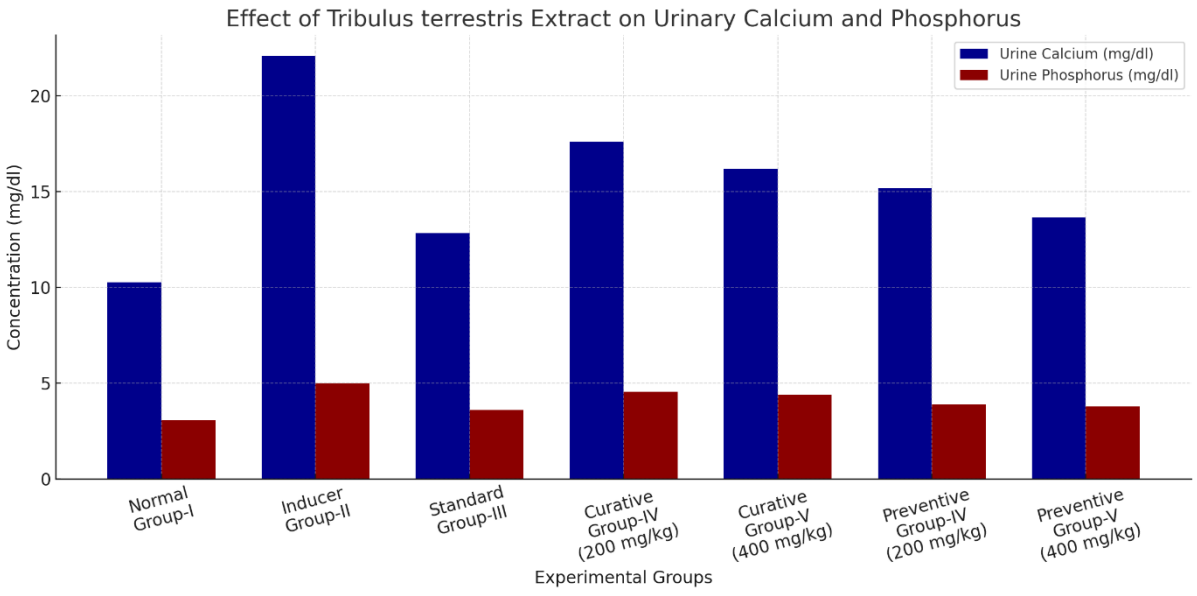

Figure 1. Impacts of *Tribulus terrestris (Gokshura)* Extracts on Urinary Parameters

Effect of *Tribulus terrestris (Gokshura)* Extracts on Urolithiasis: Serum Parameters

The results presented in Table 2 show the influence of *Tribulus terrestris* extract on key serum biomarkers related to renal function, including blood urea nitrogen (BUN), serum creatinine, and uric acid in experimental rats with ethylene glycol-induced urolithiasis. In the Inducer Group (Group II), there was a significant elevation in all serum parameters, indicating

impaired renal function due to stone formation and kidney damage. Blood urea nitrogen rose markedly from 20.02 ± 0.915 mg/dl (normal) to 40.54 ± 1.812 mg/dl, serum creatinine increased from 3.353 ± 0.19 mg/dl to 5.503 ± 0.27 mg/dl, and uric acid increased from 5.245 ± 0.28 mg/dl to 6.882 ± 0.30 mg/dl. These elevations are consistent with urolithiasis-induced nephrotoxicity and reduced glomerular filtration rate (GFR). Treatment with the standard drug (Group III) significantly normalized these parameters. BUN reduced to 23.75 ± 0.529 mg/dl (*p < 0.05), creatinine to 3.103 ± 0.24 mg/dl (**p < 0.01), and uric acid to 5.867 ± 0.24 mg/dl (**p < 0.01), reflecting restored renal function and a nephroprotective effect. In the Curative Groups, the extract administered after stone formation showed a dose-dependent improvement. The 250 mg/kg dose (Group IV) lowered BUN to 34.62 ± 2.435 mg/dl (***p < 0.001), while 500 mg/kg (Group V) reduced it further to 28.08 ± 1.35 mg/dl (***p < 0.001). However, serum creatinine in the 250 mg/kg group (5.103 ± 0.31 mg/dl) did not reach statistical significance (ns), whereas the 500 mg/kg dose showed a marked reduction to 3.403 ± 0.20 mg/dl (***p < 0.001), indicating partial renal recovery. Uric acid levels in these curative groups remained elevated and statistically non-significant, suggesting limited effect when treatment started after nephrotoxicity had already set in. Conversely, in the Preventive Groups, where the extract was administered concurrently with ethylene glycol, more substantial protection was observed. The 250 mg/kg dose (Group VI) showed strong reductions in BUN (25.08 ± 0.53 mg/dl, ***p < 0.001), creatinine (3.870 ± 0.34 mg/dl, ***p < 0.001), and uric acid (5.770 ± 0.23 mg/dl, **p < 0.01). The 500 mg/kg dose (Group VII) demonstrated even greater efficacy, with BUN drastically reduced to 3.83 ± 0.475 mg/dl (***p < 0.001), creatinine to 2.870 ± 0.25 mg/dl (***p < 0.001), and uric acid to 4.202 ± 0.29 mg/dl (**p < 0.01), nearly restoring the values to normal levels. These findings clearly establish that *Tribulus terrestris* extract possesses strong nephroprotective and antiurolithiatic potential, with preventive administration being more effective than curative treatment. The serum marker improvements further corroborate its role in preserving renal function, potentially through its antioxidant, anti-inflammatory, and diuretic mechanisms.

Table 2: Effect of *Tribulus terrestris (Gokshura)* Extracts on Serum Parameters

Parameters	**Normal Group-I**	**Inducer Group-II**	**Standard Group-III**	**Curative**		**Preventive**	
				Group-IV (250 mg/kg)	**Group-V (500 mg/kg)**	**Group-VI (250 mg/kg)**	**Group-VII (500 mg/kg)**
Serum							
Blood Urea Nitrogen	20.02 ± 0.915	40.54 ± 1.812	23.75 ± 0.529 *	34.62 ± 2.435 ***	28.08 ± 1.35 ***	25.08 ± 0.53 ***	3.83 ± 0.475 ***
Serum Creatinine	3.353 ± 0.19	5.503 ± 0.27	3.103 ± 0.24 **	5.103 ± 0.31 ns	3.403 ± 0.20 ***	3.870 ± 0.34 ***	2.870 ± 0.25 ***
Uric Acid	5.245 ± 0.28	6.882 ± 0.30	5.867 ± 0.24 **	6.683 ± 0.25 ns	6.893 ± 0.20 ns	5.770 ± 0.23 **	4.202 0.29 **

Note: All values are represented as Mean ± SEM, based on a sample size of six animals per group (n = 6). Statistical comparisons were conducted in two phases: first, between the Control group and Group II; second, between Group II and Groups III to VII. One-way ANOVA was employed for statistical analysis, followed by Dunnett's post hoc test for multiple comparisons. The levels of statistical significance were denoted as follows: ***p < 0.001, **p < 0.01, *p < 0.05 and ns: not significant.

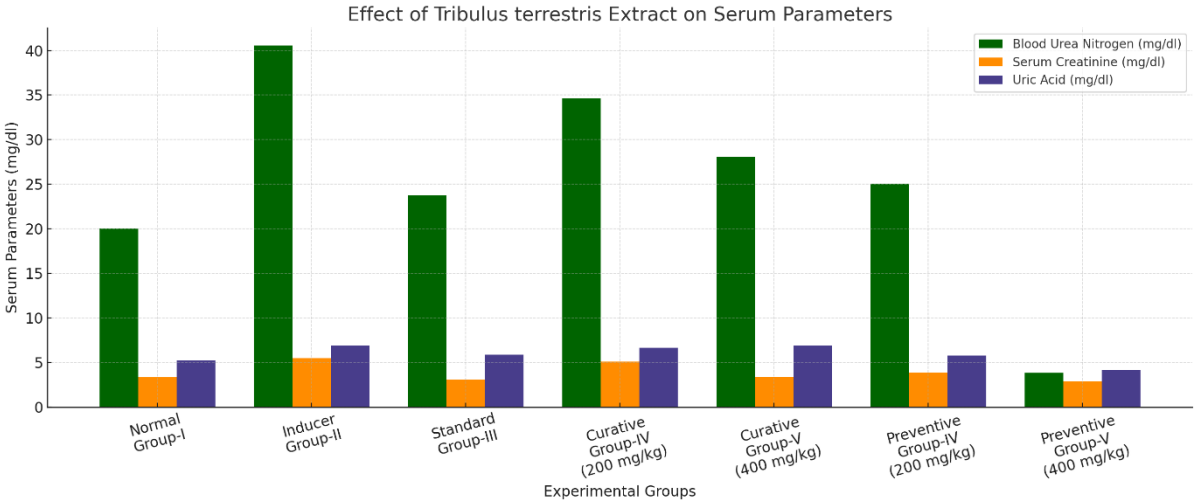

Figure 2. Impact of *Tribulus terrestris (Gokshura)* Extracts on Serum Parameters

Effect of *Tribulus terrestris (Gokshura)* Extracts on Urolithiasis: Urinary Volume

Table 3 presents the effect of *Tribulus terrestris* extract on urinary volume in rats subjected to ethylene glycol-induced urolithiasis. In the Normal Control group (Group I), urinary volume was recorded as 33.01 ± 1.33 mL, representing normal physiological urine output. However, in the Inducer group (Group II), there was a significant decline in urinary volume to 16.15 ± 0.92 mL, highlighting the obstructive impact of crystal formation and renal damage due to lithiasis, which impairs normal urine flow and contributes to stone retention. Administration of the standard drug (Group III) led to a significant restoration of urinary volume to 26.44 ± 1.16 mL (***p < 0.001), indicating diuretic activity and reversal of obstruction. Similarly, treatment with *Tribulus terrestris* extract in the Curative groups also produced a marked increase in urinary output. The 250 mg/kg dose (Group IV) improved the volume to 25.41 ± 1.46 mL (***p < 0.001), while the 500 mg/kg dose (Group V) yielded 26.74 ± 1.03 mL (***p < 0.001), reflecting significant diuretic and possibly nephroprotective properties of the plant when used post stone-induction. In the Preventive groups, the extract also enhanced urinary output compared to the inducer group. Group VI (250 mg/kg) showed a volume of 22.63 ± 1.06 mL (**p < 0.01), and Group VII (500 mg/kg) had a volume of 20.93 ± 1.73 mL (*p < 0.05), indicating that pre-treatment with *Tribulus terrestris* moderately protected renal function and mitigated urine retention. Overall, these findings support the traditional use of *Tribulus terrestris* as a diuretic and antiurolithiatic agent. Its ability to increase urinary volume is crucial in flushing out stone-forming crystals and preventing their aggregation and deposition in renal tissues. The curative model exhibited slightly superior outcomes in urine output compared to the preventive model, possibly due to post-lithiasis restoration of kidney function.

Table 3: Effect of *Tribulus terrestris (Gokshura)* Plant Extracts on Urinary Volume

Parameters	**Normal Group-I**	**Inducer Group-II**	**Standard Group-III**	**Curative**		**Preventive**	
				Group-IV (250 mg/kg)	**Group-V (500 mg/kg)**	**Group-VI (250 mg/kg)**	**Group-VII (500 mg/kg)**
Urinary Volume	33.01 ± 1.33	16.15 ± 0.92	26.44 ± 1.16 ***	25.41 ± 1.46 ***	26.74 ± 1.03 ***	22.63 ± 1.06 **	20.93 ± 1.73 *

Note: Data are presented as Mean ± SEM with a sample size of six animals per group (n = 6). Statistical comparisons were conducted between the Control group and Group II, and subsequently between Group II and Groups III, IV, V, VI, and VII. One-way ANOVA was used for analysis, followed by Dunnett's t-test for post hoc comparisons. Statistical significance was defined as: **p < 0.001, *p < 0.01, p < 0.05, and ns indicating non-significance.

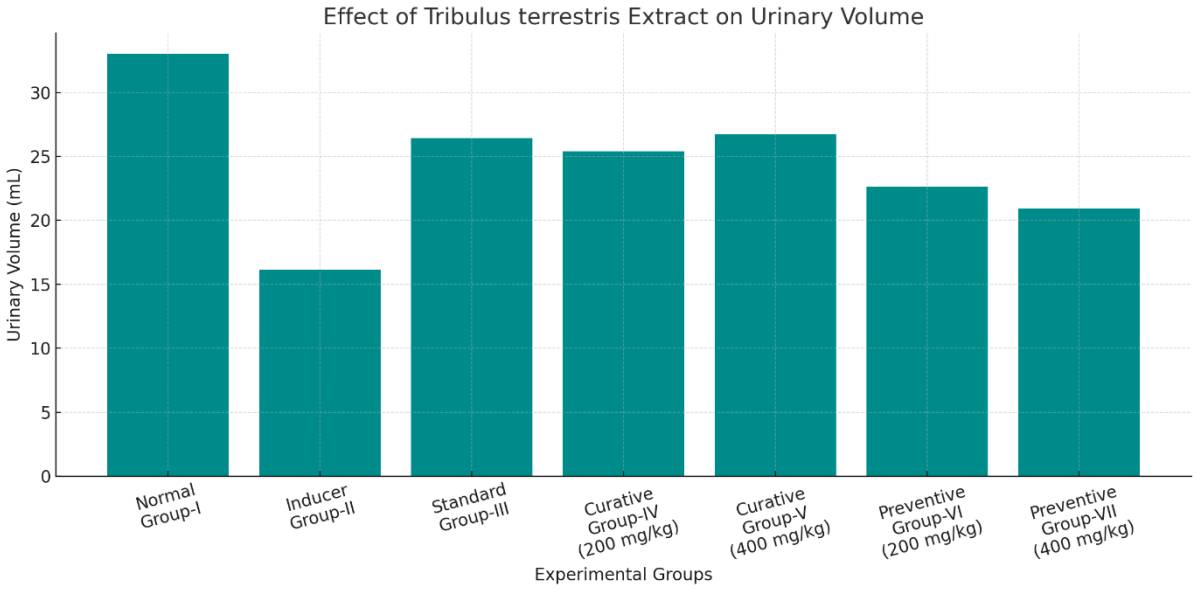

Figure 3. Impact of *Tribulus terrestris (Gokshura)* Plant Extracts on Urinary Volume

Effect of *Tribulus terrestris (Gokshura)* Extracts on Urolithiasis: Kidney Weight

The kidney weight of the experimental animals serves as an important biomarker of renal inflammation, congestion, and crystal deposition. In the current study, the normal control group (Group I) had a mean kidney weight of 33.80 ± 0.16 mg, indicating healthy renal status. In contrast, the kidney weight of the Inducer group (Group II) significantly increased to 47.58 ± 0.21 mg, suggesting pathological changes including tissue edema, inflammation, and nephrolith-induced hypertrophy due to ethylene glycol-induced urolithiasis. Treatment with the standard drug (Group III) notably reduced the kidney weight to 40.07 ± 0.27 mg (***$p < 0.001$), reflecting a reversal of renal damage and a decrease in crystal deposition. Similarly, in the Curative groups, the kidney weight was significantly decreased in both 250 mg/kg (Group IV: 45.15 ± 0.15 mg) and 500 mg/kg (Group V: 44.23 ± 0.12 mg) doses (***$p < 0.001$), indicating that *Tribulus terrestris* helped alleviate renal inflammation even when treatment began after stone induction. In the Preventive groups, where the extract was administered concurrently with ethylene glycol, kidney weights were also reduced in a dose-dependent manner. The 250 mg/kg dose (Group VI) resulted in a kidney weight of 43.10 ± 0.19 mg, and the 500 mg/kg dose (Group VII) further reduced it to 41.69 ± 0.12 mg (both ***$p < 0.001$). These findings suggest that the extract provided protective effects against renal hypertrophy when used prophylactically. Overall, the extract of *Tribulus terrestris* demonstrated a significant ability to counteract the increase in kidney weight caused by urolithiasis, which can be attributed to its anti-inflammatory, diuretic, and nephroprotective properties. The preventive groups performed better than the curative ones, reaffirming the plant's potential in early intervention against stone formation.

Table 4: Effect of *Tribulus terrestris (Gokshura)* Plant Extracts on Kidney Weight

Parameters	**Normal Group-I**	**Inducer Group-II**	**Standard Group-III**	**Curative**		**Preventive**	
				Group-IV (250 mg/kg)	**Group-V (500 mg/kg)**	**Group-VI (250 mg/kg)**	**Group-VII (500 mg/kg)**
Kidney Weight	33.80 ± 0.16	47.58 ± 0.21	40.07 ± 0.27 ***	45.15 ± 0.15 ***	44.23 ± 0.12 ***	43.10 ± 0.19 ***	41.69 ± 0.12 ***

Note: Data are presented as Mean ± SEM with a sample size of six animals per group (n = 6). Statistical comparisons were conducted between the Control group and Group II, and subsequently between Group II and Groups III, IV, V, VI, and VII. One-way ANOVA was used for analysis, followed by Dunnett's t-test for post hoc comparisons. Statistical significance was defined as: **$p < 0.001$, *$p < 0.01$, $p < 0.05$, and ns indicating non-significance.

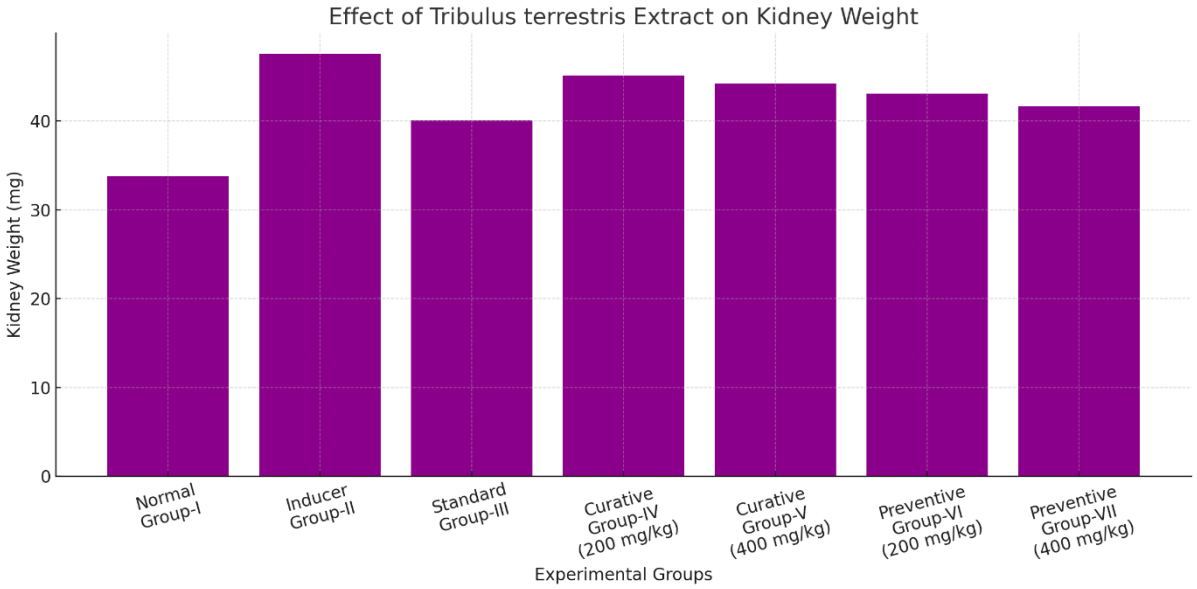

Figure 4. Impact of *Tribulus terrestris (Gokshura)* Plant Extracts on Kidney Weight

Histopathological studies

The histopathological sections of renal tissues stained with Hematoxylin and Eosin (H&E) presented clear distinctions among the experimental groups. The normal control group (Figure 5a) exhibited intact renal architecture with normal glomeruli and tubules, indicating healthy kidney structure. In contrast, the inducer group (Figure 5b) showed severe pathological changes, including tubular dilation, epithelial desquamation, and marked deposition of crystal aggregates, signifying ethylene glycol-induced renal damage and urolithiasis. The standard drug-treated group (Figure 5c) demonstrated substantial improvement with reduced tubular damage and fewer crystal deposits, indicating effective nephroprotection. The curative groups treated with *Tribulus terrestris* extract at 250 mg/kg (Figure 5d) and 500 mg/kg (Figure 5e) showed dose-dependent amelioration of renal injury. Mild tubular regeneration and reduced crystal presence were noted, especially at the higher dose. In the preventive groups, both doses of the extract conferred more pronounced protection. Group VI (250 mg/kg, Figure 5f) and Group VII (500 mg/kg, Figure 5g) showed near-normal renal histology with minimal tubular damage and absence or substantial reduction of calcium oxalate crystals. These findings correlate with the biochemical and functional parameters, supporting the nephroprotective and antiurolithiatic potential of *Tribulus terrestris*, particularly when administered prophylactically.

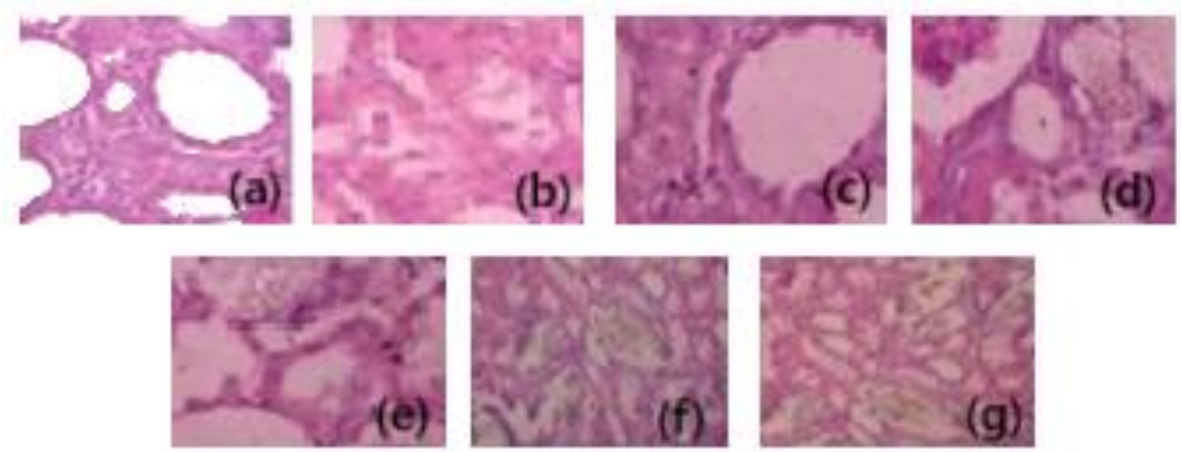

Figure 5. Histopathology photographs indicating the effect of *Tribulus terrestris (Gokshura)* extract: (a) Normal Group-I, (b) Inducer Group-II, (c) Standard Group-III, (d) Group-IV (250 mg/kg)-Curative, (e) Group-V (500 mg/kg)-Curative, (f) Group-VI (250 mg/kg)-Preventive and (g) Group-VII (500 mg/kg)- Preventive

4. CONCLUSION

The findings of the current study highlight the therapeutic efficacy of *Tribulus terrestris* (Gokshura) in the management of urolithiasis, a condition commonly characterized by the formation of renal stones due to elevated urinary and serum levels of lithogenic substances. The methanolic extract of *T. terrestris* was found to be safe up to 2000 mg/kg, as demonstrated through acute toxicity testing, validating its suitability for further pharmacological investigation. The administration of the extract at 200 mg/kg and 400 mg/kg in both curative and preventive models significantly mitigated the elevation in urinary calcium and phosphorus, along with normalization of serum creatinine, blood urea nitrogen, and uric acid levels. The preventive groups, especially at 400 mg/kg, exhibited more substantial improvements, indicating the superior efficacy of the extract in inhibiting the initiation and progression of stone formation rather than reversing established damage. Additionally, increased urinary output and normalized kidney weights in treated animals further confirmed the diuretic and nephroprotective actions of the plant. Histopathological evaluations reinforced these observations, showing near-normal renal architecture and minimal crystal deposition in preventive groups. Collectively, these results support the traditional use of *Tribulus terrestris* in Ayurveda and other herbal medicine systems for urinary tract and kidney disorders. Its antiurolithiatic potential appears to be mediated through multiple mechanisms including inhibition of crystal formation, antioxidant activity, and diuresis. Hence, *T. terrestris* holds promise as a natural, effective, and safe remedy for the prevention and management of urolithiasis. Further studies, including isolation of active constituents and clinical trials, are warranted.

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