# The Effect of *Tribulus Terrestris* Fruits and *Urtica dioica* Leaves Extracts on Renal Calculus Induced by Sodium Oxalate in Experimental Rats

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## ABSTRACT:

*A common urological disorder is kidney stone formation. Previous research has shown that long-term exposure to oxalate damages renal epithelial cells and stress caused by oxidation. The current investigation evaluated the effect of aqueous extracts of Tribulus Terrestris fruits, and Urtica dioica leaves against renal calculus induced by sodium oxalate in male albino rats. The injection with sodium oxalate (70 mg/kg) intraperitoneally for ten days to cause renal calculi increased serum creatinine, urea, uric acid, and serum electrolytes, urinary electrolytes, urinary components, serum tumor necrosis factor-alpha, as well as malondialdehyde in kidney tissue. A decrease in creatinine clearance and antioxidant enzyme activity (superoxide dismutase and catalase) was observed. Treated groups with Tribulus Terrestris and Urtica dioica aqueous extracts (250 and 500 mg / Kg body weight), respectively, for 28 days improved body weight gain (BWG), Feed intake (FI), feed efficiency ratio (FER), relative kidney weight, kidney functions, serum electrolytes, urinary electrolytes, urinary components, serum tumor necrosis factor-alpha, antioxidant enzymes activity. Histopathological examination confirmed the biochemical analysis. It was concluded that Tribulus Terrestris and Urtica dioica aqueous extracts affect kidney stone modeling caused by sodium oxalate by reducing tissue degeneration and the number of stones because they possess antioxidants.*

***Keywords:*** *Tribulus Terrestris; Urtica dioica; Sodium Oxalate; kidney functions; antioxidant enzymes.*

## INTRODUCTION

Because the chronic renal disease is now considered a risk factor, nephrolithiasis is no longer an isolated disorder. Kidney stones are a common disorder, and numerous studies have demonstrated that this ratio has remained constant over the past twenty years. Kidney stones are linked to metabolic syndrome, hypertension, coronary artery disease, and decreased bone mineral density, making them markers of metabolic imbalance that can predict various comorbidities **(Beara-Lasic and Goldfarb, 2019).**

Urolithiasis has common names such as renal calculus/ calculi or kidney stone **(Jagtap *et al*., 2023).** Urolithiasis is the occurrence of calculus anywhere in the renal system, including the kidneys, bladder, and ureter. Most renal calculi are composed of calcium phosphate and oxalate crystals **(Alomair *et al*.,2023).** Most infections and problems with the prostate can lead to kidney stones (KSs), the most common urinary tract disease. KSs comprise organic and inorganic crystals and proteins **(Winoker *et al*., 20 19).** Approximately 80% of kidney stones are calcium oxalate (Ca Ox) and calcium phosphate. Most kidney stones are calcium stones **(Evan, 2010)**. Lifestyle, race, genetic background (heritability 45-60%), gender, and diet are all connected with the development of KS. Diabetes, obesity, inactivity, gout, hyper-parathyroidism, hyperoxaluria, elevated calcium, and changes in urine pH significantly influence the outcome of KS **(Coe *et al*., 2016; Yoshioka *et al* ., 2010).**

People with KSs have severe colic pain. The obstruction caused by these stones reduces urine output and sometimes causes blood in the urine, kidney damage, kidney failure, and urinary tract infections **(Nimavat *et al* ., 2022).**

Disodium oxalate (sodium oxalate) is the disodium salt of oxalic acid. Despite its severe toxicity, it is operated in various industrial settings, including metal cleaning, leather tanning, electroplating baths, etc. **(Parekh *et al*., 2008).** Increased oxalate amount in the urine is linked to biochemical pathways of sodium oxalate-induced lithiasis. When sodium oxalate is administered intraperitoneally to rats, hyperoxaluria develops. Due to its

low solubility, this results in oxalate precipitation as calcium oxalate in urine. Experimental animals' renal tubules develop calcium oxalate crystal aggregation due to the damage that a large amount of calcium oxalate and oxalate levels, particularly inside the nephron, cause to epithelial sections **(Pawar and Vyawahare, 2017).** Induction of calculi with disodium oxalate intake causes hyperoxaluria. Kidney stones develop more frequently as a result of chronic hypercalcemia. Furthermore, Multiple organs may accumulate calcium oxalate due to hyperoxaluria**(Golla *et al*., 2023).**

*Tribulus Terrestris* (*TT*), also known as puncture vine, goat head, and devil's thorn, is an annual plant. The *Tribulus* genus is part of the *Zygophyllaceae* family. It can be found in the subtropical desert regions and the Mediterranean. Because of its bioactive components, such as alkaloids, tannins, saponins, vitamins, glutamic acid, and aspartic acid, Numerous diseases have been treated with *TT*. It is used to treat gonorrheal rheumatism with cystitis, gleet, and gout, and its ash is beneficial for external application in rheumatic arthritis **(Al-Harrasi *et al*., 2023).** The aqueous extract of *TT* treatment kidney stones and fruit hinders the nucleation and growth of calcium oxalate crystals. Following administration of *TT* extract, the amount of oxalate, citrate, glycosaminoglycan, and proteins in renal stone patients was reduced, indicating the benefits of *TT* extract for the management of kidney stones in animal experiments; it prevents the formation of stones, aids in the reversal of premature urolithiasis, and prevents calcium oxalate induced renal injury **(Abbas *et al*., 2022).**

The Urticaceae family's stinging nettle (*Urtica dioica L*.) (UD)grows wild in North Africa, North America, Europe, and Asia **(Devkota *et al*.,2022).** Its derivatives, such as crude dried powder, infusion (herbal tea), dry extract, decoction, or fresh juice, are widely used in phytotherapy. Nettle contains a variety of biochemicals, including formic acid, histamine, and acetylcholine, as well as valuable compounds such as flavonoids, tannins, phytosterols, saponins, proteins, and amino acids. Nettle contains flavonoids such as flavonols,

flavanones, and flavonoid glycosides. Thiamine, riboflavin, pyridoxine, folic acid, nicotinic acid, and ascorbic acid are all found in nettle water and alcoholic extracts **(Raimova *et al*., 2021).** Several compounds have been isolated from the nettle herb, including quercetin, trans-ferulic acid, beta-sitosterol, erucic acid, dotriacontane, ursolic acid, scopoletin, and rutin **(Tarasevičienė *et al*., 2023).** Nettle has been used since antiquity and was one of Dioscorides', "the father of pharmacognosy's" favorite domestic plants. According to various studies, *UD* is a plant with numerous therapeutic potential effects in multiple disorders, including prostatic hyperplasia, rheumatoid arthritis, allergies, anemia, internal bleeding, kidney stones, and burns. It also has anti-proliferative and antimicrobial properties and has been shown to cure infectious diseases **(Samakar *et al*., 2022).** Therefore, the current investigation's objective was to evaluate the effectiveness of *TT* and *UD* as prophylactic agents against experimentally-induced nephrolithiasis in rats.

## MATERIALS AND METHODS

### *Plant and Chemicals*

The Agricultural Seeds, Herbs, and Medicinal Plants Company in Cairo, Egypt provided the Tribulus Terrestris fruits and Urtica dioica leaves used in this study. From the native market, starch and corn oil were bought. Dextrin, L-cysteine, Casein, minerals, vitamins, and cellulose were purchased from the Cairo Corporation for Chemical Trade in Cairo, Egypt. Sigma Chemical Company provided the sodium oxalate that was purchased.

### *Preparation of plant extract*

According to the procedure employed by **Kamboj *et al*., (2020).** the plant's fruit was weighed and immersed in two-fold distilled water (5% w/v) overnight at 4°C. After filtering through a muslin cloth, the extract will be centrifuged at 3,000 rpm for 10 minutes at 4$^{o}$C. The resulting supernatant was known as an aqueous extract. *UD* was made by washing the leaves and letting them dry in the shade for fifteen days. After dehydrating the leaves, they were ground into a fine powder (1500 g) and mixed with distilled boiled water (2.5 L) in a glass flask for fifteen minutes. The

combination was then left to cold. It was twice done. The mixture was poured through filter paper. The liquid that came out was collected, put in a rotary evaporator, and evaporated at a constant temperature of 45ºC while the pressure was lowered. The extract was then frozen and dried at –55ºC. The aqueous extract dried (86 g) was reserved at 2-8ºC **(Eise *et al.*, 2022).**

### *Phenolic compounds of plants*

HPLC was used to separate and identify phenolic and flavonoid compounds in plant extract polyphenolic compounds **(Kim *et al*., 2006).**

### *Experimental design*

Thirty-six adult male *Sprague Dawley* rats weighing approximately 150 ±10g were obtained from the Laboratory Animal Colony, Helwan, Cairo - Egypt, and were housed in well-aerated cages under hygienic conditions, fed on a basal diet *ad libitum*, and had free access to water, according to **Reeves *et al.*, (1993).** For one week, the animals were acclimatized. After this week, the rats were divided into two groups: the first (6 rats) was fed a basal diet and served as a negative control group. The second group (30 rats) was injected intraperitoneally with sodium oxalate (70 mg.kg-1) for ten days to induce renal calculi **(Shehzad *et al.*, 2020)**. The second group was divided into five subgroups: Group (1) was only fed a basal diet (and served as positive control). Groups 2 and 3 were fed a basal diet plus oral *TT* (250 and 500 mg. Kg-1 body weight) for 28 days **(Kaushik *et al.*, 2019).** According to **Keleş *et al.*, (2020),** group (4,5) was fed a basal diet plus oral *UD* (250 and 500 mg Kg-1 body weight).

After 28 days, the creatinine clearance was calculated by placing the rats in metabolic cages for 24 hours and recording the total urine produced. Before being euthanized by exsanguination, the animals were weighed and fasted overnight. According to **Chapman *et al.*, (1959),** relative kidney weights were calculated. Blood was drawn from each rat's hepatic portal vein and placed in dry-clean centrifuge tubes. Serum was carefully separated from blood samples by centrifugation at 4000 rpm for 10 minutes at room temperature, transferred into dry clean Eppendorf tubes, and frozen at - 20

C for later determinations. All rats' kidneys were carefully separated, washed with 0.9 percent saline solution, dried with filter paper, and individually weighted. The right kidney was frozen at (-20°C) to prepare tissues homogenate for determining antioxidant activities. The left kidney was fixed in a neutral buffering formaldehyde solution with a pH of 7.5 for histopathological examination.

***Biological evaluation***

During the experiment, feed intake, body weight gain, relative organ weight, and feed efficiency ratio were estimated according to **Chapman *et al*., (1959).**

***Biochemical analysis***

Serum samples were used to determine creatinine, urea nitrogen, and Uric acid according to **(Bartels *et al.*, 1972; Patton and Crouch, 1977; Fossati *et al.*, 1980)** respectively. Urinary calcium, potassium, oxalate, sodium, magnesium, and phosphate were determined according to **Marshall and Robertson, (1976).** A urine dipstick test was performed along with 24-hour urine, and samples were determined according to **kyle, (1990).** Creatinine clearance (Cr Cl) was calculated from S Cr and U Cr levels and 24-hour urinary volumes. The following equation: Cr Cl = [ (U cr) mg/dL x (V) L/day] ÷ S Cr mg/dL according to **Inker *et al* ., (2019).** The pH of urine using a pH meter and the total volume using a measuring cylinder were determined according to **Cheng *et al*., (1998).** A complete urine examination was carried out to screen the presence of RPCs, according to **Evans, (1989).** Pus cells **(Ormond, 1948),** and specific gravity in urine **(Burkhardt *et al.*,1982*)*.** Electrolytes in serum, such as sodium**,** potassium, and calcium, were also measured by **Frazer *et al*., (1972).** Inflammatory bio-marker serum tumor necrosis factor-α TNF-α was measured **(Luo *et al*., 2005).** Antioxidant indications of kidney tissue were determined, such as Superoxide dismutase (SOD) (**Nishikimi *et al*., 1972).** Catalase (CAT) was measured by the method of **Sinha, (1972).** Malondialdehyde (MDA) was assessed by the colorimetric assay as maintained by **Buege and Aust, (1978),** and nitric oxide (NO) was formed according to **Cortas and Wakid, (1990 ).**

### *Histopathological examination*

The kidney was dissected, fixed in 10% neutral buffering formaldehyde solution with a pH of 7.5, cleaned in xylol, and finally fixed in paraffin. A piece 4-5 m thick was cut and stained for histological examination using hematoxylin and eosin (H&E) **(Drury and Wallington, 1980).**

### *Statistical analysis*

To determine the difference between means at $P < 0.05$, a one-way analysis of variance (ANOVA) was used in SPSS software (18), followed by the Duncan test. The data were presented as a mean ± standard deviation (SD) **(Snedecor, 1969).**

## RESULTS

*TT* was examined for its phenolic compounds. Catechin, Syringic, Gentisic, and Protocatechuic content were higher in *TT*, as shown in Table 1. while documenting a lower range of Vanillic and Cinnamic. On the other hand, *UD* was analyzed for its phenolic Compounds. *UD* recorded higher content of Catechin, p-hydroxybenzoic, and Rosmarinic while recording lower content of Vanillic and Gallic shown **in Table 1.**

**Table 2** displays that feed intake (FI), body weight gain percentage (BWG), and feed efficiency ratio (FER) have all decreased significantly in (G2) compared to (G1). The other treated groups significantly increased compared to the positive control group. The best results were obtained in TT and UD groups (500 mg/kg BW). In contrast, the positive control group's relative kidney weight increased compared to the negative control group. It was, however, significantly lower in all treated groups. TT (500 mg/kg BW) produced the best results.

**Table 3** indicates that the positive control group's mean serum creatinine, urea, and uric acid levels were significantly increased than the (G1). Compared to (G2), all indicators in the remedy categories decreased significantly at ($P<0.05$). The best urea and uric acid findings were found in *TT* and *UD* (500 mg /kg BW). At the same time, the preferable serum creatinine findings were established in *TT* (500 mg/kg BW).

**Table 4** shows that serum $Na^{+}$, $K^{+}$, and $Ca^{++}$ levels were higher in the unhealthy group (G2)

than in )G1). It was significantly lower in all treatment groups compared to the untreated group. TT and UD (500 mg /kg BW) were the most effective outcomes in Serum $Na^{+}$. In comparison, preferable findings in serum $K^{+}$ and $Ca^{++}$ were found in *TT*(250 and 500 mg /kg BW) and *UD* (500 mg /kg BW).

According to the data in **Tables (5 and 6),** the (+ve) control group's mean values of the urine electrolytes $Ca^{++}$, O-, $Na^{+}$, $ph^{-}$ and $K^{+}$ increased significantly when compared to the (G1) control group. Groups treated with extracts recorded a significant decline in all parameters compared with the control (G2). Rats receiving *TT* (500 mg /kg BW) recorded the best result, while urine $Mg^{++}$ in the positive control group was significantly decreased compared to the normal rat group. Highest level of urine $Mg^{++}$ was recorded by a treated group with *TT* (500 mg /kg BW).

**Table (7)** demonstrate that urine creatinine, urine volume, and creatinine clearance mean in the ( G2) significantly decayed compared with the (G1). However, all other examined groups recorded a significant increase compared to (G2). The maximum urine creatinine and volume levels were noticed in the treated group with *TT* (500 mg /kg BW). In comparison, the maximum C.clearance was-recorded in groups *TT* and *UD* (500 mg /kg BW).

According to **Table 8**, the positive control group had higher urine (PH, Specific gravity, RBCs, and Pus cells) than (G1). However, compared to (G2), it was significantly lower in some treated groups. The best results in specific gravity, PH, and Pus cells were recorded in *TT* (250 and 500 mg/kg BW), *UD* (500 mg/kg BW), and the best results in RBcs were recorded in *TT* and *UD* (500 mg/kg BW).

**Table 9** reveals that the mean values of tumor necrosis factor–TNF- α were significantly more significant in (G2) than in (G1). In contrast, their values were significantly lower in the other groups. *TT (*500 mg /kg BW) showed promising results for TNF-α.

**Table 10** illustrates the activities of kidney catalase (CAT), and superoxide dismutase (SOD) significantly declined in (G2)compared with (G1). At the same time, they rose in other

groups compared with (G2). The best finding in SOD was noticed in *TT* (500 mg /kg BW), while the best findings in CAT were seen in groups treated with *TT* and *UD* (500 mg /kg BW). **Table 10** also detects that the mean value of MDA and NO were significantly higher in the positive control group than in the negative control group; however, their values were significantly lower in other groups than in (G2). The best findings were recorded in *TT* and *UD* (500 mg /kg BW).

***Histopathological examination of kidney tissue.***

Microscopically, the kidney of rats from the group (1) Control (–ve) shows normal control group (A) stained renal sections showing normal tubules, glomeruli, and interstitial tissue. In contrast, renal cells from the sodium oxalate group (2) control +ve group (B) – show prominent tubular hydropic degeneration (black arrows) and coagulative necrosis (blue arrow), congested in intratubular capillaries (red arrows) and congested glomerular tuft (red arrowheads), few perivascular mononuclear cells infiltration (black arrowheads). However, renal sections of group (3) ( *TT* 250 mg / Kg BW) (C) show moderate diffuse tubular hydropic degeneration (black arrows). Moreover, renal sections from group (4) ( *TT* 500 mg / Kg BW) (D) showed it retained a typical histological picture of tubules and glomeruli. Meanwhile, stained renal sections from group (5) (*UD* 250 mg / Kg BW) (E) show decreased tubular hydropic degeneration (black arrows) and few dilated tubules with mild cast formation (arrowhead). Renal sections from group (6) ( *UD* 500 mg / Kg BW )(F) show mild tubular dilation (black arrows) with dilated Bowman's capsule (red arrowhead).

***Histopathological examination of the urine sample***

Microscopically, the urine of rats from group (1) (G1) shows normal control group (A) normal urine without crystals; in contrast, a urine sample from the sodium oxalate group (2) (G2) (B) shows Ca Ox crystal nucleation and aggregation for the increase in concentration and stone formation. However, the urine sample of group (3) ( *TT* 250 mg / Kg BW) (C) shows a slight reduction of stone formation. Moreover, the urine sample from group (4) ( *TT*

500 mg / Kg BW) (D) shows the extract significantly reduces nucleation by increasing the unstable limit of oxalate in the urine and preventing the precipitation of CaOx crystals. Meanwhile, a stained urine sample from group (5) ( *UD* 250 mg / Kg BW) (E) shows Slightly reduced stone formation. A urine sample from group (6) ( *UD* 500 mg / Kg BW)(F) shows to inhibitory for *UD* effect on crystal nucleation and aggregation.

## DISCUSSION

Researchers have turned to experimental investigations as there is no operative medical therapy for kidney stone production. Looking at the literature, the present outcomes can observe that kidney stone illness is treated with traditional herbal remedies because they have potent flavonoid and antioxidant qualities **(Yılmaz *et al*.,2022).** According to **Zhang and Li, (2014),** the total phenolics found in the *Urtica* species and identified by HPLC were predominantly phenolic compounds, such as caffeic acid, 2-O-caffeoyl malic acid, and flavonoids like quercetin, isorhamnetin glycoside and chlorogenic acid s. According to **Laoufi, (2020),** the *UD* leaves are high in total tannins, gallic tannins, flavonoids, mucilage saponins, and alkaloids. However, they contain a moderate amount of anthocyanins, coumarins, starch, and glucosides. On the other hand, catechin tannins, iridoids, free quinones, and sennosides are absent. **(Paulauskienė *et al.*, 2021)** reported that *UD* leaves contain several significant phenolic compounds, including quercetin, catechin, pelargonidin, and apigenin in both glycosidic and non-glycosidic forms, as well as caffeic acid, hydroxybenzoic acid, cinnamic, vanillic acid, coumaric acid, gentisic acid and protocatechuic. **Behiry *et al*., (2022)** revealed that *UD* contains ascorbic acid and it derivates, protocatechuic acid glucoside, coumaroyl glucaric acid isomers, di-caffeoylquinic acid, 5-caffeoylquinic acid, caffeoylmalic acid, p-coumaric acid, ferulic acid, quercetin-3-rutinoside, caffeoylglucaric acid (dimer) , and feruloyl malate . Flavonoids, tannins, volatile chemicals, fatty acids, polysaccharides, vitamins, isolectins, sterols, terpenes, protein, and minerals were

discovered to be some of the principal chemical ingredients of *UD* by **Bhusal *et al*., (2022).**

**Tian *et al*., 2020** showed that *TT*, including saponins, has many biological activities. **Mubarik *et al*., (2022)** reported that polyphenol compounds identified in *TT* fruit by HPLC are Flavonoids such as ( Naringin, Hesperidin, Narenginin, Quercetin, Rutin, Kaempferol, Apigenin, Salicylic acid. Phenolic acids include (Iso-ferulic acid, p-hydroxy benzoic acid, Chlorogenic acid, Gallic acid, Catechol, Ellagic acid, Catechin, Ferulic acid, Coumaric acid, p-Coumaric acid, Resveratrol, Cinnamic acid, Vanillic acid, Coumarin, Caffeic acid). The ethyl acetate and butanol fractions from *TT* demonstrated the most phenolic component and flavonoid recovery, according to **Oliveira *et al, (2022*** **).** Compared to the healthy control group, the ethylene glycol-administered rats' kidney weight showed a significant elevation; this increase may result from irritation, fluid buildup, and crystal formation in the kidney **(Ahmed *et al*., 2020 ).** The results of the present work agree with **Zhang and Li, (2014)** mentioned The *UD* alcoholic extract was established to reduce kidney weight in treated groups.

The current study results of work agree with **DeAraújo *et al*.,( 2020)** revealed that injection of NaOx (70 mg/k g/day) induced a significant reduction of feed intake(FI) and body weight gain( BWG) in hyperoxaluria rates (the control group) reduced body weight gain may be attributed to the direct toxic effects of Naox. These findings agree with **Kumar *et al*., (2022)** showed that induction of NaOx causes renal stone formation leading to a significant decrease in body weight in the induced control group when compared with the normal control group as a result of stress and toxicity.

The dose-dependent weight gain of rats with kidney stones disease compared to a positive control group showed that TT aqueous extract improved metabolic activity, according to **Sutar and Kamble, (2019)**. TT contains flavonoids, which improve health.

The results of the present work agree with **Khalesi *et al*., (2022),** significantly increased body weight in treated groups with

*UD* aqueous extract compared with the positive control group because it contains sterols, triterpenes, coumarins, phenols, lignans, ceramides, and fatty acid.

Another study sustained by **Ibrahim *et al*. (2019)** revealed that intraperitoneal injection of sodium oxalate mice caused nephrotoxicity manifested by significant urea level and creatinine increases compared with the negative control group. The present study was supported by **Golla *et al*., (2023)** reported that sodium oxalate-treated rats indicated a significant rise in serum creatinine and uric acid, resulting in a reduction in kidney filtration rate, which in turn leads to the blood's accretion of nitrogenous compounds such urea, creatinine, and uric acid.

Results are supported by **Hussein *et al*., (2022)** showed that treatment with *TT* decreases the creatinine levels, blood urea nitrogen, and uric acid in treated groups compared with ethylene glycol. Our study found that uric acid concentration was reduced after treating the aqueous extract of *TT*, reiterating the antilithic efficacy. Our results agree with **Kaushik *et al*., (2019),** who found that therapy with an aqueous extract of *TT* can reduce uric acid levels. The increased uric acid concentration was not good because it dramas a vital role in the increased stones. It helps Ca Ox buildup by absorbing glutamic acid and other organic compounds. An increase in glomerular filtration rate (GFR) and a decrease in serum nitrogenous waste accumulation were both seen after treatment with( *TT)* extract **(Kaushik *et al*., 2019). Sharma e*t al*., (2019)** stated the belongings of the hydroalcoholic section of (*TT*) in cisplatin-treated mice. Induced nephrotoxicity treatment of *TT* decreased serum creatinine levels compared to the cisplatin group.

**Kilany *et al*., (2020)** found that *TT* extract improves plasma parameters such as creatinine and blood urea nitrogen, improving renal blood flow, and relieves nitric oxide from blood vessel endothelium. Vascular smooth muscles become hyperpolarized, leading to vasodilation in treated groups instead of gentamicin groups. **Saoudi *et al*., (2020)** mentioned that rats treated with extract aqueous of *UD* showed significant reductions in creatinine, urea, and uric acid markers compared to the positive group

because UD contains antioxidant compounds that can decrease the degree of toxicity. The present study is stayed by **Keleş *et al* ., (2020)** cleared that *UD* treatment reduced the serum levels of creatinine and uric acid in treated groups, contributing to its curative efficacy in impaired kidney function**. Vasanthi *et al*., (2017)** reported that hyperoxaluria caused by ethylene glycol is a risk factor in the development of urolithiasis than in the healthy control group. It causes damage to the kidney membranes, which lets calcium and potassium into the bloodstream. In our investigation, the electrolyte balance was theoretically restored after treatment with *TT* extract compared to the hyperoxaluric rats, which agrees with **Maharana and Dadhich, (2015).** The results of the present work agree with **Keleş *et al*., (2020)** mentioned that catechin and ferulic acid in *urtic dioca* ethanolic extract reduced serum sodium and potassium levels compared with ethylene glycol groups.

Calculi-inducing therapy increases urine phosphate, calcium, and oxalate excretion while decreasing urinary magnesium excretion, according to **Pawar and Vyawahare, (2017).** Increased urinary calcium and oxalate concentrations may enhance the nucleation and precipitation of calcium oxalate from urine, generating supersaturation of urinary colloids, which functions as a nidus when trapped, resulting in further crystal development. Our results confirm **Pawar and Vyawahare, (2017)** hypothesis that an early calcium phosphate concretion, also known as Randall's plaque, forms in the terminal collecting ducts where most calcium oxalate stones develop. This calcium phosphate concretion functions as a nidus for calcium oxalate deposition. Renal phosphate levels were significantly higher in lithiasis control rats, indicating the formation of calcium phosphate concretions and the emergence of calcium oxalate-calcium phosphate mixed stones.

The present outcomes supported by **Kamboj *et al*., (2020)** showed treatment with an aqueous extract of *TT* significantly decreased the urinary oxalate, calcium, and phosphate excretion compared with the positive control group. **Gupta and Dubey, (2020)** found that treated animals with an

aqueous extract of *TT* showed decreased calcium levels compared to the hyperoxaluric rats. The aqueous extract also reduced levels of phosphorous and increased magnesium levels. Magnesium has the ability to process a stable compound with oxalate, which lowers the concentration of CaOx and prevents the formation of CaOx stones in the renal tubules.

Our study goes along the same lines as **(Keleş *et al*., 2020)** mentioned that *UD* treatment decreased urinary oxalate levels in the treated groups compared to the positive control group. Ethanol extract of *UD* leaves used for the preventive medication of CaOx kidney stone.

**Zhang and Li, (2014)** suggested that hyperoxaluria induced with NaOx causes renal damage, which may cause reduced urine excretion. Results are supported by **Bawari *et al*., (2023)** reported that sodium oxalate-treated rats showed a significant decrease in urine volume, urinary creatinine, and creatinine clearance as the index of glomerular filtration rate when compared with the treated group.

In this study, *TT* aqueous extract increased urine volume. **Chhatre *et al*., (2014)** said that *TT* is a well-known immune-modulatory, diuretic, and anti-urolithic agent because it contains diuretic substances such as saponins. **Sudheendran *et al*., (2021)** observed that treatment with *TT* elevated diuresis, producing a larger amount of urine in the treated group than the infected group, hastened the process of dissolving the preformed stones due to a decrease in ion saturation in the urine. **Hajihashemi *et al*., (2020)** showed that rats administrated extract leaves of *UD* caused significantly increased urine volume and creatine clearance compared to the unhealthy group.

In the results of the current study, calcium oxalate stones were produced at a high PH, where the high acidity leads to crystallization to form calcium phosphate stones as a starting point for the formation of calcium oxalate stones. The role of urine pH in developing calcium oxalate stones is highly controversial. Utmost authors found that calcium oxalate stones procedure in any urine pH **(Carvalho, 2018).** The ongoing study findings agree with **Pandhare *et al*., (2021*),*** *who*

found that the administration of NaOx increased urine pH compared with untreated groups.

The results of studies show there is an increase in RBCs in the urine of are similar to **Al-Assaf *et al.*, (2020)** discovered that chemicals such as ethylene glycol liberation in an overdose of some of the toxic components have strong DNA destruction effects on bone marrow DNA and thus lower all blood components. The ongoing study's findings on phenolic acid and organic acid found in species *TT* and *UD* probably equilibrium urine pH by their acidifier properties. Results supported by **Saoudi *et al*., (2020)** cleared that *UD* those activities of decrease in the number of RBCs could be due to the inhibition of erythropoiesis and chemosynthesis in treated groups compared with the positive group. In addition to a significant increase in urine uric acid proteins, red blood cells, and oxalate levels in ethylene glycol-treated rats, urine specific gravity was significantly higher than in standard control rats **(Ahmed *et al*., 2020).**

These results agree that **Mulay *et al.*, (2016)** indicated that prolonged exposure to Na Ox can cause various pathophysiological conditions, including inflammatory illnesses, by interfering with natural killer (NK) cells and TNF levels**.** These results agree with **Gu *et al.*, (2022)** reported that sodium oxalate stimulates cytokines such as TNF-α, which alters the inducible nitric oxide synthase (INOS) metabolism. **Saleem *et al*., (2020)** found that *TT* methanol extract reduced the elevated levels of proinflammatory cytokines TNF- in treatment groups compared to a model group by inhibiting neuroinflammation and oxidative stress. These outcomes are consistent with **Keleş *et al.*, (2020)** noting that ethanolic *UD* treatment significantly caused to fall in TNF-α.

The current study's findings are consistent with those of **Saha and Verma, (2014)** found that intraperitoneal injection of sodium oxalate lead to decreasing antioxidant defense in the kidney tissues due to a decrease in the action of antioxidant enzymes such as SOD and CAT in the Sodium oxalate group compared to normal controls. Resulting in renal tubular injury ROS (reactive oxygen species) causing oxidative stress.

Renal cell injury caused by hypercalcemia and oxalate accumulation resulted in membrane lipid peroxidation, increased MDA levels, and free radical generation.

In the current study, results agree with **Kamboj *et al*., (2020)** who showed that *TT* lowered the levels of free radicals that cause lipid peroxidation, lowering the malondialdehyde. This is because *TT* can eliminate free radicals and lessen the damage that oxalate causes to free radicals. Hyperoxaluria decreased SOD and GST activity as well as their mRNA expression in kidney tissues. The balance of enhanced antioxidant enzyme activity and gene expression by *TT* therapy verified the plant extract's preventive properties against free radical-induced oxidative stress.

These findings are consistent with those of **Kilany *et al*., (2020)** discovered that the methanolic extract of *TT* inhibited the generation of NO phenolic amides in *TT* fruits and inhibited NO in treatment groups compared to positive control groups. These findings are consistent with those of **Hussein *et al*., (2022)** discovered that mice fed *TT* aqueous extract had higher levels of SOD activity in their kidneys and lower levels of malondialdehyde. They attributed these findings to *TT* antioxidant and free radical scavenging properties, which include phenolic compounds.

This study agrees with **Caglar *et al*., (2019)** found that *UD* increased levels of CAT, SOD, and reduced MDA levels in kidney tissue in the treated group compared with the positive control group. These results agree with **Saoudi *et al*., (2020)** mentioned that rats treated with an aqueous extract of *UD* showed reductions in malondialdehyde levels compared to the positive group. *UD* contains antioxidant compounds that it can decrease malondialdehyde. The presented results align with **Al-Assaf *et al*., (2020)** showed *UD*. MDA is reduced in male rabbet compared with the positive control because it contains some phenolic compounds such as Catechin and gallic acids, which have been shown to have a high ability to protect against oxidative stress and programmed cell death**.**

**Shehzad *et al*., (2020)** said that the positive control group had a buildup of CaOx crystals,

significantly widening the tubules and causing congestion and inflammation. **Pandhare *et al.*, (2021)** said that NaOx therapy significantly increased the number of kidney stones. Histological changes in the kidneys include tubular dilatation and early changes to the cysts, tubular atrophy, calcium oxalate crystal deposits, and interstitial mononuclear cell infiltration**.. Farokhi *et al.*, (2022)** showed that *TT* extract decreases histological damage, and oxidative stress, which improves kidney function. Oxalate injured renal epithelial cell lines to increase cell viability and reduce cell death**.** The presence of antioxidant and anti-inflammatory compounds in the aqueous extract, such as Cinnamic, Syringic, Gentisic, Protocatechuic Vanillic, and Catechin, improves renal epithelial cells and reduces oxidative stress. These results agree tha**t Al-Assaf *et al.*, (2020)** found that *UD* extract significantly enhanced the histological features of kidneys, characterized by degenerative and necrotic alterations of epithelial cells lining renal tubes, decreased oxalate accumulation, and a minor infiltration of inflammatory cells in the interstitial tissue.

The presented results align with **Lordumrongkiat *et al.*, (2022)** showed that injection with Naox caused calcium oxalate crystals to appear in the urine due to supersaturation and increased promotors.

These outcomes align with **Devi, (2017)** indicated that calcium oxalate crystallization inhibition in urine after giving TT has an inhibitory effect *on* CaOx nucleation, crystal growth, and aggregation in the urine. These results agree with **Belmamoun *et al.*, ( 2022)** showed that the main bioactive of *UD* are flavonoids, anthocyanins, and saponins which could inhibit calcium and oxalate deposition and crystal growth in urine by disintegrating mucoproteins.

## CONCLUSION

The current study's findings suggest that *Tribulus Terrestris* and *Urtica dioica* aqueous extracts have potent antioxidants; they can inhibit kidney stone development owing to crystal formation and aggregation when administered as a therapeutic agent. *Tribulus Terrestris* and *Urtica dioica* are effective in treating kidney stones,

and the benefits may be due to their antioxidant characteristics. Further research is needed to determine their mechanism of action in preventing kidney stone development.

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## REFERENCES

**Abbas MW; Hussain M; Akhtar S; Ismail T; Qamar M; Shafiq Z and Esatbeyoglu T (2022):**
Bioactive compounds, antioxidant, antiinflammatory, anti-cancer, and toxicity assessment of *Tribulus Terrestris—In vitro* and *in vivo* studies. *Antioxidants*, *11*(6), 1160.

**Ahmed OM; Ebaid H; El-Nahass ES; Ragab M and Alhazza IM (2020):**
Nephroprotective effect of Pleurotus Ostreatus and Agaricus Bisporus extracts and carvedilol on ethylene glycol-induced urolithiasis: roles of NF-κB, p53, bcl-2, bax, and back. *Biomolecules*, *10* (9), 1317.

**Al-Assaf IN; Al-Dabbagh AG; Al-Abachi RQ; and Al-Bajari SA (2020)**:
Protective role of Urtiea dicica on the pathological alteration induced by ethylene glycol in male rabbits. *Systematic Review Pharm*, *11*(12), 1180-1183.

**Al-Harrasi A; Bhatia S; Chigurupati S; Behl T and Kaushik D (2023):**
Plant Profile, Phyto-chemistry, and Ethno-pharmacological Uses of Swertia chirality, Tribulus Terrestris, and Plumbago zeylanica. In *Recent Advances in Natural Products Science* (pp. 173-210). CRC Press.

A**lomair MK; Alobaid AA; Almajed MAA; Alabduladheem LS; Alkhalifah E A; Mohamed M E; and Younis N S (2023):**
Grape Seed Extract and Urolithiasis: Protection Against Oxidative Stress and Inflammation *Pharmacognosy Magazine*, 09731296221145069.

**Araújo L; Costa-Pessoa JM; De Ponte MC and Oliveira-Souza M (2020):**
Sodium oxalate-induced acute kidney injury associated with glomerular and tubulointerstitial damage in rats. *Frontiers in Physiology*, *11*, 1076.

**Bartels H; Bohnzer M and Heierli C (1972):**
Serum creatinine determination without protein precipitation. *Clinical chemistry Acta*, 37: 193.

**Bawari S; Sah A N; Gupta PK; Zengin G; and Tewari D;(2023):**
Himalayan Citrus jambhiri juice reduced renal crystallization in nephronlithiasis by possible inhibition of glycolate oxidase and matrix metalloproteinases. *Journal of Ethnopharmacology*,11615

**Beara-Lasic L and Goldfarb DS;( 2019):**
Recurrent Calcium Kidney Stones. *Clin J Am Soc Nephrol*, 14 (9):1388-1390.

**Belmamoun AR; Ammam A; Chadli R; Mhamdia C; & Bakdi A (2022):**
Phytotherapy of urinary calculi, the mass reduction of calcium oxalate stones in vitro by the aqueous extract of Urtica dioica l Vol. 8 (2): 36- 42.

**Behiry SI; Philip B; Salem MZ; Amer MA; El-Samra IA; Abdelkhalek, A; and Heflish A (2022):**
Urtica dioica and Dodonaea viscosa leaf extracts as ecofriendly bioagents against Alternaria alternata isolate TAA-05 from the tomato plant. *Scientific Reports*, *12*(1), 16468.

**Bhusa KK; Magar S; Thapa R; Lamsal A; Bhandari S; Maharjan R and Shrestha (2022):**
Nutritional and pharmacological importance of stinging nettle (Urtica dioica L.): A review. *Heliyon*, e09717.

**Buege JA; and Aust SD (1978):**
Microsomal lipid peroxidation, *Methods in Enzymology*, 52: 302–310.

**Burkhardt AE; Johnston KG; Waszak CE; Jackson CE and Shafer SR (1982):**
A reagent strip for measuring the specific gravity of urine. *Clinical chemistry*, *28*(10), 2068-2072.

**Caglar HG; Selek S; Koktasoglu F; Koyuncu I; Demirel M; Sarikaya A and Meydan S (2019):**
Effect of Camellia sinensis, Hypericum perforatum, and Urtica dioica on kidney and liver injury induced by carbon tetrachloride in rats. Cellular and Molecular Biology, 65(5), 79-86.

**Carvalho M (2018):**
Urinary pH in calcium oxalate stone formers: does it matter? *Brazilian Journal of Nephrology*, *40*, 6-7.

**Chapman DG; Gastilla R; and Campbell TA (1959):**
Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. *Can. J. Biochem. Physio*. 1(37) 679-686.

**Cheng DS; Rodriguez RM; Rogers J; Wagster M; Starnes DL and Light RW (1998):**
Comparison of pleural fluid pH values obtained using blood gas machine, pH meter, and pH indicator strip. *Chest*, *114*(5), 1368-1372.

**Chhatre S; Nesari T; Somani G; Kanchan D and Sathaye S (2014):**
Phytopharmacological overview of *Tribulus Terrestris*. *Pharmacognosy reviews*, 8 (15), 45.

**Coe FL; Worcester EM and Evan AP (2016):**
Idiopathic hypercalciuria and formation of calcium renal stones. *Nat. Rev. Nephrol*. 12:519.

**Cortas NK and Wakid NW (1990):**
Determination of inorg-anic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem.*36: 1440-3.

**Devkota H P; Paudel K R; Khanal S; Baral A; Panth N;**

**Adhikari-Devkota A and Hansbro PM (`2022):**
Stinging nettle (*Urtica dioica L.*): Nutritional composition, bioactive compounds, and food func-tional properties. *Molecules, 27* (16), 5219.

**Devi C (2017):**
Calcium oxalate crystallization inhibition by Pedalium murex and Tribulus Terrestris fruit extracts. *International Journal of Chem. Tech Research*, 10 (3), 128-136.

**Drury RA; and Wallington EA (1980):**
Carton's histological technique. 5th ED., Oxford Univ.

**Eise NT; Simpson JS; Thompson PE; and Ventura S (2022):**
Aqueous extracts of *Urtica dioica* (stinging nettle) leaf contain a P2-purinoceptor antagonist—Implications for male fertility. *PloS One, 17*(7), e0271735.

**Evan AP (2010):**
Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatr. Nephrol*. 25:831-41.

**Evans EA (1989):**
Structure and deformation properties of red blood cells: Concepts and quantitative methods. *Methods in enzymology*, *173*, 3-35.

**Farokhi F; Peeri M; Azarbayjani MA and Hosseini SA (2022):**
The Effects of Tribulus Terrestris Extract and Endurance Training on Oxidative Stress and Apoptotic Markers in the Kidney Tissue of Rats, Poisoned with Arsenic. *Gene, Cell and Tissue*, (In Press) 76 (6): 564 -722.

**Fossati P; Prencipe L and Berti G (1980):**
Use of the 3,5 – dichloro – 2 – hydroxybenzene - sulfonic acid /4-aminophenazone chromogenic system in the direct enzymatic assay of uric acid in serum and urine. Clinical chemistry, 26: 227-231.

**Frazer A; Secunda SK and Mendels J (1972):**

A method for the determination of sodium, potassium, magnesium, and lithium concentrations in erythrocytes. *Clinica chimica acta*, *36* (2), 499-509.

**Golla S; Pasala PK; Sura S; Nainita K and Katabathina D (2023):**
Anti urolithiatic activity of Cyperus rotundus tubers: In silico, In vitro and In vivo approaches. *Brazilian Journal of Pharmaceutical Sciences*, 58 (2), 904-981.

**Gu Y; Shen Y; Chen W; He H; Ma Y; Mei X and Liu H (2022):**
Protective effects of interleukin-22 on oxalate-induced crystalline renal injury via alleviating mitochondrial damage and inflammatory responsese. *Applied Microbiology and Biotechnology*, *106* (7), 2637-2649.

**Gupta D and Dubey PK (2020):**
Effect of ethanolic extracts of Tribulus Terrestris, Phyllanthus niruri and combination on calcium oxalate urolithiasis in rats. *International Journal of Pharmacy & Life Sciences*, *11*(2).

**Hajihashemi S; Ahmadi M; Chehrei A and Ghanbari F (2020):**
Ameliorative effect of cotreatment with the methanolic leaf extract of Urtica dioica on acute kidney injury induced by gentamicin in rats. *Avicenna Journal of Phytomedicine*, *10*(3), 273.

**Hussein SA; Osman RH; Abuelgasim AI and Ali SA (2022):**
Biochemical Effects of *Tribulus-Terrestuis* on Ethylene glycol-induce Nephrolithiasis in Albino rats. *Journal of The Faculty of Science and Technology*, (9 (2)), 1-9.

**Ibrahim H M; Moustafa AE; and Elgebaly HA (2019):**
Effect of black and green tea on experimental sodium oxalate-induced nephrotoxicity in adult male mice. *Egyptian*

*Journal of Histology*, *42*(2), 358-368.

**Inker LA; Perrone RD; Sterns RH and Forman JP (2019):**
Calculation of the creatinine clearance. *Up To Date Waltham (MA); Wolters Kluwer.*

**Jagtap PN; Shriwas S and Patel R (2023):**
Investigation Of Potential Anti-Urolithiatic Activity of Some Herbs by Using Ethylene Glycol Induced Urolithiasis In Animals. *European Journal of Molecular & Clinical Medicine*, 10 (01), 2210-2236.

**Kamboj P; Aggarwal M P; uri1, S and Singla SK (2020):**
Effect of aqueous extract of *Tribulus Terrestre's* on oxalate induced oxidative stress in rats. Indian Journal of Nephrology. Vol 21 / Issue 3: 154-159.

**Kaushik J; Tandon S; Bhardwaj R; Kaur T; Singla SK; Kumar J and Tandon C (2019)**:
Delving into the Antiurolithiatic potential of Tribulus Terrestre's extract through–In Vivo Efficacy and Preclinical Safety investigations in Wistar Rats. *Scientific Reports*, *9*(1), 1-13 .

**Keleş R; Şen A; Ertaş B; Kayali D; EKER P; ŞENER TE and Şener GÖ (2020):**
The effects of *Urtica dioica L*. ethanolic extract against urinary calculi in rats. *Journal of Research in Pharmacy*, *24*(2), 205-217.

**Kilany OE; Abdou RH; El-Beltagy MA and Mohammad HM (2020):**
Protective effects of *Tribulus Terrestre* against gentamicin mediated nephrontoxicity, oxidative damage and apoptosis in male rats. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, *12*(1), 41-58.

**Kim KH; Tsao R; Yang R and Cui SW (2006):**
Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry., 95, 466-473.

**Khalesi B; Fatemi SA; Torabi Goudarzi M; Ghorashi S A; Motamed N and Andalib F (2022).**
Effects of *Urtica dioica* Hydroalcoholic Extract on the urinary Tract of Broilers. *Egyptian Journal of Veterinary Sciences*, *53*(2), 147-156.

**Kumar A; Nandi MK; Kumar B; Kumar A; Kumar R; Kailashiya, V and Singh AK (2022):**
Toxicity (acute and subacute) assessment and *in-vivo* antiurolithiatic activity of ethanolic extract of Caesalpinia bonducella seed in albino Wistar rat. *Journal of Applied Pharmaceutical Science*, *12*(2), 187-197.

**Kyle RA (1990):**
The sequence of testing for monoclonal gammopathies: serum and urine assays. Archives of Pathology and Laboratory Medicine, 123(2), 114-118.

**Laoufi R (2020)**:
The beneficial effect of the *Urtica dioica* aqueous extract on the crystallization of calcium oxalate in urine. *International Journal of Green Pharmacy (IJGP)*, *14*(1).

**Luo L; Zhang Z and Lifeng M (2005):**
Determination of recombinant human tumor necrosis factor-α in serum by chemiluminescence imaging. *Analytica Chimica Acta*. 539, (1–2); 277-282.

**Lordumrongkiat N; Chotechuang N; Prasanth MI; Jindatip D; MaOn C; Chuenwisad K and Boonla C (2022):**
HydroZitLa inhibits calcium oxalate stone formation in nephrolithic rats and promotes longevity in nematode Caenorhabditis elegans. *Scientific Reports*, *12*(1), 5102.

**Maharana L and Dadhich OP (2015):**
Review on the role of Herbal Drugs in the Prevention and Mana-

gement of Kidney Stones. *Journal of Ayurveda*, 9 (2).

**Marshall RW and Robertson WG (1976):**
Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid, sodium acid urate, ammonium acid urate, and cystine. *Clinica Chimica Acta*, *72*(2), 253-260.

**Mubarik F; Khalil AA; Akhtar MN; Khalid A; Basharat S; Farooq F and Siddiqa A (2022):**
Assessing the antioxidant characteristics and polyphenol content of *Tribulus Terrestris* microwave-assisted fruit extract. *Phytopharmacology Research Journal*, 1 (3), 1-10.

**Mulay SR; Desai J; Kumar SV; Eberhard JN; Thomasova D; Romoli S and Anders HJ (2016):**
Cytotoxicity of crystals involves RIPK3-MLKL-mediated necroptosis. *Nature communications*, *7*(1), 10274.

**Nimavat A; Trived A; Yadav A and Patel P (2022):**
A Review on Kidney Stone and Its Herbal Treatment. *Journal of Pharmacy and Pharmacology*, *10*, 195-209.

**Nishikimi M; Dunmore OD; Chidiebere OE; Igube NA; Kalu AU; Rae NA and Yagi K (1972):**
The occurrence of superoxide anion in the action of reduced phenazine methosulphate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46: 849-853.

**Oliveira NNPM; Pereira AC; Aazza S; Germano CM; de Assis R MA; Mendonça SC and Bertolucci SKV ( 2022):**
Preparações de frutas Tribulus terrestris afetam os perfis químicos de HPLC e as atividades antioxidantes, lipoxigenase e inibidoras de α-glucosidase. Research, Society and Development, 11(17), e17111738751.

**Ormond JK (1948):**
Bilateral ureteral obstruction due to envelopment and compression by an inflammatory retroperitoneal process. *The Journal of urology*, *59* (6), 1072-1079.

**Pandhare RB; Shende RR; Avhad MS; Deshmukh VK; Mohite PB; Sangameswaran B and Daude RB (2021):**
Anti-urolithiatic activity of Bryophyllum pinnatum Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. *Journal of Traditional and Complementary Medicine*, *11*(6), 545-551.

**Parekh BB; Vyas PM; Vasant SR and Joshi MJ (2008):**
Thermal FT-IR and dielectric studies of gel-grown sodium oxalate single crystals. *Bulletin of Materials Science*, *31*, 143-147.

**Patton CJ and Crouch SR (1977):**
Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia: *Analytical Chemistry*, 49(3):464-469.

**Paulauskienė A; Tarasevičienė Ž and Laukagalis V (2021):**
Influence of harvesting time on the chemical composition of wild stinging nettle (Urtica dioica L.). *Plants*, 10(4), 686.

**Pawar AT and Vyawahare NS (2017):**
Protective effect of ethyl acetate fraction of Biophytum sensitivum extract against sodium oxalate-induced urolithiasis in rats. *Journal of Traditional and Complementary Medicine*, *7*(4), 476-486.

**Raimova KV; Berdiev NS; Ishimov UZ; Turdieva Z V; Abdullajanova, NG and Matchanov AD (2021):**
Comparative study of the total protein and free amino acids of plants Urtica dioica and Crataegus pontica. Asian Journal of Multidimensional Research, 10(9), 504-510.

**Reeves PG; Nielsen FH and Fahey GC (1993):**
AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of nutrition, 123(11): 1939–1951.

**Saha S and Verma R J (2014):**
Polyphenolic extract of Piper nigrum inhibits sodium oxalate-induced oxidative stress in rat kidneys. *Toxicology and Environmental Health Sciences*, *6*, 164-169.

**Saleem U; Chauhdary Z; Raza Z; Shah S; Rahman MU; Zaib P and Ahmad B (2020):**
Anti-Parkinson's Activity of Tribulus Terrestre's via Modulation of AChE, α-Synuclein, TNF-α, and IL-1β. *ACS omega*, *5*(39), 25216-25227.

**Samakar B; Mehri S and Hosseinzadeh H (2022):**
A review of the effects of *Urtica dioica* (nettle) in metabolic syndrome. Iranian Journal of Basic Medical Sciences, 25(5), 543.

**Saoudi M; Rahmouni FEl; Aroui M; Boudaya M; Jamoussi K; Kallel C and El Feki A; Ameliorative (2020):**
Effects of *Urtica dioica* Aqueous Extract against Hepatotoxicity and Nephro-toxicity Induced by Insecticide Mixture in Adult Male Rats. Entomology and Applied Science Letters 7(3),98-110.

**Sharma US; Yadav HN; Singh S; and Gupta YK (2019):**
Hydroalcoholic extract of Tribulus Terrestris protects against the accumulation of platinum in rat kidneys. *Innov. Pharm. Pharmacother*, *7*, 7-14.

**Shehzad A; Saleem U; Shah MA; Cruz CV; Khan AH and Ahmad B (2020):**
Antiurolithic evaluation of *Cucurbita pepo* seeds extracts against sodium oxalate induced renal calculi. Phcog Mag 16: S174-80.

**Snedecor GW (1969):**
Statistical methods "Fourth Ed.; The lowa state, college press, Ames lowa.

**Sinha KA (1972):**
Colorimetric Assay of Catalase. *Analytical Biochemistry*, 47:389-394.

**Sudheendran A; Shajahan MA and Premlal S (2021):**
A comparative diuretic evaluation of fruit and root of Gokshura (*Tribulus terrestris Linn*.) in albino rats. *AYU (An international quarterly journal of research in Ayurveda), 42* (1), 52.

**Sutar VS and Kamble NA (2019).**
Phytoremediation of ethylene glycol induced renal calculi in the vertebrate model Rattus norvigicus. GSC Biological and Pharmaceutical Sciences, 8 (1), 01-011.

**Tarasevičienė Ž; Vitkauskaitė M; Paulauskienė A and Černiauskienė J (2023):**
Wild Stinging Nettle (*Urtica dioica L*.) Leaves and Roots Chemical Composition and Phenols Extraction. *Plants*, *12*(2), 309.

**Vasanthi AHR; Muthulakshmi V; Gayathri V; Manikandan R; Ananthi S and Kuruvilla (2017):**
Antiurolithiatic effect of Sirupeelai Samoola Kudineer: A polyherbal siddha decoction on ethylene glycol-induced renal calculus in experimental rats. *Pharmacognosy magazine,* 13 (Suppl 2), S273.

**Winoker JS; Bamberger JN; Chandhoke RA; Atallah W and Gupta M (2019):**
What Factors Drive Staghorn vs No staghorn Pattern Growth in Patients with Metabolic Stones? J. Endourol.33:954.

**Yılmaz H; Ekinci N; Ömerli A; Nisari M; Yay A H; Ülger H and Taştan M (2022):**
The protective effect of *Myrtus communis L.* against experimental kidney stone in rats. *Advances in Traditional Medicine,* 1-9

**Yoshioka I; Tsujihata M; Momohara C; Akanae W; Nonomura N and Okuyama A (2010):**

Effect of sex hormones on crystal formation in a stone-forming rat model. Urol. J. 75:907-13.

**Zhang H; Li N; Li K; and Li P (2014):**

Protective effect of *Urtica dioica* methanol extracts against experimentally induced urinary calculi in rats. *Molecular medicine reports*, *10*(6), 3157-3162.

**Table 1. The phenolic compounds in aqueous extracts of *TribulusTterrestris and Urtica dioica***

| Phenolic Compound | *Tribulus Terrestris(µg/ml)* | *Urtica dioica (µg/g)* |
|---|---|---|
| **Gallic** | 0.56 | 1.736 |
| **Protocatechuic** | 1.13 | 8.413 |
| ***p*-hydroxybenzoic** | 0.61 | 14.928 |
| **Gentisic** | 1.42 | - |
| **Catechin** | 4.71 | 16.463 |
| **Chlorogenic** | 0.33 | - |
| **Syringic** | 1.70 | 2.004 |
| **Vanillic** | 0.09 | 1.723 |
| **Ferulic** | - | 9.725 |
| **Apigenin-7-glucoside** | - | 12.757 |
| **Rosmarinic** | - | 14.318 |
| **Cinnamic** | 0.12 | 7.561 |
| **Chrysin** | - | 2.186 |

**Table (2): Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on feed intake, body weight gain, feed efficiency ratio, and relative kidney weight in hyperoxaluria rats (M±SD)**

| Parameters / Groups | FI (g / 28days) | BWG (%) | FER | Kidney (%)Row |
|---|---|---|---|---|
| **Control –ve (G1)** | 557.00± 2.55$^{a}$ | 23.80 ± 2.68$^{a}$ | 0.0682 ±0 .003$^{a}$ | 0.98±.04$^{e}$ |
| **Control +ve (G2)** | 398.80±4.81$^{e}$ | 8.00 ± 2.55$^{d}$ | 0.0326 ± 0.006$^{d}$ | 1.69± .05$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 507.00 ± 2.55$^{c}$ | 14.40±2.88$^{c}$ | 0.0458 ± 0.005$^{c}$ | 1.44±.09$^{b}$ |
| ***Tribulus terrestris* 500 mg(G4)** | 532.20 ± 3.35$^{b}$ | 20.40 ± 2.96$^{b}$ | 0.0605 ± 0.004$^{b}$ | 1.14±.08$^{d}$ |
| ***Urtica dioica* 250 mg (G5)** | 489.00 ± 2.24$^{d}$ | 14.00 ± 2.24$^{c}$ | 0.0487 ± 0.004$^{c}$ | 1.52± .09$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 529.20 ±7.11$^{b}$ | 19.32 ± 1.81$^{b}$ | 0.0578 ± 0.005$^{b}$ | 1.31±.1$^{c}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (3): Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on serum Creatinine, Urea, and Uric acid in hyperoxaluria rats (M±SD)**

| Parameters<br>Groups | Creatinine (mg/dl) | Urea (mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|
| **Control –ve (G1)** | 0.52±.11$^{d}$ | 13.20± 2.44$^{e}$ | 1.7± 0.016$^{e}$ |
| **Control +ve (G2)** | 1.13±.15$^{a}$ | 49.19± 2.98$^{a}$ | 2.90 ± 0.026$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 0.88±.07$^{b}$ | 26.35± 3.74$^{c}$ | 2.47± 0.026$^{c}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 0.63±.09$^{cd}$ | 18.56± 2.39$^{d}$ | 1.88± 0.032$^{d}$ |
| ***Urtica dioica* 250 mg (G5)** | 0.95±.09$^{b}$ | 32.66±2.24$^{b}$ | 2.65± 0.019$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 0.70±.08$^{c}$ | 18.70±2.51$^{d}$ | 1.9± 0.026$^{d}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (4)**: Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on serum (Sodium, Potassium, Calcium) in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | Sodium ($Na^{+}$) mmol/L | potassium ($K^{+}$) mmol/L | Calcium ($Ca^{++}$) mg/dl |
|---|---|---|---|
| **Control –ve (G1)** | 133.56±2.75$^{e}$ | 5.02±0.08$^{d}$ | 8.69±0.31$^{d}$ |
| **Control +ve (G2)** | 147.44±1.73$^{a}$ | 5.84±0.11$^{a}$ | 12.75±1.2$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 140.70±1.86$^{bc}$ | 5.37±0.23$^{c}$ | 10.02±0.36$^{bc}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 137.32±0.53$^{d}$ | 5.27±0.06$^{c}$ | 9.60±0.17$^{c}$ |
| ***Urtica dioica* 250 mg (G5)** | 142.84±1.07$^{b}$ | 5.68±0.10$^{b}$ | 10.45±0.27$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 139.14±1.12$^{cd}$ | 5.40±0.08$^{c}$ | 9.75±0.11$^{bc}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (5):** Effect of *Tribulus Terrestris* and *Urtic dioica* aqueous extracts on Urine (Calcium, Oxalate, and Sodium) in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | Calcium ($Ca^{++}$) (mg/24hr) | Oxalate ($O^{-}$) (mg/24hr) | Sodium ($Na^{+}$) (mmol/24hr) |
|---|---|---|---|
| **Control –ve (G1)** | 1.21±0.079$^{e}$ | 204 ±3.01$^{f}$ | 14.4 ±1.28$^{e}$ |
| **Control +ve (G2)** | 3.17±0.184$^{a}$ | 310±2.70$^{a}$ | 41.3±3.32$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 2.46±0.223$^{c}$ | 262 ±2.55$^{c}$ | 27.7±3.26$^{c}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 1.88±0.129$^{d}$ | 228±3.28$^{e}$ | 18.8±1.11$^{d}$ |
| ***Urtica dioica* 250 mg (G5)** | 2.82±0.191$^{b}$ | 283±3.16$^{b}$ | 33.8±2.69$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 2.36±o.195$^{c}$ | 239±1.58$^{d}$ | 25.0±3.02$^{c}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (6):** Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on Urine ( Magnesium, Phosphor, and Potassium ) in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | Magnesium($Mg^{++}$) (mg/24hr) | Phosphor ($ph^{-)}$ (mg/24hr) | potassium ($K^{+}$) (mmol/24hr) |
|---|---|---|---|
| **Control –ve (G1)** | 2.73± 0.080$^{a}$ | 7.64± 1.01$^{e}$ | 19.28± 2.38$^{e}$ |
| **Control +ve (G2)** | 1.78 ± 0.105$^{e}$ | 30.27 ± 2.79$^{a}$ | 33.36 ± 1.91$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 2.09 ± 0.102$^{c}$ | 27.41± 1.48$^{b}$ | 28.39 ± 1.55$^{c}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 2.41±0.090$^{b}$ | 13.26± 1.68$^{d}$ | 25.18± 0.99$^{d}$ |
| ***Urtica dioica* 250 mg (G5)** | 1.97±0 .074$^{d}$ | 24.62 ± 2.58$^{c}$ | 30.96 ± 1.19$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 2.19±0 .082$^{c}$ | 25.34± 1.38$^{bc}$ | 27.47± 1.33$^{c}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (7):** Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on urine creatinine, Urine volume, and creatinine clearance in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | Urine creatinine (mg/dl) | Urine volume (ml/24hr) | Creatinine clearance (ml/min) |
|---|---|---|---|
| **Control –ve (G1)** | 22.01± 1.82$^{a}$ | 11.87± 1.55$^{a}$ | 0.35± 0 .07$^{a}$ |
| **Control +ve (G2)** | 10.0±1.58$^{e}$ | 5.06± 0 .70$^{d}$ | 0.03±0 .009$^{c}$ |
| ***Tribulus terrestris* 250mg (G3)** | 13.25±2.13$^{d}$ | 8.16± 0.83$^{c}$ | 0 .08±0.027$^{c}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 20.21±1.03$^{ab}$ | 10.64±3.02 $^{ab}$ | 0 .23± 0.052$^{b}$ |
| ***Urtica dioica* 250 mg (G5)** | 15.73±0.66$^{c}$ | 7.62±1.18$^{c}$ | 0 .09± 0.026$^{c}$ |
| ***Urtica dioica* 500 mg (G6)** | 18.88±1.40$^{b}$ | 9.47 ±0.88$^{bc}$ | 0.18 ± 0.043$^{b}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (8):** Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on urine (PH, Specific gravity, RBCs, and Pus cells) in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | PH | Specific gravity | RBCs | Pus cells |
|---|---|---|---|---|
| **Control –ve (G1)** | 5.16 ± 1.26$^{c}$ | 1020 ±0.29$^{d}$ | 1.50 ±0.50$^{e}$ | 0.80±0.10$^{c}$ |
| **Control +ve (G2)** | 8.50 ±1.0$^{a}$ | 1027±2.5$^{a}$ | 7.50±0.50$^{a}$ | 5.50±0.50$^{a}$ |
| ***Tribulus terrestris* 250mg (G3)** | 7.0 ± 1.0$^{abc}$ | 1024 ±0.50$^{bc}$ | 4.50± 0.50$^{bc}$ | 3.00±1.0$^{b}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 6.27± 1.17$^{bc}$ | 1022±0.50$^{c}$ | 2.77 ±0.25$^{d}$ | 1.70± 0.26$^{c}$ |
| ***Urtica dioica* 250 mg (G5)** | 7.60±1.15 $^{a b}$ | 1025±0.00$^{b}$ | 5.00 ±1.0$^{b}$ | 3.0±1.0$^{b}$ |
| ***Urtica dioica* 500 mg (G6)** | 6.50± 1.0$^{abc}$ | 1023 ±1.0$^{bc}$ | 3.50 ±0.50$^{cd}$ | 2.86±0.06$^{b}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (9):** Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on serum tumor necrosis factor (α –TNF) in hyperoxaluria rats (M±SD)

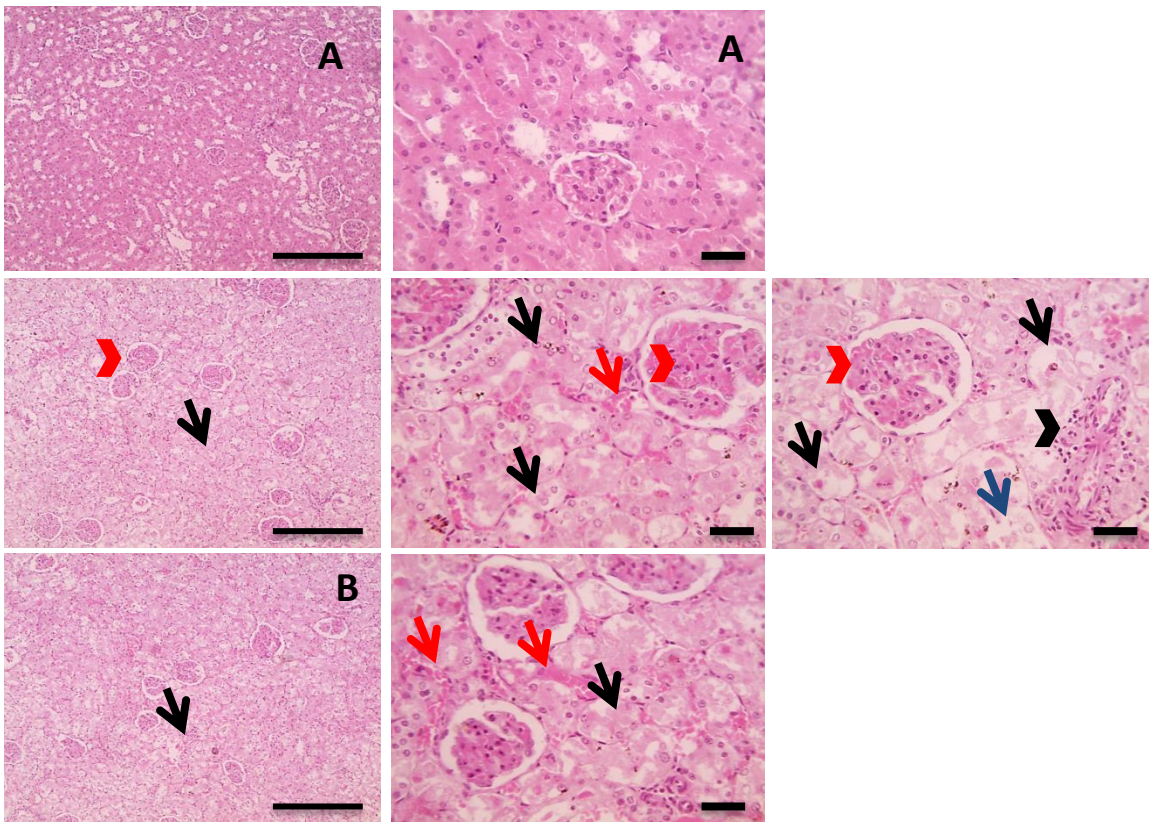
| Parameters<br>Groups | (α–TNF) (pg/ml) |
|---|---|
| **Control –ve(G1)** | 20.66± 2.80$^{f}$ |
| **Control +ve(G2)** | 83.40±2.23$^{a}$ |
| ***Tribulus terrestris* 250 mg** | 46.660±2.29$^{c}$ |
| ***Tribulus terrestris* 500 mg** | 27.18±4.41$^{e}$ |
| ***Urtic dioca* 250 mg** | 55.42±1.52$^{b}$ |
| ***Urtic dioca* 500 mg** | 32.72±2.23$^{d}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*

**Table (10):** Effect of *Tribulus Terrestris* and *Urtica dioica* aqueous extracts on antioxidant enzymes (CAT, SOD), (MDA) and NO in hyperoxaluria rats (M±SD)

| Parameters<br>Groups | MDA (nmol/g.t) | NO (µmol/g.t) | CAT (U/g.t) | SOD (U/g.t) |
|---|---|---|---|---|
| **Control –ve (G1)** | 10.59±0.78$^{c}$ | 0.98±0.09$^{c}$ | 2.94±0.04$^{a}$ | 176.21±2.89$^{a}$ |
| **Control +ve (G2)** | 35.58 ±1.47$^{a}$ | 2.31±0.16$^{a}$ | 1.05±0.03d | 56.36±2.71$^{f}$ |
| ***Tribulus terrestris* 250mg (G3)** | 18.09±1.43$^{b}$ | 1.69±0.09$^{b}$ | 1.77±0.19$^{c}$ | 99.85±3.07$^{d}$ |
| ***Tribulus terrestris* 500 mg (G4)** | 12.74±1.91$^{c}$ | 1.19±0.22$^{c}$ | 2.31±0.20$^{b}$ | 135.19±2.23$^{b}$ |
| ***Urtica dioica* 250 mg (G5)** | 20.46±2.21$^{b}$ | 1.82±0.14$^{b}$ | 1.57±0.18$^{c}$ | 90.41±2.09$^{e}$ |
| ***Urtica dioica* 500 mg (G6)** | 12.71±1.21$^{c}$ | 1.22±0.07$^{c}$ | 2.25±0.06$^{b}$ | 129.54±2.88$^{c}$ |

*This means that the same column with completely different letters is significantly different at p<0.05.*


A
A
B

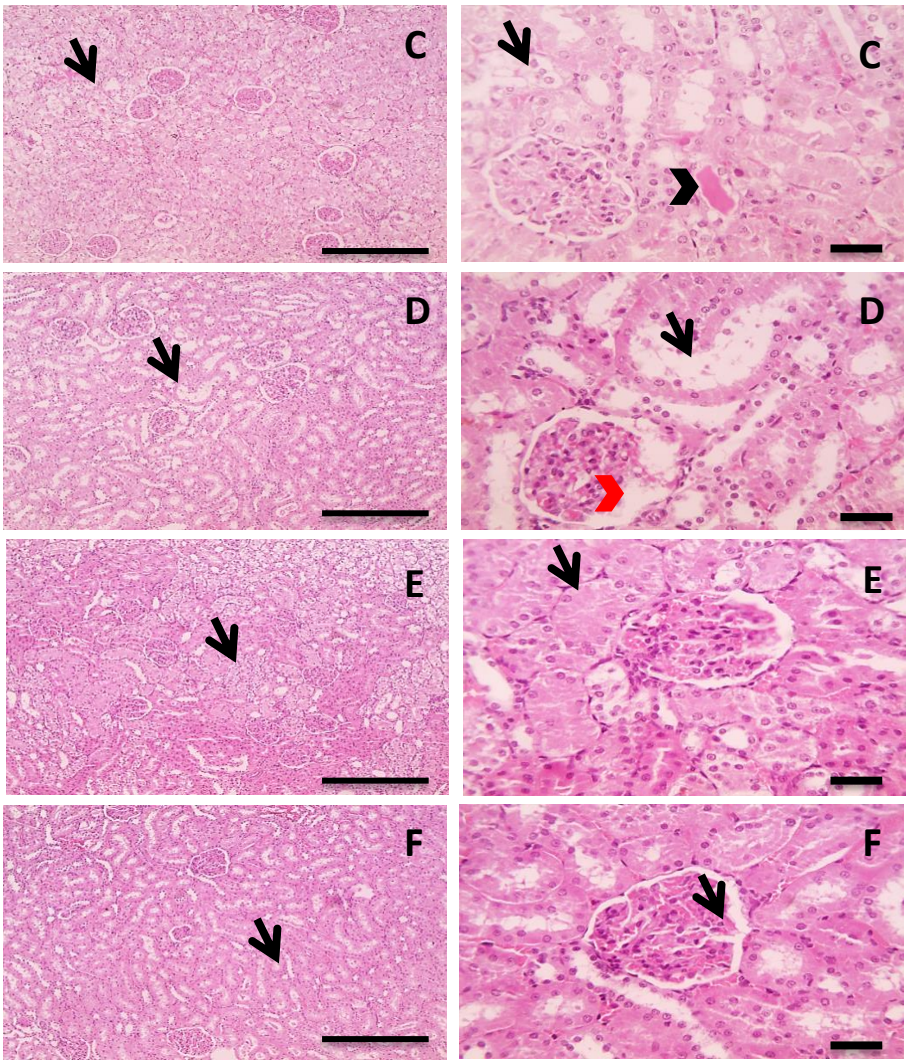



**Fig. 1. Representative images of Haematoxylin and Eosin stained kidney sections (x400) from A Normal Control group, B Urolithiasis control group, C *Tribulus Terrestris* 250 mg treated group, D *Tribulus Terrestris* 500 *Urtica dioica* 500 mg treated group.**

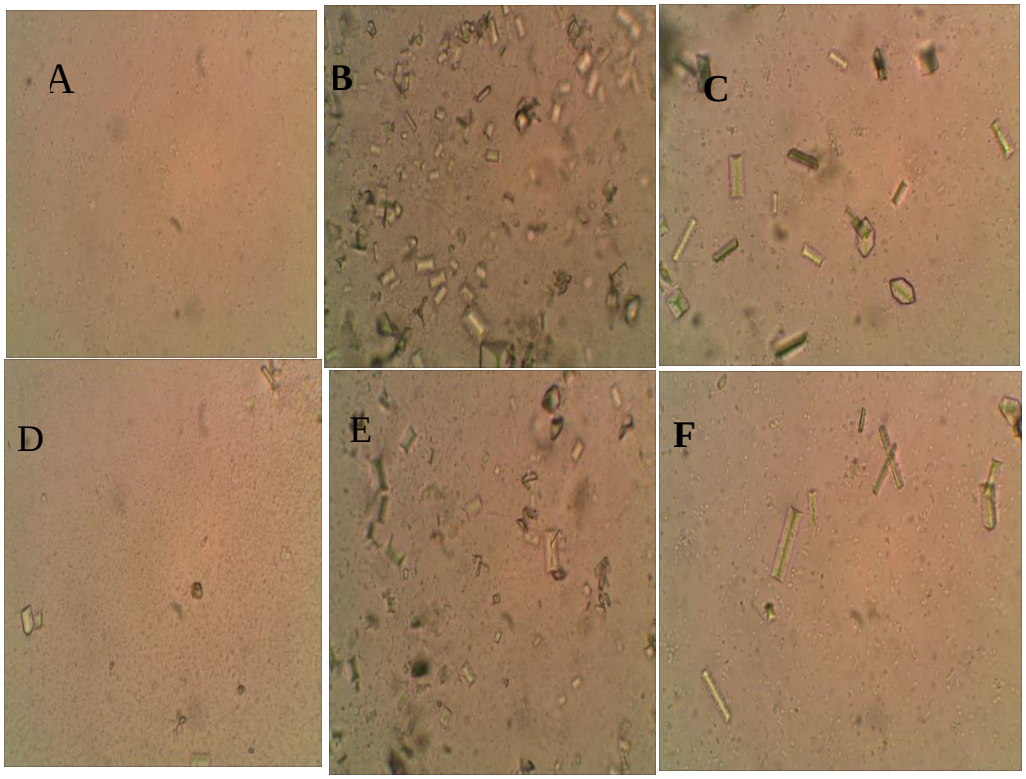



Fig. 2. Representative photographs of CaOx crystals from urine samples of experimental rats as observed under a light microscope (x1000) in A Normal Control group, B Urolithiasis control group, C *Tribulus Terrestris* 250 mg treated group, *D Tribulus Terrestris* 500 mg treated group, E *Urtica dioica* 250 mg treated group, F *Urtica dioica* 500 mg treated group.

# تأثير مستخلصات فاكهة القرطب الارضي واوراق القراص علي حصوات الكلى الناتجة عن أوكسالات الصوديوم في جرذان التجارب

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**المستخلص العربي**

يعد تكوين حصوات الكلى أحد أكثر اضطرابات المسالك البولية شيوعًا. أظهرت الدراسات السابقة أن التعرض طويل الأمد للأوكسالات سام للخلايا الطلائية للكلي وينتج عنه إجهاد تأكسدي. أجريت الدراسة الحالية لمعرفة تأثير المستخلصات المائية لثمار نبات القرطب الأرضي وأوراق نبات القراص ضد حصوات الكلي المتكونة بواسطة أوكسالات الصوديوم في جرذان التجارب .تسبب الحقن بأكسالات الصوديوم (70 مجم / كجم) داخل الصفاق لمدة عشرة أيام في تكوين حصوات الكلى وادي الى زيادة في مستويات المصل من الكرياتينين واليوريا وحمض البوليك وإلكتروليتات المصل والكتروليت البول ومكونات البول وعامل نخر ورم المصل ألفا ، وكذلك المستويات الكلوية من المالون داي الدهيد وعملت على تقليل تصفيه الكرياتين والاقلال من نشاط الانزيمات المضاده للاكسده(ديسموتاز الفائق والكتاليز) . المجموعات المعالجة بالمستخلصات المائية للقرطب الارضي والقراص (250 و 500 مجم / كجم من وزن الجسم) ، لمدة 28 يومًا على التوالي عملت على التحسن في المأخوذ الغذائي, الزيادة المكتسبة في الوزن, معدل كفاءة الغذاء و الوزن النسبي للكلي وظائف الكلى ، إلكتروليتات المصل ، الكتروليتات البول ، والمكونات البولية ، وعامل نخر الورم في المصل ، ونشاط الأنزيمات المضادة للأكسدة ،والفحص الهستوباثولوجي للكلي . اكدت نتائج التحسن البيوكميائيه إلى أن المستخلصات المائية للقرطب الارضي والقراص لها تأثير مضاد لحصوات الكلي التي يسببها أوكسالات الصوديوم لأنها أدت إلي انخفاض في تدهور أنسجة الكلي وعدد الحصوات المتكونة بها بسبب احتوائهم علي مضادات الاكسده.

**الكلمات المفتاحية:** القرطب ،القراص ، اوكسالات الصوديوم ، وظائف الكلى ، المعلمات الحيوية