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Impact of Almond (*Terminalia catappa*) Ethanolic Leaf Extracts on an Ethylene Glycol-Induced Urolithiasis Rat Model

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Abstract

The disease of renal stones has been recognized for centuries. It is one of the most com-mon disorders, characterized by calcifications in the kidneys, bladder, or urethra. Phy-tomolecules are effectively used in traditional medicine. The current study aimed to evaluate the effects of high and low doses of *Terminalia catappa* (*T. catappa*) leaf extracts on renal stone formation in a rat model of urolithiasis. The rats werehoused individually in metabolic cages and were given drinking water containing 0.75% Ethylene Glycol (EG) and 1% Ammonium Chloride (AC) to induce the production of kidney stones. EG and AC elevated the levels of molecules indicative of renal efficiency, including citrate, oxalate, urobilinogen, and microalbumin. Additionally, they reduced urine volume and urinary pH. After administering (200 or 400 mg/kg body weight) of Cystone or ethanolic extracts of *T. catappa* leaves orally, renal function parameters returned to normal ranges. Additionally, the urinary electrolytes were diluted, which may have contributed to a re-duced risk of calculus formation. Histological analyses were consistent with the bio-chemical data. This study demonstrated that Cystone and ethanolic extracts of *T. catappa* leaves exhibited protective properties against urolithiasis induced by EG in rats. The higher dose of *T. catappa* extracts showed a more significant effect compared to the lower dose.

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Introduction

The term urolithiasis refers to a disorder better known as kidney stone disease. Over the last 20 years, this pathology has had a significant effect on public health. Multiple types of calculi are implicated, including calcareous stones, i.e., calcium oxalate (CaOx) mon-ohydrate (COM), calcium oxalate dihydrate or apatite, and noncalcareous stones, such as uric acid, struvite, and cysteine. Approximately 80% of patients have stones formed from CaOx,

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whereas uric acid accounts for only 5%–10% of the cases. The National Health and Nutrition Examination Survey reported that the prevalence of renal calculi is 7.1% in fe-males and 10.6% in males.[1] In developed countries, approximately 10%–12% of the pop-ulation experience urolithiasis. A notable issue relating to health care is that urolithiasis recurrence rate is more than 50%. Recurrence per se or the resulting multiple therapeutic courses can ultimately give rise to renal impairment.[2]

The underlying causes of renal calculi are complicated and often unpredictable. Indeed, the pathogenesis is influenced by a disturbance of the equilibrium among renal promoters and inhibitors. Several intrinsic and extrinsic factors, together with diverse pathogenetic pathways, are implicated. Intrinsic elements include impaired calcium, oxalic acid, phosphorus, and uric acid metabolisms as well as an influence of nitrogenous waste me-tabolites such as urea. Extrinsic contributors encompass diet, dehydration, warm cli-mates, and drinking hard water.[3]

Kidney stones are generally categorized according to whether they are attached or not to tissues. Attached stones have a significant COM content and are connected to the renal papilla. They contain a center situated in close proximity to the place of attachment, known as the concave zone, and radially striated concentrically laminated layers toward their edge.[4] Unattached stones have no identified connection to the papilla. They arise within renal luminal areas, where urodynamic functions are diminished, and exhibit a broad spectrum of configurations and conformations.[5]

Clinical management of urolithiasis is dictated by the dimensions and localization of the calculus within the renal tract. Sizable stones that are initially unable to navigate through the renal tract require disintegration before elimination. Urine contains stone inhibitors, e.g., magnesium citrate, which combines with calcium ions to form a soluble complex and diminishes urinary CaOx supersaturation. The efficacy of this process differs among individuals.[6] Additional treatments include diuretics, probiotics, citrate, and chelating compounds. However, these options have pharmacological restrictions and long-term uti-lization might induce adverse effects and failure to eradicate calculi.

Surgical intervention can also have deleterious consequences, including complications such as hypertension, tubular necrosis, hemorrhage, and renal fibrosis.[7] Thus, it is essen-tial to use prophylactic methods and definitive treatment strategies for the management of renal stone disease. However, no efficacious agents allowing to eliminate calculi in their entirety are currently available. In several countries, numerous medicinal herbs have been employed to relieve renal stones. They have a broad range of nontoxic biological effects, i.e., antioxidant, diuretic, anti-microbial, anti-inflammatory, analgesic, antispas-modic, litholytic, and anticalcifying actions, without presenting any significant adverse effect.[8]

Ethylene glycol (EG) is toxic to the renal epithelium and can therefore precipitate renal stone formation, mostly by inducing the accumulation of oxalate crystals, which cause oxidative injury. The EG rat model is not entirely appropriate for the study of urolithiasis but provides insights into renal papillary calculus formation, particularly for stones that arise from cellular oxidative injuries.[9]

Kidney stone formation can occur when stones develop in the free solution within the re-nal collecting system. These stones can form relatively quickly, often accompanied by abnormal values in blood and urine, indicating the initial events leading to stone for-mation.[10, 11] Another model suggests a slower process, where stones form and attach to plaques in the renal interstitium. This process can take decades before the stones become clinically detectable.[11]

Kidney stones develop when the concentration of stone-forming minerals in urine sur-passes the level that can be dissolved. This process, known as nucleation, involves the in-itial formation of tiny crystals that may grow over time if the conditions remain favora-ble. Factors such as dehydration, diet, and certain medical conditions can all influence the formation of kidney stones.[12, 13]

CaOx renal calculi originate from an interstitial calcium phosphate plaque, named Ran-dall's plaque. Insoluble CaOx and soluble magnesium oxalate complexes arise through the attraction between oxalate and the respective cations. Insoluble CaOx deposits on Randall's plaque.

COM crystals bind to the renal papilla and crystal growth causes epi-thelial injury.[14] Hyperoxaluria then induces nucleation, crystal perpetuation, and growth, and finally, retention within the renal tubular system. Epithelial cell damages lead to free radical release that provokes a wide variety of crystal nucleation processes and crystal accumulation.[15]

Medicinal herbs contain ingredients enhancing serum antioxidant potency. Antioxidants are either natural or manmade. Natural antioxidants tend to not have adverse effects and might constitute ideal agents to diminish the consequences of free radical formation.[16]

Terminalia catappa (*T. catappa*), also referred to as tropical or sea almond, belongs to the Combretaceae or white mangrove family. Typically, it is grown in tropical climates for its ornamental value and its edible fruits and seeds.[17] Various parts of this plant are uti-lized in folk medicine against diarrhea or fever or for its hemostatic functions. It is found in several nations, e.g., India, the Philippines, Malaysia, and Indonesia.[18] Annually, be-tween 20 and 30 kg of fruits can be harvested per tree and 700,000 tons were picked worldwide in 2004.[19] The leaves have high oil content, about 30%–60%, and thus are an excellent lipid source. *T. catappa* foliage is richer in oil than frequently used soybeans, palms, and peanuts. Additionally, leaves from *T. catappa* are valued for their nutritional constituents, which are associated with antioxidant, hypoglycemic, gastroprotective, antimicrobial, antidiabetic, and anticancer properties. The foliage has high carbohydrate (76%) and low lipid (3%) contents.[20] In particular, antioxidant compounds and polyphe-nols, such as gallic acid, citric acid, and punicalagin, have been found.[17]

In view of the numerous medical attributes and biological effects of the different parts of *T. catappa*, considerable research has been performed and has demonstrated the efficacy of the traditional medical use of the leaves, bark, kernel, and fruits for the management of conditions such as dermatitis, helminthiasis, hepatitis, diarrhea, antioxidants, diabetes, and infections caused by various pathogens.

The present study aimed to explore the potential of using *T. catappa* as a prophylactic agent against the development of renal calculi.

Materials and Methods

Preparation of Ethanolic Extract of *T. catappa* Leaves

Leaves from *T. catappa* came from a local market vendor. They were dried in a dark room at ambient temperature and pulverized with an electric grinder. A Soxhlet extractor was used for the reflux of 100-g leaf powder and 1 liter of 99.5% ethanol at 70°C for 48 h. Vacuum filtration was performed through a number 1 Whatman filter paper to dissociate the liquid extract from the solid residue. The liquid extract was then reduced to a small volume using a rotary evaporator. The samples were then freeze dried to keep a constant mass and the product was stored in airtight conditions until needed.

Phenolic Compound Identification

The total phenolic components of the extract were obtained using a validated method.[21] Briefly, 250 µl of extract diluted in dimethyl sulfoxide was combined with 1.25 ml of Fo-lin–Ciocalteu (F–C) reagent, diluted 1:1 in distilled water, in a test tube and incubated for 10 min. A further 30-min incubation in the dark was conducted following the addition of 1 ml of 7.5% sodium bicarbonate solution. Spectrophotometric measurements were per-formed at 650 nm.

Flavonoid Compound Identification

The total flavonoid content was determined using a Method modified from the previous work.[22] A calibration curve was produced from a mixture of aluminum chloride and quercetin standard. An initial 0.32-mg/ml quercetin solution was used and diluted at dif-ferent concentrations. A calibration curve was obtained by spectrophotometry analyses at 415 nm of each quercetin dilution. We used 10% aluminum chloride and 1-M potassium acetate. Indeed, when aluminum chloride encounters flavonoid groups, it typically yields stable acid complexes either through the C-4 keto group or between the C-3 and C-5 OH groups. It may also create acid labile compounds through the orthodihydroxyl groups at the flavonoid A- or B-ring sites.

Drugs and Chemicals

Sigma Aldrich Chemical Co. (St. Louis, Missouri, United States) was the vendor for EG and ammonium chloride (AC). Cystone was purchased from the Himalaya Drug Company Makali, Bangalore 562, 123, India. Cystone is a polyherbal formulation,

predominantly comprised of pharmaceuticals derived from plants. It is extensively employed for its an-tilithic properties in traditional medicine. The impact of Cystone at a dose of 750 mg/kg body weight was investigated on urolithiasis induced in an experimental rat model.

Experimental Animals

A total of 35 adult male albino rats, mean bodyweights ranged from 97.8 to 100.2 g. Rats were divided into five groups with similar average bodyweights and kept singly in meta-bolic cages with a wire mesh base, following the National Institutes of Health Guiding Principles for the Care and Use of Animals. Temperature and humidity were kept con-stant at 25°C ± 5°C and 50% ± 10%, respectively, and animals were housed under a 12/12 h light/dark cycle. The study was conducted in accordance with the Declaration of Hel-sinki, and approved by Standing Committee for Scientific Research (REC-46/07/968).

EG Model of Urolithiasis

Rats were divided into five groups of seven animals each. The groups were assigned the following protocols:

Group 1: Controls, fed a routine rat chow diet with free access to tap water for 28 days.
Group 2: Urolithiasis rats, fed a normal diet. Drinking water contained 0.75% EG v/v and 1% AC for 28 days in order to provoke urolithiasis.
Group 3: As group 2, but with the administration of Cystone at a dose of 750 mg/kg body weight from day 14 to day 28.
Group 4: As group 2, but with the administration of a low dose (200 mg/kg body weight) ethanolic extract from *T. catappa* leaves from day 14 to day 28.[23]
Group 5: As group 2, but with the administration of a high dose (400 mg/kg body weight) ethanolic extract from *T. catappa* leaves from day 14 to day 28.[23]

Collection of Urine and Serum Samples

At day 28, the rats were placed in individual metabolic cages for 24 h. Total urinary volume, in ml, was evaluated using a measuring cylinder.[24] A pH meter was used to as-sess the urinary pH. After a 12-h starvation period, the animals were sacrificed by cervi-cal decapitation. Blood samples were obtained by retroorbital puncture and centrifuged at 10,000 rpm for 20 min in order to isolate the serum for biochemical assays. The kidneys were removed, weighed, and stored for histopathological assessment.

Assessment of Antiurolithic Activity: Urine and Serum Analyses

Urine specimen was acidified with 10% hydrochloric acid in order to block bacterial and fungal replication. The samples were then placed into cold storage at a temperature below 4°C. Commercial testing kits from BioVision Co. (Milpitas, USA) were utilized to meas-ure creatinine, urea, and uric acid concentrations in urine. Randox diagnostic kits were used to detect microalbumin, following the manufacturer's protocol. Citrate levels were assessed according to the method published by Rajagopal[25] and oxalate levels were measured using the protocol suggested by Hodgkinson.[26] The amounts of sodium, potas-sium, calcium, and chloride were determined with an automated analyzer. Analyzer kits from MyBioSource Inc. (San Diego, CA, USA) were used for urobilinogen measurement. Serum assays of creatinine, the blood urea nitrogen profile, and uric acid were performed using a Biovision Kit (CA, USA).

Histopathological Studies

Kidneys obtained from the rats were weighed and then quickly immersed then shortly fixed in 10% formalin. Paraffin-embedded organs were thinly sliced in to microtome and stained with eosin and hematoxylin. They were then studied to evaluate any histopatho-logical alterations.

Statistical Analyses

One-way analysis of variance was used for statistical analyses. The Software Package for Social Sciences software was employed (version 15.0 Inc., Chicago, IL, USA). Statistical significance was defined for p values ≤ 0.05. All data are presented as means ± standard deviations (SDs).

Results

Total Flavonoid and Phenolic Contents in Ethanolic Extracts from *T. catappa* Leaves

Total flavonoid and phenolic contents in ethanolic extracts from *T. catappa* leaves are presented in Table 1. *T. catappa* leaves contained significant amount of flavonoid and phenolic compounds.

Table 1. Total phenolic and flavonoid contents of ethanolic extracts from *T. catappa* leaves

Parameter	Flavonoid test	Phenolic compound test
Ethanolic extract	77.21 ± 0.83	260.10 ± 3.67

Data are presented as means ± SDs.

Effects of Ethanolic Extracts on Kidney Weight and Urinary Parameters

The effects of *T. catappa* leaf ethanolic extracts on kidney weight, urinary pH, and urine volume are presented in Figure 1. A significant increase in kidney weight and decrease in urinary pH and urine volume were observed in urolithiatic rats (group 2) compared with those of controls. The kidney weight was significantly decreased and the urinary pH and urine volume were increased in urolithiatic rats treated with Cystone compared with those of nontreated rats. Ethanolic extracts from *T. catappa* leaves at low and high doses significantly reversed the effects of urolithiasis on kidney weight, urinary pH, and urine volume. The effects were comparable to those obtained with Cystone. Importantly, there was no significant difference between the rats treated with low or high doses of extracts, indicating the high efficacy of extract even at lower concentration.

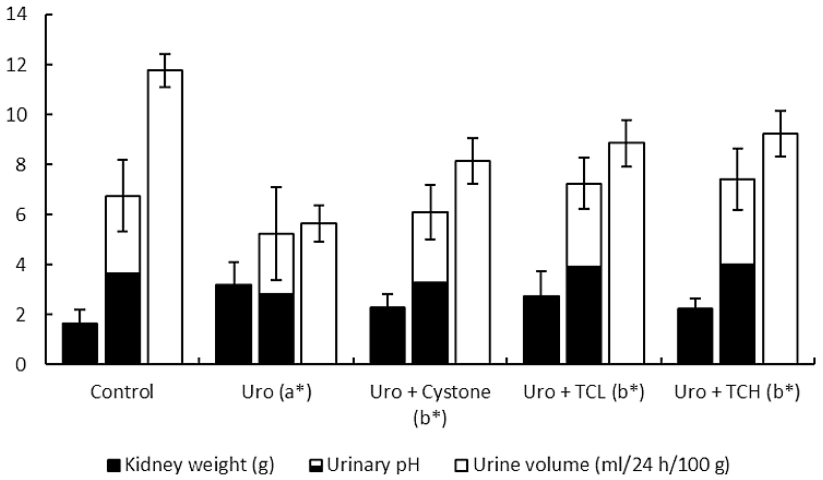

Fig. 1. Effects of the different treatments on kidney weight, urinary pH, and urine volume.
Uro, urolithiatic rats; Uro + Cystone, urolithiatic rats treated with Cystone; Uro + TCL, uro-lithiatic rats treated with a low dose of *T. catappa* leaf ethanolic extract; Uro + TCH, urolithiatic rats treated with a high dose of *T. catappa* leaf ethanolic extract. [a]Compared with controls, [b]compared with Uro group; *p < 0.05. Data are presented as means ± SDs (n = 8).

Effects of Ethanolic Extracts on Serum Levels of Kidney Functional Markers

The serum levels of kidney functional markers measured in the different treatment groups are provided in Figure 2. As expected serum levels of creatinine, urea nitrogen, and uric acid were significantly elevated in urolithiatic rats compared with those in con-trols. In contrast, these serum levels were significantly decreased in rats treated with Cystone or with low or high doses of ethanolic extracts compared with those found in urolithiatic rats. The low and high concentrations of extracts had identical effects on se-rum creatinine levels, whereas urea nitrogen and uric acid levels were significantly lower in rats treated with a high dose of extract than those measured in rats treated with a low dose of extract.

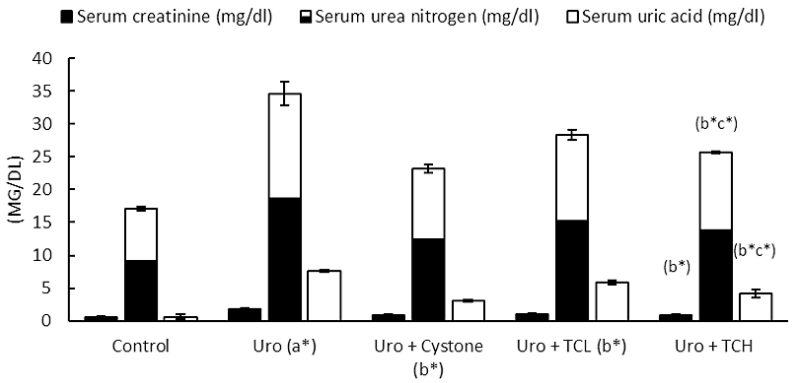

Fig. 2. Effects of the different treatments on serum levels of creatinine, urea nitrogen, and uric acid

Uro, urolithiatic rats; Uro + Cystone, urolithiatic rats treated with Cystone; Uro + TCL, urolithiatic rats treated with a low dose of *T. catappa* leaf ethanolic extract; Uro + TCH, urolithiatic rats treated with a high dose of *T. catappa* leaf ethanolic extract. [a]Compared with controls, [b]compared with Uro group, ccompared with Uro + TCL group; *p < 0.05. Data are presented as means ± SDs (n = 8).

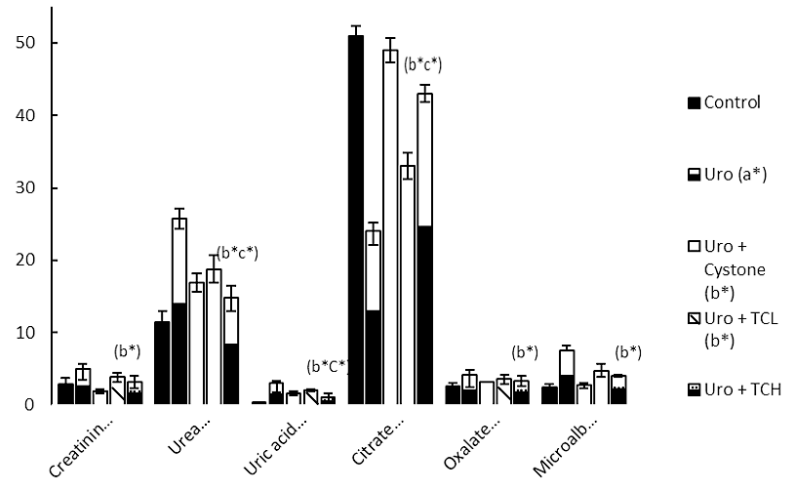

Fig. 3. Effects of the different treatments on urine levels of kidney functional markers

Uro, urolithiatic rats; Uro + Cystone, urolithiatic rats treated with Cystone; Uro + TCL, urolithiatic rats treated with a low dose of *T. catappa* leaf ethanolic extract; Uro + TCH, urolithiatic rats treated with a high dose of *T. catappa* leaf ethanolic extract. [a]Compared with controls, [b]compared with Uro group, ccompared with Uro + TCL group; *p < 0.05. Data are presented as means ± SDs (n = 8).

Effects of Ethanolic Extracts on Urine Levels of Kidney Functional Markers

The urine levels of kidney functional markers measured in the different treatment groups are presented in Figure 3. Urine levels of creatinine, urea nitrogen, uric acid, oxalate, and microalbumin were significantly increased and citrate levels were significantly decreased in urilithiatic rats compared with those measured in control rats. In contrast, significantly lower urine levels of creatinine, urea nitrogen, uric acid, oxalate, and microalbumin and higher levels of citrate were found in lithiatic rats treated with Cystone or low or high concentrations of ethanolic extracts than those measured in untreated lithiatic rats. Cre-atinine, oxalate, and microalbumin levels in rats treated with low or high doses of ex-

tracts were comparable, whereas the decrease in urea nitrogen and uric acid and increase in citrate levels were more significant in rats treated with a high dose of extract compared with those in rats treated with a low dose of extract.

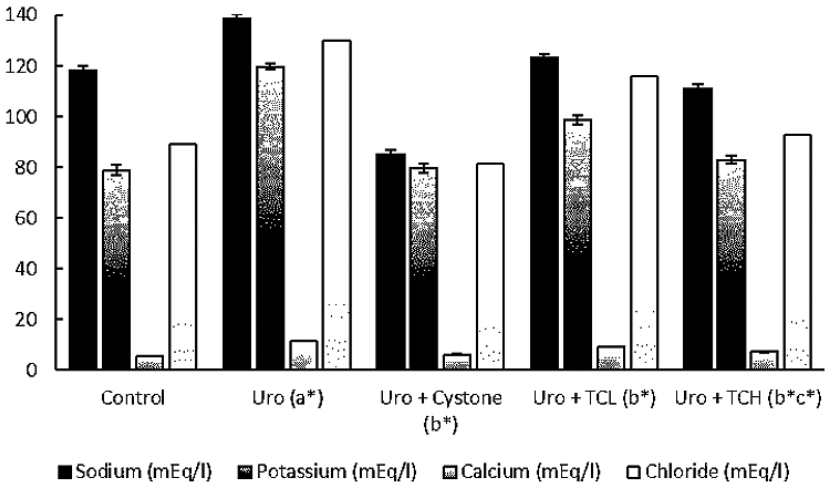

Fig. 4. Effects of the different treatments on urine electrolyte levels

. Uro, urolithiatic rats; Uro + Cystone, urolithiatic rats treated with Cystone; Uro + TCL, urolithiatic rats treated with a low dose of *T. catappa* leaf ethanolic extract; Uro + TCH, urolithiatic rats treated with a high dose of *T. catappa* leaf ethanolic extract. [a]Compared with controls, [b]compared with Uro group, ccompared with Uro + TCL group; *p < 0.05. Data are presented as means ± SDs (n = 8).

Effects of Ethanolic Extracts on Urine Electrolyte Levels

The urine levels of electrolytes measured in the different treatment groups are provided in Figure 4. A significant increase in sodium, potassium, chloride, and urobilinogen lev-els was found in urolithiatic rats compared with those in controls. The treatment of uro-lithiatic rats with Cystone or low or high doses of ethanolic extracts resulted in a signifi-cant decrease in the levels of all measured electrolytes. Importantly, the high dose of ex-tract had significantly stronger effects on all electrolyte levels compared with those of the low dose.

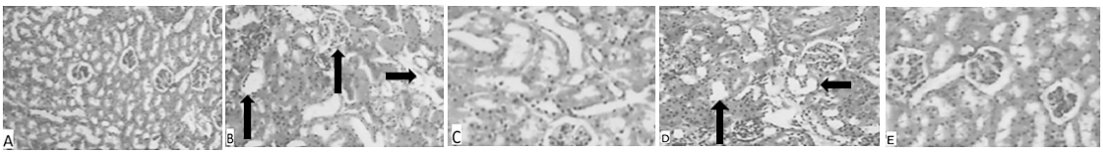

Fig. 4. Microscopic images of kidney tissue sections (a) Normal glomerular structure observed in the control group (b) Renal tubular damage (red arrows) present in the urolithiatic group (c) Dilation in some renal tubules and normal glomerular structure observed in urolithiatic rats treated with Cystone (d) Tubular dilation with some damage in renal tu-bules in urolithiatic rats treated with a low dose of *T. catappa* leaf ethanolic extract. (e) Normal renal tubular structure in urolithiatic rats treated with a high dose of *T. catappa* leaf ethanolic extract compared with the kidney histology in the urolithiatic group

Effects of Ethanolic Extracts on Kidney Histology

Histological changes in kidneys in response to different treatments are presented in Fig-ure 1. Marked pathological changes including renal tubular damage, dilation of renal tu-bules, and altered glomerular structure, were observed in tissues

sections from urolithiat-ic rats compared with the normal histology found in sections obtained from control rats. On the other hand, tissue sections from rats treated with Cystone showed dilation in few renal tubules and a normal glomerular structure. Similarly, tubular dilation and partial damage to renal tubules were found in tissue sections from rats treated with a lower con-centration of ethanolic extract from *T. catappa* leaves. Kidneys of rats treated with a high dose of extract presented a marked decrease in tubular dilation and normal tubular and glomerular structures.

Discussion

Renal stones arise as a consequence of the interaction between a number of biological occurrences stimulated by genetic vulnerability, dietary components, and lifestyle altera-tions, which disturb the equilibrium between renal inhibitors and promoters.[27] Several in vivo experimental models have been generated to explore kidney stone pathogenesis.

In the present study, male rats were chosen as models of renal stone disease because their urinary system is analogous to that of humans.[28] An accelerated model consisting of the administration of 0.75% EG and 1% AC was selected. This combination acidifies urine and consequently potentiates CaOx stone formation.[29] Earlier research reported that treatment of male albino rats for 28 days with 0.75% EG induces the formation of renal stones predominantly formed from CaOx.[30]

Here, an increased renal weight, lower urine volume, and decreased urinary pH were found in rats receiving EG compared with the control group, suggesting the presence of CaOx calculi. These data are in agreement with previous research from.[31] who used 1% v/v EG in albino rats to induce CaOx urolithiasis. In this study, the rats presented elevat-ed urine levels of oxalate, calcium, and phosphate. Hyperoxaluria is considered a greater risk factor for renal stone formation then hypercalciuria.

Several acidic byproducts, i.e., oxalic, benzoic, formic and hippuric acids, arise from the metabolism of EG and induce a metabolic acidosis, leading to increased urine acidity. Typically, a pH between 5.0 and 6.5 favors CaOx calculus formation [32] by facilitating CaOx nucleation, growth, accumulation, and crystallization. Ultimately, the calculi are retained in the renal tissue, which leads to toxicity to renal mitochondria in a manner analogous to CaOx stones.[33] CaOx calculus development is also influenced by renal out-put. In the present study, the reduced urine volume suggested a urinary tract obstruction caused by calculi. These findings are in line with previously published data.[29]

EG also caused renal impairment. Indeed, compared with the untreated control cohort, higher urine and serum titers of creatinine, uric acid, and urea as well as increased levels of urine citrate, sodium, potassium, calcium and chloride oxalate minerals, microalbu-min, and urobilinogen were found in rats receiving EG. A significant kidney injury was indicated by the greater amounts of waste metabolites, i.e., creatinine and uric acid, in the serum.[34] previous study have postulated that an increased elimination of these com-pounds is associated with calculus formation by providing ideal conditions for crystalli-zation.[35] The stone-forming potential of EG is predominantly due to increased oxalate concentrations and the consequent oxidative injury. Augmented renal deposition of cal-cium and oxalate is consistently found with EG and AC-provoked renal stone formation.[36] Here, rats with renal stones had elevated serum and urine concentrations of urea and uric acid. In the presence of kidney stones, the glomerular filtration rate is reduced be-cause of the physical obstructive effect of the calculi. Therefore, more waste products are entering the circulation, especially in the form of the nitrogen-based chemicals, urea, creatinine, and uric acid. Enhanced lipid peroxidation and reduced antioxidant capacity have also been described in kidneys of rats fed a calculus-inducing regime.[8]

Interestingly, the present data are not in agreement with a study by Wanger and Mohebbi [37] showing that urine rinses out calcium and uric acid and consequently diminishes the risk of salt formation and deposition and development of calculi. However, the progres-sive dilution of calcium and uric acid in urine seen in rats with provoked urolithiasis was in agreement with previous studies.[38]

EG is quickly absorbed and broken down into glycolic and glyoxylic acids before oxida-tion-reduction pathways transform these metabolites into glycolate and oxalate. This process is catalyzed

by enzymes such as glycolic acid oxidase and lactate dehydrogenase.[39] Cystone was utilized as a standard control in this experiment. It was selected because of its polyherbal constituents and its wide use in traditional medicine for the management of patients with kidney stones. Its anticalculus properties are numerous and encompass crystal breakdown, diuretic effects, and smooth muscle relaxation within the urinary tract. Because of its various activities, Cystone effects can be compared with those of a test drug to assess the effectiveness and mode of action of the latter.[40]

The present data parallel findings of two studies, which demonstrated that, the dimin-ished serum and potassium levels were returned to the normal range after Cystone inges-tion.[41,42] Cystone might act by inhibiting the production of the hepatic enzyme, glycolate oxidase.[43] Furthermore, after Cystone administration, the urinary pH returns to normal, patients report lesser dysuria, and urinary bacteria are kept at bay. previous work have described a relief from an irritable bladder as a consequence of Cystone antiinflammatory actions.[44]

Globally, numerous plants are used for urolithiasis patient relief, and they are inexpen-sive pharmaceutical agents. Phytotherapeutic compounds have been evaluated in in vivo, in vitro, and clinical settings as a treatment on their own or in conjunction with other therapies.[45] These plant-derived chemicals offer a spectrum of actions on the different components of the renal calculus formation pathway. These actions include initiation of diuresis, inhibition of CaOx crystal precipitation, augmentation of glycosaminoglycan ti-ters, reduction in renal oxalate and calcium elimination, and enhancement of urinary cit-rate excretion. All these mechanisms might contribute to the prophylaxis and manage-ment of renal stone disease.[46]

From a prophylactic perspective, phytochemicals have additional modes of action, in-cluding the inhibition of crystallization and crystal amassment as a consequence of en-hanced magnesium excretion but also antioxidant, renoprotective, cytoprotective, and an-tispasmodic properties.[47] In the present work, high and low doses of ethanolic extracts from *T. catappa* leaves were administered to urolithiasis rats. Another work have inves-tigated the use of both aqueous and ethanolic extracts from almond tree.[48] They reported that the aqueous extract has four active compounds, i.e., punicalagin, punicalin, gallic acid, and ellagic acid. *T. catappa* leaf extracts reduced calcium and uric acid concentra-tions, thus inhibiting stone formation, which was in agreement with previous study.[49] These actions might reflect the antioxidant effects and consequently the prophylactic impact on calculus development.[50] Furthermore, the data presented here on the renopro-tective properties of *T. catappa* concur with the work from Divya and Anand.[51] Numer-ous publications have shown an antistone formation action of various phytomolecules in in vitro nucleation assays, suggesting that such pharmaceuticals have potential prophy-lactic benefit.[52]

An interesting work reported similar findings and postulated that the almond tree family offers protection to the kidneys.[53] It has been suggested that the different effects induced by different doses of T.[54] catappa leaf extracts result from their capacity to diminish ox-idative stress. Oxidative stress potentiates crystallization and crystal aggregation, which leads to the development of calculi.[15] Urinary calcification is induced by numerous ele-ments. However, several studies have described the contribution of oxygen free radicals to cellular damages, thus reinforcing the CaOx persistence within the renal tubules and subsequent urolithiasis. The natural substances from *T. catappa* have been isolated.[55] They documented the presence of triterpenoids, i.e., ursolic and asiatic acids and squalene but not of caffeine, flavonoids, such as isovitexin, vitexin, and rutin, gallic acid, hydrolyzed tannins predom-inantly in the form of punicalagin anomers, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granato B, and corilagin. A number of studies have proposed that saponins exert prophylactic effects against kidney stones through diuresis and disintegration of mucoprotein suspensions, which enhance crystallization.[56]

A vitro indicated that gallic acid exhibits an anticrystallization action by chelating cal-cium.[57] The tannin components of *T. catappa* have been shown to improve microvascu-lature functions and to safeguard the tubular epithelium in an in vivo murine model of sepsis-induced acute renal damage.[58] Additionally, tannins exert anticrystallization ef-fects by enhancing the formation of calcium

complexes, thus hindering the development of CaOx crystals.[59] It was investigated that the antioxidant properties of hydrolyzed *T. catappa* leaf extracts.[60] These authors documented a positive association between the quantity of phenols and the antioxidant capacity of the extracts, suggesting that the ex-tract polyphenolic components are major actor in this activity. Punicalagin is one of the many polyphenolic substances contained within hydroalcoholic *T. catappa* extracts, and it has been associated with antiinflammatory effects responsible of diminishing oxidative stress and inhibiting renal cell death.[61] These results were in agreement with a previous finding.[62] that noted that almond leaves could be consumed as part of a diet to relieve symptoms related to oxidative stress and tissue pathologies.

Our histopathological analysis demonstrated that the kidneys from the rats receiving EG and AC had different tubular structures than that of the control group and presented an irregular mononuclear inflammation. These histological characteristics might represent oxalate-provoked lipid peroxidation and kidney tissue injury.[63] Adding Cystone or *T. catappa* extracts to the rats' diet prevented these histological alterations as a marked re-duction in the damage index of the kidney structure was found, which was consistent with the diuretic properties of these two compounds. These data are also in agreement with previous studie.[64, 65]

Conclusion

Ethanolic extract of *T. catappa* leaves had a notable prophylactic effect against the for-mation of renal calculi in the presence of urolithiasis-inducing substances. It inhibited stone development, decreased calculus frequency, and dispersed renal CaOx stones. Thus, this experimental in vivo rat model was successfully utilized to investigate the possible antiurolithic properties of ethanolic *T. catappa* leaf extract on CaOx renal caculi.

It is essential to recognize that the effectiveness of a dose can vary depending on the ob-jectives. Research on (*T. catappa*) leaf extract shows that different dosages can produce different results. For example, one study found that a dose of 200 mg/kg significantly in-creased certain liver enzymes. In contrast, a higher dose of 400 mg/kg caused an even more substantial increase in multiple liver enzymes, Which highlights the need for cau-tion when considering higher doses, as they may raise the risk of side effects. Therefore, it is essential to approach dosing thoughtfully, considering the potential effects on the body.[62] In fact, It is important to identify the specific effects the desire to achieve and watch for any negative reactions. Seeking advice from a healthcare professional or re-searcher in this field can offer more personalized guidance. Further studies are necessary to fully understand these effects and determine the optimal dosage for safety and efficacy. It is important to monitor renal markers, as well as other potential biomarkers and physiological responses, to gain a comprehensive understanding of the extract's impact.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

All data included in this study are available from the corresponding author upon reasona-ble re-quest.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by Standing Committee for Scientific Research (REC-46/07/968).

Informed Consent Statement

This study did not involve human participants, or any material requires ethical approval.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

- **Ghalia Shamlan:** Conceptualization, Methodology, Analysis and Interpretation of Data, Writing – Original Draft, Supervision, Review and Editing the Manuscript.
- **Wafa Suliman Alansari:** Data Collection, Methodology, Analysis and Interpretation of Data, Supervision, Review and Editing the Manuscript.

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