American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

The Antibacterial and Antiuroletiasis Activities of Cissus Rotandifolia Extract on Urolithiasis Rats Induced by Ethylene Glycol and its Mechanism as Antiurolithiasis Remedy.

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To Cite This Article: *Hussein S Gumaih, Afrah A Alasbahy, Salem H Alharethi, Fayed Al-Yousofy, Elsayed N Salamah. The Antibacterial and Antiuroletiasis Activities of Cissus Rotandifolia Extract on Urolithiasis Rats Induced by Ethylene Glycol and its Mechanism as Antiurolithiasis Remedy.. Am J Biomed Sci & Res. 2022 - 16(1). AJBSR.MS.ID.002184.* DOI: 10.34297/AJBSR.2022.16.002184

Received: March 22, 2022; **Published:** April 01, 2022

Abstract

Cissus rotandifolia (CR) is a medicinal plant widely used in the southern region of Saudi Arabia and Yemen. The present study aimed to evaluate the antibacterial and antiurolithiasis effect of CR and its mechanism. Thirty rats divided into five groups: Group A (negative control), Group B were treated with EG and ammonium chloride (AC) and served as positive group. Group C and D served as treated groups and treated as B group with 200 mg/kg and 400 mg/kg of CR respectively for 28 days and group E fed as group B for 28 days and then treated for28 days by 1000 mg CR extract and served as curative group. Another 10 rats divided into two groups: group F took normal diet and served as negative control group and group G took normal diet with 500 mg/kg of CR to investigate the mechanism of CR as antiurolithiatic substance.

At the end of the experiment blood samples were collected from rats. Kidney rats were removed to histopathological examined.

We found a significant decrease in serum urea, creatinine, and MDA of CR groups. Also the kidneys of CR treated group appeared mostly to be calculi-free compared to positive control. A significant increase in water intake, urine volume, urinary magnesium, citrate and urinary pH of CR treated rats when compared to negative control. These results proved the antibacterial activity of CR

The present study emphasized the safe herbal remedies of CR as antioxidants, antibacterial, nephroprotective as well as its antiurolithiatic role. We recommended to use it in pharmaceutical forms as it is safe and effective as antiurolithiasis is remedy.

Keywords: Cissus Rotandifolia; Antiuroletiasis; Antibacterial; Urolithiasis

Introduction

Kidneys have a critical function of the urinary system. They are involved in the excretion of metabolic waste products and chemicals, are responsible for the production of certain hormones and vitamins, and have a key role in blood pressure regulation. They are the maintenance of normal composition and volume of body fluid. This is accomplished by glomerular filtration, tubular reabsorption, and tubular secretion [1].

Urolithiasis (UL) means the accretion of a solid, hard mass of nonmetallic minerals inside the urinary tract. Stone formation is culmination of a series of physicochemical events like super-saturation, nucleation, growth and aggregation of the crystal [2]. Nearly 4-15% of the human populations suffer from urinary stone problem all over the globe [3].

Urolithiasis is generally composed of calcium as calcium oxalate (CaOx) (75-80%), magnesium as ammonium magnesium phosphate (struvite) (10%), uric acid (5-10%), and 0.5-1% is composed of cystin [4].

Oxalate induce injury of renal epithelial cell lines through its cytotoxic effects mediated by apoptosis, necrosis, release of cellular enzymes and membrane lipid peroxidation [5].

Common mechanisms of urolithiasis are superstauration, crystallization, change of urinary pH, metabolic alterations such as hypercalciuria and hyperuricosuria and deficiency of stone-inhibiting factors like citrate and magnesium [6].

There are a number of practices for treatment of urinary calculi, including surgery, endoscopic procedures such as ureter-scopy, extracorporeal shock wave lithotripsy (ESWL) and synthetic drugs. Medical management of urolithiasis is still challenging for modern medical practice. ESWL causes complications like decreased renal function, subcapsular hematomas, inflammation, ischemia, renal fibrosis, hemorrhage, hypertension and steinstrasse "multiple small stones blocking ureter" [7], while surgical methods are costly and require longer recovery times [8].

Although, some drugs used to prevent and treat urolithiasis, the overuse of synthetic drugs, results in higher incidence of adverse drug reactions and not completely solve the problem. Therefore, alternative treatments using natural resources showing antiurolithiatic activity are important. Medicinal plants are used worldwide, and there is increasing interest in treating kidney stones using medicinal plants [9].

Herbal medicines have many phytoconstituents which may exert their beneficial effect in kidney stone treatment. Plant extracts contain phytochemicals that inhibit stone formation by inhibiting synthesis and agglomeration of crystals [10]. Wild edible plants are species of plants that grow freely in the wild habitat without any agricultural treatments and can be consumed as a food [11]. Cissus rotundifolia (CR; Halas) is a wild edible plant grows extensively in the southern region of Saudi Arabia and Yemen, their leaves are widely consumed after cooking by local people as leafy vegetables [12]. Halas is used traditionally in Yemen for the treatment of gastrointestinal troubles [13],

Several studies suggested that CR extracts are beneficial as antiinflammatory and hepato-protective [14,15]. Most of the remedies are very useful, but their mechanisms of action remain unclear and need more investigations.

Materials And Methods

Experimental Animals

Male albino rats Rattus rattus (Rattus norvegicus albinus) each weighing about 200 - 250g were used in this study. The rats were reared in the animal house of Biology Department, Faculty of Science at Sana'a University. They were housed in a standard metallic cages under the same environmental conditions with an alternate 12 h light-dark cycle at room temperature (20±2°C). The animals had ad libitum access to a commercial diet and water. The bedding of the animal cages was changed every 48hrs. Animals were left seven days prior to the experiment for adaptation.

Animals were fed on a diet with the formula which was kindly supplied by the department of animal production, Faculty of Agriculture, Sana'a University.

Leaves of CR were collected from Taiz governorate, Yemen and were identified and authenticated at Botany Department, Faculty of Science, Sana`a University. Plant was carefully washed with tap water, rinsed with distilled water, chopped into small pieces and shade dried at room temperature, and then they were grinding into fine powder. The extraction of bioactive material from the powder was carried out with 70% methanol and 30% distilled water using Soxhlet apparatus. The extract was concentrated by a rotary evaporator and subjected to freeze drying in a freezer [16]. The percentage yield was found to be 10.50%. The extract was preserved in refrigerator until further use.

Experimental Design

Stone Induction: In this study, hyperoxaluria was induced by administration of ethylene glycol(EG)v/v (0.75%) in drinking water for 21 days and1% ammonium chloride (AC) v/w. 1% of AC was given only for the first 7 days, as administration of for more than 7 days lead to death of the rats [17,18].

Dose Preparation: The methanolic extract of CR was dissolved in distilled water at a dose mg/kg body weight and shacked until completely dissolved.

Experimental Animals: Thirty male rats were randomly divided into five groups, each of six rats. Group-A: fed with normal diet and serve as negative control. GroupB: took normal diet with EG (0.75%) and AC (1%) for 28 days and serve as a positive control. Group-C and D: took the same substances as groupB with 200 mg/kg and 400 mg/kg of CR respectively for 28 days and reserve as prophylactic and group E as group B, took normal diet with EG (0.75%) and AC (1%) for 28 days and then treated for28 days by 1000 mg CR extract and served as curative group.

Assessment of Antiurolithiatic Activity

Collection and Analysis of Blood: In the last day of the experimental period, all animals were fasted overnight and blood was collected from orbital veins. Serum was separated by centrifugation at 3,000 r.p.m for 15 mins and analyzed. Blood plasma was separated by centrifugation at 3,000 r.p.m for 15 mins. After centrifugation.

Estimation of Lipid Peroxidation (LPO): Malondialdehyde (MDA) was determined according to the method of [19].

Biochemical Analysis: The serum levels of creatinine and urea were measured by kinetic UV assay colorimetric methods using kits supplied by Roche diagnosis attached with Roche/Hitachi analyzer machine according the method obtained by [20].

Histopathological Study: Kidneys, liver and heart were weighted, fixed in formalin 10% and processes through graded alcohol series and xylene. Then embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for histopathological examination under light microscope.

Investigates the Mechanism of Antiurolithiasis of CR

Additional 10 male rats were randomly divided into 2 groups, each of 5 animals were used to investigate the mechanism of CR as antiurolithiatic substance. Group F: were fed with normal diet and left as negative control group whereas, group G: took normal diet with 500 mg/kg of CR and allowed free access to food and drinking water [21].

Determination of Water Intake, Urine Volume and PH: The rats were kept separately in metabolic cages. Water intake was measured and 24hs and urine samples were collected. A drop of concentrated hydrochloric acid was added to the urine prior to storage at 4 °C, to measure its urine volume. pH of the fresh urine samples from all rats was measured with the help of a calibrated pH meter (Model: WTW-Series pH-720) [22].

Determination of Urine Magnesium and Citrate: The determination of the magnesium concentrations and citrate in urine samples by colorimetric procedure was determined according [23].

Antibacterial Activity: Five types of bacteria were used for this study. Gram-positive bacteria included Bacillus cereus, staphylococcus aerus and Gram negative bacteria included Escherichia coli, Proteus and Kelibsella pneumoniae. All the tested strains were local isolates and were obtained from Department of Biology, Division of Microbiology, Faculty of Science, Sana'a University. These bacteria served as test pathogens for antibacterial activity assay. Three different concentrations of each extract of selected plants (50, 100 and 150 mg/ml) were dissolved in 10% dimethyl-sulfoxide (DMSO) in purified water to be used in antibacterial activity test. Extract solutions were prepared just before carrying out the test. Antibacterial activity of the extracts was determined by agar well diffusion method.

The bacterial suspensions containing 106 CFU/ml of bacteria were spread on petri dishes plates with a sterile swab moistened with the bacterial suspension. In each of these plates, five wells were cut out using a standard corn borer (7mm). About 60µl of each extract was added into different wells (duplicate each concentration), DMSO was used as a negative control. Positive control antibiotic wells were placed in the plate.

All the plates were incubated for 24hs at 37 °C. After incubation, bioactivity was evaluated by measuring the zone of inhibition. The experiment was performed in two of antibiotics standard Gentamycin (10 mcg) and Neomycin was used as reference to determine the sensitivity of each bacterial species tested and used as control positive. The antibacterial activity of CR extract was determined by agar diffusion method according to Ma et al., (2018).

Statistical Analysis

The data were collected and expressed as a mean±SE/. The statistical significances between groups were analyzed using one-way analysis of variance (ANOVA). A p value < 0.05 considered significant.

Results

Effect of CR extract on the plasma level of MDA

The mean levels of MDA in different groups were: 1.339±0.03001, 3.818±0.1156, 2.335±0.0949 and, 2.058±0.7518 for groups A, B,C&D respectively (Table 1 & Figure 1).

Table 1: Effect of CR on plasma MDA level.

Group	MDA (nmol/ml)
A negative control	1.339±0.03001
B positive control	3.818±0.1156a****
C (200mg/kg) CR	2.335±0.0949 a*** b****
D (400mg/kg) CR	2.058±0.7518 a** b****

The values are expressed as mean±SE. statistically significant at p<0.05 a- Significant difference as compared to negative control, (A). b- Significant difference as compared to positive control (B).

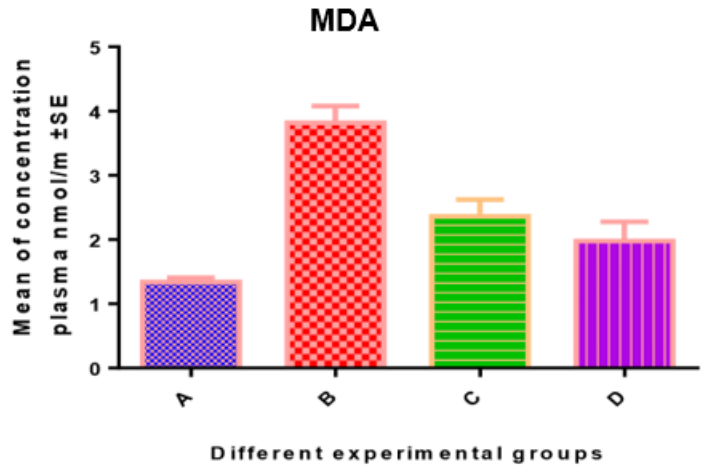

Figure 1: MDA Level in different groups.

Effect of CR extract on the serum level of creatinine and urea

The mean values of urea were 10.20±0.68, 12.14±0.41, 9.02±0.30 and 7.94±0.30; the mean values for creatinine were 28.28±1.31, 39.22±2.12, 29.78±1.63 and 26.70±2.02 for groups A,B,C and D respectively (Table 2 & Figure 2).

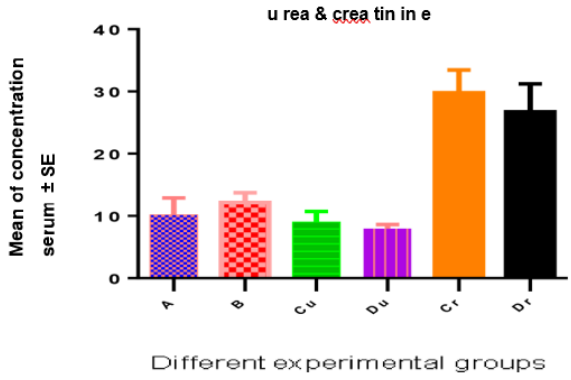

Figure 2: Serum urea and creatinine.

Table 2: Effect of CR and CS on the serum level of creatinine and urea:

Group	Urea(mmol/L)	Creatinine (umol/L)
A	10.20±0.68	28.28±1.31
B	12.14±0.41	39.22±2.12 a**
C	9.02±0.30 b***	29.78±1.63 b**
D	7.94±0.30 a* b****	26.70±2.02 b***

The values are expressed as mean±SE. statistically significant at p<0.05 a- Significant difference as compared to A. b- Significant difference as compared to B .

Effects of methanolic extract of CR on water intake, urine volume, PH , magnesium and citrate

There is significant increased in water intake, urine volume, pH, urinary Mg and citrate in group G compared to group F (Table 3 & Figure 3).

Effect of methanolic extract of CR as antibacterial

CR extract has antibacterial activity and this is dose dependent in all bacteria tested. This activity is better than gentamycin for proteus (Table 4 & Figure 4).

Table 3: Effect of methanolic extract of CR on Water intake, urine volume and PH, urinary Mg and citrate.

Parameter Group	Water intake ml/24hrs	Urine volume ml/24hrs	PH	Urine Mg (mg/dl)	Citrate (mg/dl)
F negative control	12,4±1.37	5.5±0.3	6.5±0.4	6.84±0.82	0.70±0.16
G(500mg/kg)	22,20±1.39a****	14,2±0.7a***	8±0.2a**	13,7±0.72a***	1.49±0.32a*

a, significant compared to negative control group
Water intake, urine volume and pH: a****p<0.0001, a***p<0.0001,a**P<0.001 respectively.
Urine magnesium and citrate: a***p<0.0001. Urine citrate a*p<0.01

Table 4: Antibacterial activity of methanolic extract of CR.

Type of Bacteria	Inhibitory zone in mm				
	CR extract concentration mg			Gentamycin	Placebo
	50mg	100mg	150mg		
E . Coli	5mm	11mm	14mm	19mm	0
Bacillus cereus	13mm	14mm	17mm	23mm	0
S .aureus	13.5mm	16mm	19mm	25mm	0
Klebsiella pneumoniae	5mm	7mm	9mm	17mm	0
proteus	15mm	16mm	19mm	15mm	0

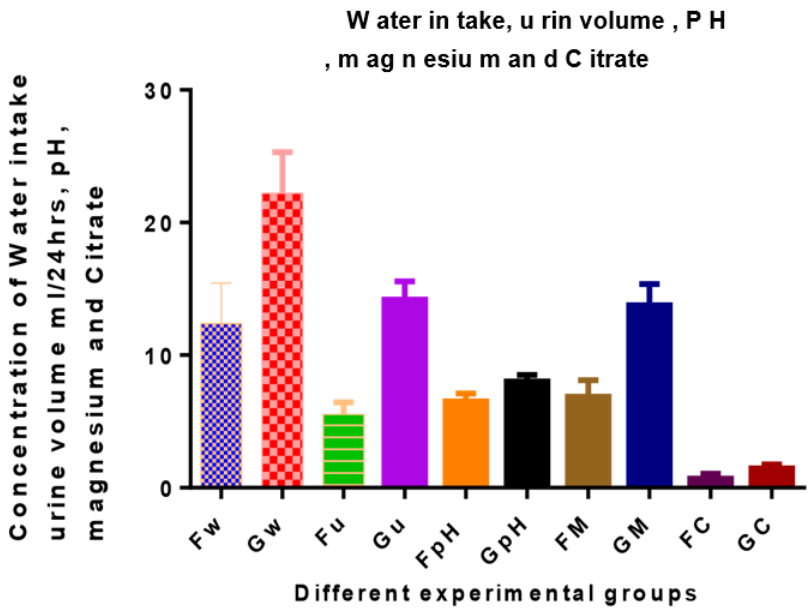

Figure 3: Water intake, urine volume, urinary pH, Mg and citrate in CR treated rats.

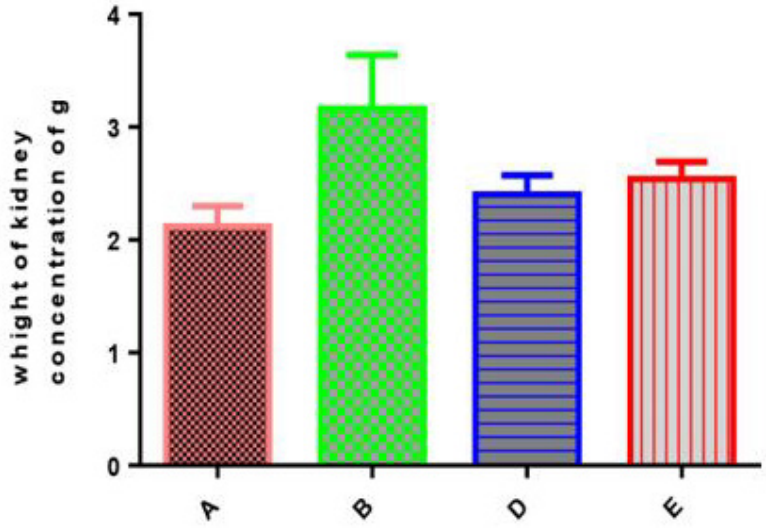

Figure 4: Kidney weight in different groups.

Pathological Study

change in kidney weight in different groups: The mean kidney weights in different groups were: 2.12±0.08, 3.10±0.197, 2.46±0.08 and 2.54±0.06 in groups A, B. C and D respectively (Table 5 & Figure 5).

Effect of methanolic extract of CR oncalcium oxalate density and integrity of kidney: Histological examination of kidneys of different groups revealed compete absence of oxalate crystals in groups A, C,D and E. The positive control group (group B) is the only group shows crystals. This group also show some adaptive responses in their kidneys like massive dilation of Bowmans space, hyperceleularity of glomeruli and dilatation of renal tubules. The latter are blocked by oxalate crystals. The casts were seen intratubular and extratubular with interstitial hemorrhage (Figure 6 & 7).

Table 5: kidney weight in different groups.

Group	Kidney weight
A negative control	2.12±0.08
B positive control	3.10±0.197 a****
D (400mg/kg CR)	2.36±0.08b****
E (1000mg/kg CR)	2.50±0.06b****

a- Significant difference as compared to negative control. b- Significant difference as compared to positive control.

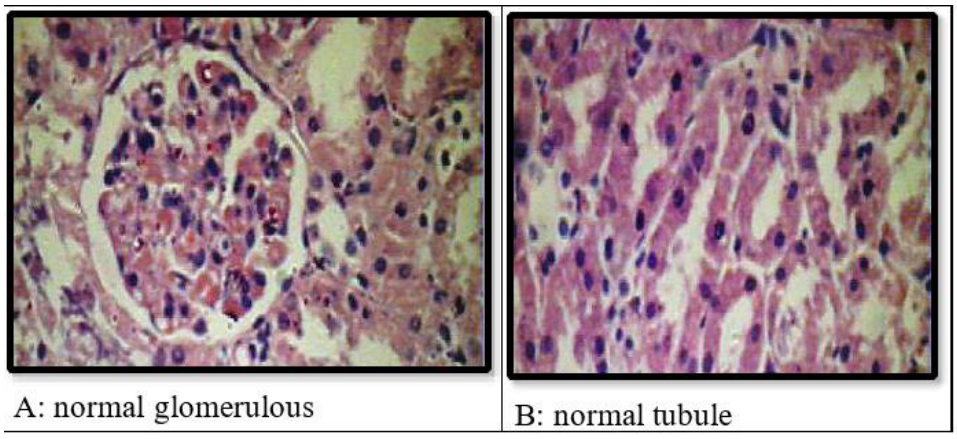

Figure 5: Normal kidney (group A/ negative control).

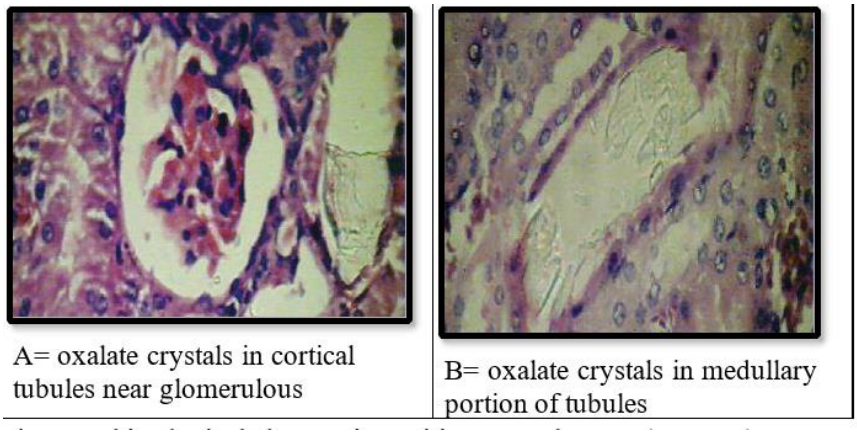

Figure 6: Histological changes in positive control group (group B).

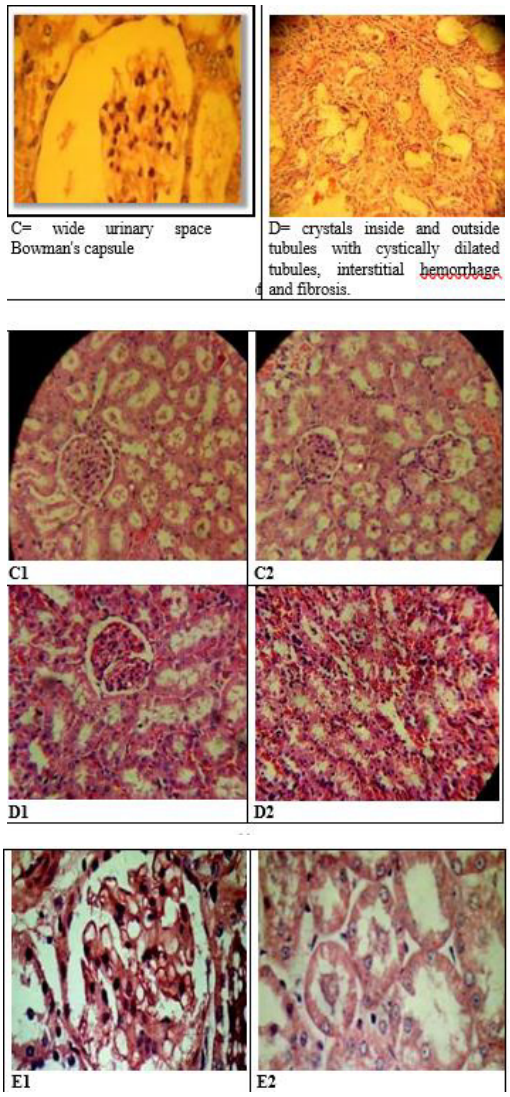

Figure 7: Kidnyes of CR treated groups (C, D and E) show no oxalte crystals. Increased vascularity with intratubular hemorrhage in group D also noted.

Discussion

The present study investigated the impact of CR experimentally induced urolithiasis in rats and the associated biochemical changes. This study found statistically significant changes in all measured parameters when compared to the normal group. Ethyline glyocle (EG) and ammonium chloride (AC) combination is a widely used experimentally for inducing hyperoxaluria by CaOx stones [17,25].

EG induced CaOx crystals in urinary tubules can produce damages in ECs by many mechanisms like production of free radicals, hence disruption of renal cellular membrane integrity probably by inducing lipid peroxidation (LPO), hence renal epithelial injury that increases the areas available for crystal attachment and eventual retention within the kidney [26-28].

Medicinal plants have a wide variety of phenolic compounds such as flavonoids and alkaloids that act potentially as antioxidants scavenge reactive oxygen species (ROS) and inhibit LPO [29]. Moreover, the increase of LPO and decrease of antioxidant potential have been reported in the kidneys of rats supplemented with a calculiproducing diet. In this context, oxalate has been reported to induce LPO and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane [30].

The current work revealed a significant increase in plasma TBARS levels in urolithiasis rats group indicates the pathological changes in tissues which increase the production and liberation of LPO into the circulation. This result is agreed with [31]. On the contrary, the decreased levels of plasma TBARS observed in the treatment group by CR indicates its strong antioxidant activity and this is consistent with others [32].

EG poisoning can lead to acute renal failure which is characterized by proximal tubular necrosis and an accumulation of (CaOx) monohydrate crystals in the urine and kidney tissues [33]. The estimation of serum urea and creatinine gives idea about the extend of affection of renal damage function. Our study showed significant change in serum urea and creatinine and these indicating that there is a deterioration renal function in group B compared to negative control and treated groups (A,C&D). The deterioration in renal function in group B may be through direct toxicity by EG or by OxCa or both, consistent with others [30,33]. Furthermore, the significant decrease in urea and creatinine in CR treated group (D) indicating the protective effect of CR against EG/CaOx induced renal damage, hence preservation of normal renal function. The protective effect of CR may be due to prevention of crystal deposition in renal tubules, and prevention of CaOx induced injury on renal tubules, and this is consistent with others [34,35].

The protective effect of CR against stone formation was obvious in our study. Kidneys of treated (curative and preventive) groups (C ,D&E) was free of crystals either intratubular or interstitial compared with positive control group (B). These findings are agreed with [36]. The exact mechanism of this protection unclear. However, the following are possible mechanisms: 1- CR methanol extract reduces the LPO level thus preventing CaOx crystal attachment and subsequent development of kidney stones. 2- saponins and flavonoids present in CR prevent calcium and oxalate deposition [37]. 3- the large amount of vitamin E present in CR [38] has also, shown to decrease the urinary excretion of oxalate and calcium and restore antioxidant ability [39].

The urolithiolytic activity of CR was promising in our study as kidneys of treated group (group E) were also free of crystals compared to the positive control group. The mechanism of such urolithiolytic effect of CR also investigated in our study. There is a significant increase of water intake, urine volume, urinary pH, urinary Mg and citrate in group G compared to group F. The increase in water intake, hence urine volume reduce supersaturaion of urine by CaOX and this is consistent with others [40]. In addition, the diuretic effect of CR may be due to inhibition of aldosterone [32].

Urinary pH is a major determinant for kidney stone formation suggested that a urine pH approximately near 6 on the pH scale reduces the risk of kidney stone formation, however, the risk of uric acid and calcium stone formation increases progressively at urinary pH<5.5 [41]. Furthermore, the slight decrease in pH lead to increase urine calcium excretion mediated by a decrease in renal tubular calcium reabsorption. In addition, the increase in systemic acidity leads to a decrease in urinary citrate excretion [42]. So, if urinary pH rises, renal citrate production does as well, thus producing a decrease in tubular citrate reabsorption, and increased citrate excretion [43]

Alterations in urinary pH might be due to genetic variants or mutations in transport pathways, lifestyle habits such as specific diets or metabolic diseases, and infections. Inappropriately acidic or alkaline urine affects the solubility of various metabolites and salts. Furthermore, alkaline urine reduces the solubility of calcium phosphate products, and promotes the formation calcium phosphate stones [44].

Mg combines with oxalate, potentially reducing oxalate absorption in the gastrointestinal tract (GIT) and decreasing CaOx supersaturation in urine [45]. Moreover, Mg has been shown to lower urinary supersaturation of CaOx and increase urinary citrate [46].

Finally, the antibacterial activity of CR was also investigated and we found acceptable antibacterial effect against some bacteria that may be a predisposing factor for urolithiasis. This antibacterial activity is concentration (dose) depended in most bacteria. However, it is effective in low concentration in some bacteria like proteus.

In conclusion, CR is good for urolithiasis as protective and curative remedy. It has diuretic activity, rising urinary pH, increase urinary citrate and magnesium. Additionally, it has antibacterial activity, renal tissue protective activity both structurally and functionally.

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