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RESEARCH ARTICLE

THE PROTECTIVE AND THERAPEUTIC EFFECT OF CORN SILK EXTRACT ON UROLITHIATIC AND HYPERTENSIVE RATS INDUCED BY ETHYLENE GLYCOL

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Abstract

Renal stone is one of the most problems worldwide. They are affected by different factors such as environmental effectors such as nutrient as well as family history. Corn silk (CS) used as antiurolythiasis. This study investigated the impact of corn silk extract as the management of renal stone formation, hypertension, and hepatoprotective. This work has been carried out on rats at Faculty of science Sana'a University, University of Science, and Technology Laboratories. Twenty-four male albino rats with weighing range between (200 g to 250g) were taken. They were divided in four groups (each group consists of 6animals). The first group of rats took normal diet and named as negative control (Co) whereas, the second group took normal diet with ethylene glycol (EG) (0.75%) and 1% ammonium chloride (AC) for 28 days and serve as positive control (Po). The third and fourth groups took the same substances as inPo group with 200 mg/kg and 400 mg/kg of corn silk (CS) for 28 days respectively.

Blood samples were collected from rats on last day of the experiment. All the tested samples showed a significant antioxidant DPPH radical scavenging activity and a notifiable decrease in serum aldosterone hormone, angiotensin comforting enzyme urea and creatinine levels comparedto positive control. Shown to significantly increased AST, ALT, and LDH in comparison to the urolithiatic group and near of normal group.

In conclusion, the present study emphasizes the safe herbal remedies of CS as anti-hypertensive and antioxidants, as well as antiurolithiatic and hepatoprotective.

Keywords: Urolithiasis, Hypertension, Corn silk and Ethylene glycol.

1. Introduction

Kidney acts as a filter of blood from poisonous substances and helps to regulate the levels of chemicals, which are important for body functions [1].

Nephrolithiasis (NL) also, known as kidney stones, renal stones, urinary stones, urolithiasis and renal calculi affects great number of patients worldwide [2]. It is characterized by formation of stones in the urinary system [3].

Renal calculi formation is one of the most common urological disorders. Urinary stones disease is a common disease, which affects 10-12% of the population in industrialized countries [4]. Moreover, NLis a universal problem, affecting patients across geographical, cultural, and racial boundaries [5].

Kidney stones are aggregates of crystal formed in the kidneys from dietaryminerals of urine. The physical process of stone formation is a complex cascade of events. It begins with urine that becomes supersaturated with stone-forming salts (e.g. calcium oxalate, uric acid and phosphate [6].

On the other hand, approximately 80% of stones are composed of calcium oxalate (CaOx) and calcium phosphate, 10% of struvite, 9% of uric acid and the remaining 1% composed of cystine or ammonium

acidurate [7]. Furthermore, patients will be affected by multiple stones throughout their lifetime, with estimated recurrence rates of 50% within 5–10 years and 75% within 20 years of urolithiasis [8].

Urolithiasis is associated with a variety of abnormalities in urinary composition, which are due to dietary indiscretions, physiological-metabolic disturbances or both [9].

In humans, CaOx is the major component of urolithiasis, and CaOx stones constitute ~80% of all stones [10]. It was also, reported that a small amount of calcium phosphate is involved in stone attachment [11].

Ethyline glycol (EG) is metabolized to four main organic acids in vivo, glycooxalic acid, glycolaldehyde, glycolic acid, and oxalic acid, which is the initiative factor for lithiasis [12].

Experimentally EG found responsible for formation of kidney stone or renal calculi, which has altered the cellular content and biochemical feature of nephrocytes in the experimental animals [13]. [14]found that in both 0.5% and 1.0% ethylene glycol increased calcium oxalate crystal in animal kidneys with pathologically disturbed nephritic cells.

On the other hand, several studies have demonstrated that urolithiasis is associated with hypertension [15]. Either the mechanisms by which urolithiasis might be associated with or cause hypertension remainsunclear [16].

Three methods that are used in treatment of kidney stones: Drugs, Extracorporeal Shock Wave Lithotripsy (ESWL) and surgical removal [17].

Although, some drugs used to prevent and treat the disease have adverse effects when using in high doses. These motivated humans to return to nature for safe remedies using phytotherapy [18]. In the same time, antihypertensive agents, such as diuretics, β-blockers, calcium-channel blockers, and blockers of the rennin-angiotensin system, such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers among others are used separately or in combination to treat this disease [19], but antihypertensive drugs have many side-effects as reduced renal function, dry cough, Angioedema among others [20]. Hence, the management of hypertension by herbal medicine is an alternative. The use of medicinal plants in treatment and prevention of diseases have increased dramatically over the last years [21].

For to inhibit this activity and as a part of phytoremideation variety of medicinal plants were used in remedial purpose against urolithiasis or kidney stone without side-effect and cost effectiveness and minimum surgical operative method [22].

Natural plant has been used for centuries in treating various illnesses play a major role in forming the basic platform of modern medicines [23]. One of these herbs is corn silk (Stigma maydis). Corn silk (CS) is a waste material from corn cultivation, but it is also, an inexpensive medical diet of plant [24].

Morphologically CS are fine soft threads 10 to 20 cm long, commonly found on the corn. When fresh, they are like silk threads of a light green or yellow; when dry, they resemble fine, dark brown, crinkled hairs. It is a collection of the stigmas from the female flowers of the maize plant [25, 26]. It has been found that CS is an excellent source of many bioactive compounds such as flavonoids, saponin, alkaloids, tannins, chlorogenic acid, phytosterols, allantoin, vitamin E and K, etc. [27]. Moreover,CS contains proteins, vitamins, carbohydrates, Ca2+, K+, Mg2+ and Na+ salts, volatile oils and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tanninsandflavonoids [28]. CS and has been used for thousands of years as a folk medicine in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones,nephritis, diabetes mellitus and prostatitis [25-28-29]. Other beneficial treatments of CS include anti-fatigue activity, anti-depressant activity and kaliuretic[30]. Furthermore, pharmacological studies have proved that CSwas found to have medicinal properties like antioxidant, anti-depressant, anti-hyperlidemic, anti-diabetic,anti-inflammatory, neuroprotective toxicity and many more properties [31]. Another study reported that CS is considered an important medicinal plant, with the function of inducing diuresis and excreting dampness[32]. In addition, itwas reported to be useful to treat urinary infection and cystitis [33].

2. Materials and Methods

Experimental Animals and Diet Preparation

This study was performed on male albino rats, initially weighing between 200gm to 250gm. Rats were obtained and reared in animal house of Biological Department, Science Faculty, Sana'a University. They were housed in stainless steel cages at a well-ventilated animal house. Rats were permitted the following diet and given water ad libitum for one week of adaptation period prior to the experimental work. The bedding of the animal cages changed every 48hrs.

Preparation of Extract

Fresh hairs CS was collected from Sana`a Government in Yemen. Plant was carefully washed with distilled water and dried at room temperature and then it was grind into fine powder. The powdered materials were stored in airtight polythene bags for future use. The dried powder was extracted by method using Soxhlet apparatus extracted with 70% methanol and 30%

aqueous, and the extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a freeze dryer till dry powder was obtained according the method of [34] The percentage yield was found to be 12%. The extract was preserved in refrigerator until further use.

DPPH Scavenging Assay

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extract. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The scavenging ability of the natural antioxidants of the CS towards the stable free radical DPPHwas measured by the method of [35].

Urolithiasis Induction

Ethylene glycol (0.75% v/v) and ammonium chloride (AC 1%)was used to induce hyperoxaluria and consequently the deposition of CaOx crystals in rat kidneys it was added in drinking water for 28 days according the method of [36].

3. Experimental Design

Dose Preparation:

Corn silk was dissolved in D. W at a dose 200 and 400 mg/kg (b.w) of rat using a stomach tube.

Animal Groups

Twenty-four rats were divided into four equal groups (each 6 rat). Group 1 received normal feeding for 28 days and served as negative control. Groups 2was fed by normal dietwith EG (0.75%) and ammonium chloride (AC 1%) for 28 days and serve as positive control (Po). Groups 3 and 4 was fed bythe same substances as in Po group, with 200 mg/kg of CSand 400 mg/kg (b.w.) respectively for 28 days according the method of [37] .

SerumCollection and Analysis:

All rats were fasted overnight. Then blood was collected from the eye canthus of each rat usingmicrohaematocrit capillary tubs in last days of experimental period. Serum was separated by centrifugation at 3,000-×g for 15 min tobe determined to following parameters.

Enzyme Immunoassay

Serum aldosterone and ACEwere estimated by microplate enzyme immunoassay, colorimetric technique.

Estimation of Aldosterone

Serum aldosterone was estimated by microplate enzyme immuno- assay, colorimetric technique using the Aldosterone Test System Product Code: 10125-300 that reported by [38].

Estimation of Angiotensin Converting Enzyme (ACE):

ACE estimated by commercially kits using a different factor calculation by the method of [39].

Determination of Liver and KidneyFunctions

The serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) as liver function parameters and creatinine and urea as kidney function parameters 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 [40- 41].

4. Statistical Analyses

All vales are expressed as mean ± S.E. Statistical analyses were performed by one-way(ANOVA) followed Tukey Multiple Comparisons methods. The values of $p < .05$ were considered statistically significant.

5. Result

To The present study investigated the impact of CS on the kidney stone formation in male rats with experimentally induced urolithiasis and the biochemical changes in liver and heart. The obtained data were calculated for the urolithiatic group (Po) according to the negative control (Co) and treated groups.

In the present study, it is of important to note that, administration of CS to treated rats produced statistically significant changes in all measured parameters when compared to the normal group.

Free Radical Scavenging Activity (DPPH Assay)

This method is based on the reduction of the stable free radical diphenypicrylhydrazyl (DPPH) in the presence of an antioxidant to the non-radical form of yellow colored DPPH-H.

Mean regarding DPPH scavenging activity of CSare presented in Table.(1)and figure.(1), that showed, the free radical scavenging activities of the studied solutions in this study was maximum in ascorbic acid (93.89%) and CS (82.37%).

Table 1: Free radical scavenging activity of ascorbic acid and CS

Parameters	DPPH (%)
Ascorbic acid	93.89 ±4.842
Corn silk	82.37±3.055

Values are expressed in mean ± SE of 3 times repeated for each set of CS extract.

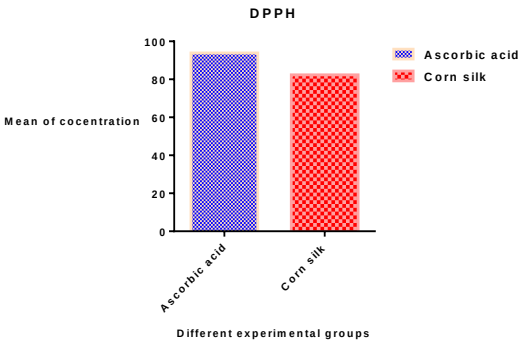

Fig.1: Free radical scavenging activity of ascorbic acid and CS

Effect of Methanolic Extract of CS on Serum Aldosterone Level:

As indicated in table(2) and fig.(2), the measured parameters were significantly increased in Po when compared with other groups whereas serum aldosterone concentration were significantly decreased in CS groups. Serum aldosterone wasnon-significantly differences in-group Awhen compared with Co group whereas still significantly increase in-group B in comparison to Co group. Moreover, there are significantly differences between group A and B as shown in table(2) where group A was significantly decrease in comparison to group B,

Table (2): Effect of CS on serum aldosterone level

parameters / Groups	Serum aldosterone level pg/ml
Co	98.25±19.15
Po	534.25± 10.74a****
A	112.75± 1.80b****
B	197.25± 4.27a****b***c***

Co = negative control, Po = positive control, A= CS 400 mg/kg treated B= CS 200 mg/kg. The values are expressed as mean±SE. a- significant difference group as compared to Co. b- Significant difference group as compared to Po

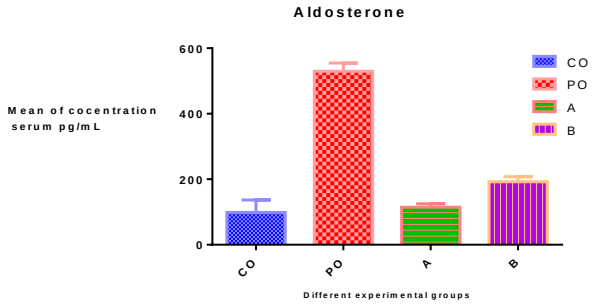

Fig. 2: Effect of CS on serum aldosterone level

Effect of Methanolic Extract of Corn Silk on Serum ACE Level

As shown in table(3) and fig.(3), ACE was non significantly increased in Po group when compared with Co group, but significantly increased in comparison to A group that was significantly decreased in serum ACE concentration when compared with Co group.

Table 3: Effect of CS on serum ACE level

parameters / Groups	Serum ACE level U/ L
Co	96.70 ± 3.64
Po	102±5.09
A	72.30 ± 3.83 a**b***

Co = negative control, Po = positive control, A= CS 400 mg/kg.The values are expressed as mean±SE a- Significant difference group as compared to Co. b- Significant difference group as compared to Po.*P <0.031

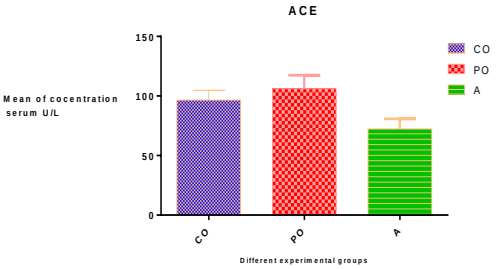

Fig.3: Effect of CS on serum ACE level

Effect of Methanolic Extract of CS on Creatinine and Urea Serum Levels

As shown in table, (4) and fig.(4 and 5), The measured parameters were significantly increased in Po when compared with Co group in creatinine whereas creatinine and urea concentration in serum were significantly decreased in CStreated A group and in serum creatinine only in B group when compared with Po group. Non-significantly differences were observed between CS treated groups in comparison to the Co group.

Table 4: Effect of CS on creatinine and urea serum level

Parameters / Group	Urea(mmol/L)	Creatinine(umol/L)
CO	10.20±0.68	28.28±1.31
PO	12.14±0.41	39.22±2.12 a**
A	8.96±0.23 b**	30.20±2.02 b*
B	9.12±2.67 b**	33.43± 1.52

Co = negative control, Po = positive control, A= CS 400 mg/kg treated.B= CS 200 mg/kg. The values are expressed as mean±SE. a- Significant difference group as compared to Co. b- Significant difference group as compared to Po.

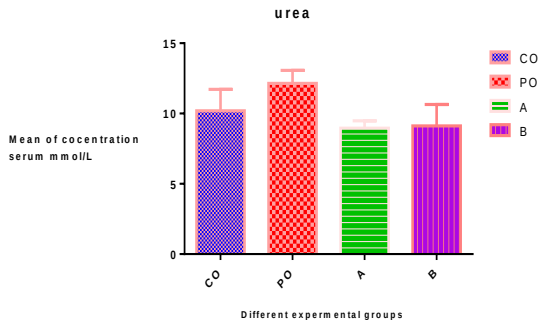

Fig.4: Effect of CS on serum urea level in different experimental groups

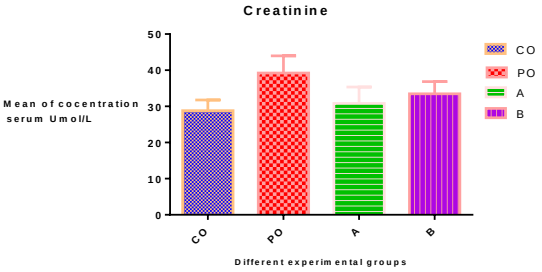

Fig.5: Effect of CS on serum creatinine level in different experimental groups.

Effect of Methanolic Extract of CS onAST, ALT and LDHSerum Level

The data observed in table(5) and fig. (6, 7 and 8), that that serum of AST, ALT and LDH levels were significantly decreased in Po when compared with Co group and the two CSgroups. Whereas, illustrated that the measured groups hadnon significantly differences in all CStreatedgroupsand Co group.

Table .5: Effect of CS on AST, ALT and LDH serum levels

Parameters / Group	AST	ALT	LDH
Co	182.2±8.55	52.24±3.04	1957±148.0
Po	125.9±10.91 a**	39.6±1.14 a*	1422±110.9 a*
A	168.3±7.18 b*	53.24±2.56 b**	2031±94.58 b**
B	176±11.03 b**	49.6±3.01 b*	1873±92.01

Co = negative control, Po = positive control, A= CS 400 mg/kg treated B= CS 200 mg/kg The values are expressed as mean±SE. a- Significant difference group as compared to Co. b- Significant difference group as compared to Po.

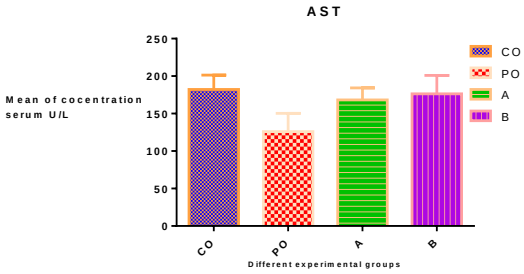

Fig. 6: Effect of CS on serum AST level in different experimental group

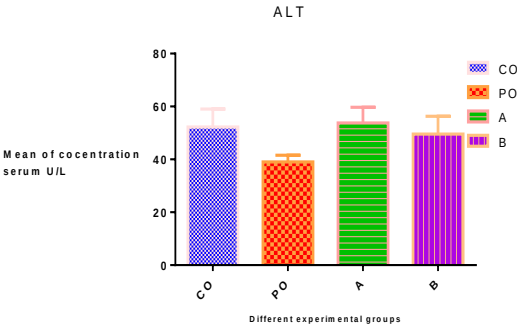

Fig.7: Effect of CS on serum ALT level in different experimental groups.

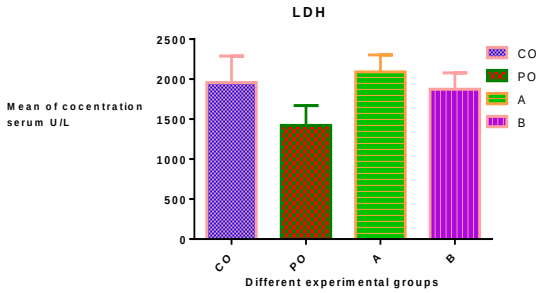

Fig.8: Effect of CS on serum LDH level in different experimental groups.

6. Discussion

This study investigated the impact of CS extract as the management of renal stone formation and decomposition.Calcium oxalate (CaOx) crystals in urinary tubules can produce damages in the epithelial cells (ECs). On the other hand, the role of tubular ECs damage and crystal retention in the nephron has been considered necessary for stone formation by CaOx crystals, which, can bind to ECs[42-43]. Moreover, hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stone than hypercalciuria[44]**.**

EG is metabolized in liver into four organic acids: glycolaldehyde, glycolic acid, glyoxalic acid and oxalic acid, which lead to hyperoxaluria, the major initiative factor for urolithiasis[45]. On the other hand, free radicals that are formed by EG cause damage in urinary tract cells [46]**.** Meanwhile,renal cell damage is also,

associated with LPO production indicating that cell injury was due to the production of free radicals [47].

Free Radical Scavenging Activity (DPPH Assay)

Antioxidants are free radical scavengers, which provide protection to living organism from damage caused by freeradicalsinducing by environmental elements[48].

Medicinal plants such as CS have a wide variety of phenolic compounds such as flavonoids that act potentially as antioxidants, scavenging reactive oxygen species (ROS) and inhibit LPO [49]

CS is rich in antioxidants including proteins, vitamins, alkaloids, tannins, mineral salts, carbohydrates, steroids and flavonoids as well as volatile chemicals [50].In the present study, DPPH radical scavenging activity showed that (CS) extract was significantly exhibited strong antioxidant activity. This result is in agreement with the findings of [28]

Serum Aldosterone and ACE Level

Renin-angiotensin-aldosterone system (RAAS) is a well-known mechanism that controls the blood pressure by regulating the volume of fluid in the body. Angiotensin-converting enzyme (ACE) is a crucial dipeptidylcarboxypeptidase in RAAS that converts angiotensin I to the active vasoconstrictor angiotensin II [51]. ACE inhibitionisa better physiological target for treatment of clinical hypertension due to its association with two types of blood pressure systems, the rennin-angiotensin system (RAS) and kinin-nitric oxide system (KNOS) a vasodilator. Therefore, ACE inhibitors, such as captopril, lisinopril and enalapril, are widely used as pharmaceutical drugs for the treatment of hypertension [52]. Due to the important roles of ACE in the regulation of blood pressure, the inhibition of this enzyme has been used to treat hypertension [53].

The kidney is the major target for the classical action of aldosterone: sodium retention. It acts on epithelial cells in the distal convoluted tubule and reorganizes the activity of ion pumps and channels accordingly. In particular, the epithelial sodium channel [54]. This mechanism lead to water reabsorption that elevate blood pressure.

Our results reported that CS has role as hypotensive effects by decreasing ACE and aldosterone lead to dieresis and decrease blood volume and in turn decreasing ACE (decreasing vasoconstrictor) and decrease blood pressure. These results agreed with [55] suggested that CS exhibited anti-hypertension effects via the inhibition of ACE, the target of anti-hypertensive drugs.

Our results also agreed with results of [56] who, observed that CS activates macrophagesthatexcrete high level of iNOS and generates large amounts of NO that has essential role in vasodilation and in turn serve as hypotension.

In our study, increases aldosterone level in urolithiatic group (po) Urolithiasis increases hypertension that may be attributed to aldosterone secretion by renin hormone. This results agreed with the results obtained by [57]

Serum Creatinine and Urea in Different Groups

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 [57]. Our study appeared the renal obstruction through the rise of serum creatinine and urea in urolithiatic group Po when compared to negative control Co. This increasing was significant but not to the level indicating renal failure which means that the doses of EG/AC used in the study were accepted and not too toxic to kidneys throughout the experiment. The increase in serum level of these parameters in group Po in our study agreed with [59]who attributed the renal tubule obstruction to CaOx crystals resulting in decreased glomerular filtration rate (GFR) and retention of waste products particularly nitrogenous substances such as urea, creatinine. Furthermore, EG administration also, leads to hypertrophy of renal papilla and increased kidney weight probably by inflammation and fluid accumulation [60].

In addition, the kidney function is affected in urolithiasis, since lowering of the glomerular filtration rate (GFR) is observed due to the obstruction to the outflow of urine by calculi deposited along the urinary system. Thereby, the waste products particularly nitrogenous substances such as urea and creatinine accumulate in blood [61].

The significant decreases in creatinine and urea in this work between group Po and CS treated group may be due to prevention ofcrystal deposition in renal tubules that induced injury on renal tubules. These results were in agreement with the finding in the studies of [62-63]. Furthermore, The nephroprotective effect of CS may be due to antioxidant activity that has curative effect againstfree radical damage induced by EG [64] Moreover the diuresis of CS important mechanisms for excreting waste [25].

Serum AST, ALT and LDH in Different Groups

Ethylene Glycol can be due to altered calcium homeostasis concomitant with a significant increase in cytosolic calcium, in liver [65]. Moreover, intracellular calcium homeostasis disturbances have been shown to be associated with a variety of toxicological and pathological processes [66] that lead to significantly decrease AST, ALT and LDH in urolithiatic group Po when compared to CS groups.

On the other hand, the treatment with CS extract has been shown to significantly increase AST, ALT and LDH in comparison to Po groupand near of normal group. These results were in agreement with the results obtained by [67]. Moreover, CS extract decreases formation of ROSand OS, resulting decreasesin lipid peroxidation. Furthermore, May this mechanism the mechanism by which CS extract induces hepatoprotectivity [68].

7. Conclusion

Urolithiasis is a complex process that results of the imbalance between the precipitate and the excrete processes of menials in the kidneys. The pharmaceutical drugs used to prevent urolithiasis have adverse effects that compromise their long-term use. Therefore, it is important to use medicinal plants to use for treating urolithiasis to minimize the side effects of drugs. In the present study, the therapeutic potential of CSwas evaluated for its preventive effects in experimental urolithiaticanimals. our results presented in this current work indicates that administration of CS increased the passage of urinary stones through the urinary tracts and played an important physical role in treatment by increasing the vasodilation of urinary tracts that led to increase the urinary output and decrease hypertension.

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مقالة بحثية

التأثير الوقائي والعلاجي لمستخلص حرير الذرة على حصى الكلى وضغط الدم المستحث بالإيثيلين جليكول في الجرذان

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المُلخّص

تهدف هذه الدراسة إلى تقييم كفاءة المستخلص الإيثانولي لحرير الذرة كمضاد للحصوات والضغط والتأثير الوقائي للكبد. حيث عوملت ذكور الجرذان بالإيثلين جليكول والأمونيوم كلورايد لإحداث الحصوات. إستخدمت هذه التجربة 24من ذكور الجرذان قسمت إلى أربع مجموعات قسمت المجاميع كالتالي: مثلت المجموعة الأولى المجموعة الضابطة في حين عوملت المجموعة الثانية بـ 75% من الإيثلين جليكول و1% من كلوريد الأمونيوم لإحداث الحصوات في كلى حيوانات التجارب. وعوملت المجموعتين الثالثة والرابعة بنفس النسب من الإيثيلين جليكول والامونيوم كلورايد مع إضافة مستخلص حرير الذرة بنسبة 200و400 ملي جرام لكل كيلو جرام. تم جمع عينات الدم في آخر يوم من التجربة لعمل التحاليل المطلوبة.

أعطت النتائج المتحصل عليها قيم معنوية عالية:

أعطى النبات قدره جيده كمضاد للأكسده، تقليل نسبة هرمون الألدوستيرون، أنجيوتنسين كومفرتنج إنزيم واليوريا والكرياتينين في الحيوانات المعالجة وبنسب معنوية مقارنة بالحيوانات المصابة.

قلة في مستوى انزيمات الكبد في الحيوانات المصابة وزيادتها في الحيوانات المعالجة بالمستخلص لتعود إلى مستواها الطبيعي.

نستنتج من النتائج المتحصل عليها من الدراسة أن النبات ذو فعالية جيدة كمضاد للحصوات والضغط ووقائي للكبد.

الكلمات المفتاحية: حصوات الكلى، ضغط الدم، الايثلين جليكول وحرير الذرة.

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