

THERAPEUTIC POTENTIAL OF *Citrus aurantium* ON UROLITHIASIS INDUCED BY ETHYLENE GLYCOL IN MALE RABBITS

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ABSTRACT: Nephrolithiasis is a complex physicochemical event which starts with urinary supersaturation and then leads to aggregation of renal crystal forming constituents within the kidneys. Researches gives much attention for better medical therapy and assess a satisfactory drug to prevent renal stone formation. The purpose of the study was to detect the effectiveness of *Citrus aurantium* (bitter orange) juice on ethylene glycol induced nephrolithiasis in male rabbits. Animals were divided into five groups (n=8) in which group 1 as control (C), group 2 as ethylene glycol non treated group (EG), group 3 as ethylene glycol group treated with bitter orange juice (EG+B.O), group 4 as ethylene glycol group treated with Citraforte (EG+C) and group 5 as ethylene glycol group treated with bitter orange juice and Citraforte (EG+B.O+C). Ethylene glycol was given to group 2-5 in drinking water for 21st days for the induction of kidney stones then the treatments were continued for successive 3 weeks. The obtained results showed a significant increase ($p \leq 0.05$) in the serum level of total protein, albumin, phosphorus, uric acid, creatinine, blood urea nitrogen (BUN), Malondialdehyde (MDA) and a significant decrease in calcium, catalase (CAT), Superoxidase dismutase (SOD) and glutathione (GSH) in the group of rabbits treated with ethylene glycol. All the estimated parameters showed an improvement in their values in the treated groups especially in group 5 which treated with bitter orange juice plus Citraforte. There was a detectable pathological lesion in the kidney tissues of rabbits in ethylene glycol group which improved with treatment by bitter orange juice and or Citraforte.

In conclusion: Co-administration of bitter orange (*Citrus aurantium*) juice with Citraforte initiated a synergistic action for curing urolithiasis induced by ethylene glycol in male rabbits.

Keywords: *Citrus aurantium*; Urolithiasis; Ethylene glycol, Calcium oxalates.

INTRODUCTION:

Kidney stones have been associated with an increase renal failure, cardiovascular diseases, diabetes and hypertension (Sigurjonsdottiret *al.*,2015).Generally, urolithiasis causes crystal aggregation, growth of insoluble particles and may be associated with systemic andmetabolic disorders. Nephrolithiasis withnephrocalcinosis is responsible for 2 to 3% of end-stage renal cases (Courbebaisseet *al.*,2016).

Various therapies and investigations are failed to trace out the exact cause and mechanisms of urolithiasis, including thiazide and alkali-citrate that applied to prevent the hyperoxaluria frequency and calculi formation but they are not enough due to low tolerability andlimited effectiveness (Rathodetal.,2012). Several drugs have been used against nephrolithiasis in solitary or in combination with other drugs. However, there is no satisfactory drug to remove the stones. So, researchers give much attention for better medical therapy and to develop a satisfactory drug to prevent stone formation in lithiatic patients (Prathibhakumari and Prasad,2018).

In addition, the surgical techniques disadvantages and limited choice of new pharmacological therapies for the management of kidney stones is worthwhile (Nirumandet *al.*,2018).Currently, there is no satisfactory drug to cure and/or prevent kidney stone recurrences. Thus, further understanding of the pathophysiology of stone formation to manage urolithiasis using new drugs is necessary (Alelign and Petros,2018).

Consequently, prophylactic management is of great importance to manage urolithiasis (Afsaret *al.*,2016). The natural role of dietary plants in the prevention of kidney stones, elaborate underlying pharmacological mechanismsand their phytochemical constituents responsible for this activity against stone formation. However, the pharmacological evidence of phytonutrients in dietary plants in the prevention of kidney stones have not been well-established yet (Nirumandet *al.*,2018).

Citrus aurantium (Rutaceae), the unripe fruit of bitter orange is used in traditional medicine to treat urolithiasis. Previous studies have shown that *Citrus aurantium* have numerous bioactive compounds, including polymethoxylated flavones (PMFs),flavonoid glycosides, alkaloids, and coumarins. These active components demonstrate pharmacological activity; antioxidant, antimicrobial, anticarcinogenic properties and neuroprotective effects (Stohs,2017).

*Citrus aurantium*known as bitter orange, is usually utilized as a flavoring and acidifying agent for food (Karabiyıklıet *al.*,2014). It preventsCaOx crystallization and promotes crystal dissolution,possibly through a combination

of increasing antioxidant levels and antimicrobial activity while decreasing urinary stoneforming constituents, OPN expression, and hypercitraturic effects. *Citrus aurantium* has attracted the attention of many researchers, and the study of this herb is gradually increasing (Li *et al.*,2015).

Therefore, this study is aim to evaluate the therapeutic aspect of *citrus aurantium* or bitter orange juice parallel to standard anti-urolithiatic drug as Citraforte in curing kidney stones and its ability to preventing further stone formation.

MATERIALS AND METHODS:

Adult male rabbits of New Zealand White (NZW) spp. (600-700 gm) are housed in standard conditions of temperature ($22\pm 2^\circ$), relative humidity ($55\pm 5\%$) and light (12 h light-dark cycles) were used. They have been fed with standard pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals. (Protocol No.: 902 dated: 26/09/2009). The experiment was conducted in accordance with internationally accepted standard guidelines for the use of animals.

Chemicals and Reagent

Ethylene glycol (EG), Citraforte[®] powder. Standard estimation kits for calcium, phosphate, uric acid, urea and creatinine(Sigma chemicals, USA). All the other reagents used for analysis were of analytical grade.

Plant material:

The fruits of *Citrus aurantium* were purchased from shop at Zagazig, Sharkia, Egypt. Then the fruits prepared to obtain the fresh juice of bitter orange daily during the period of treatment and diluted (1:2) using distilled water (Karoui and Marzouk, 2013).

Ethylene glycol induced urolithiasis model

Fourty male rabbits (600-700 g) were divided into five groups having eight animals in each. All groups except control were received 0.75% ethylene glycol in distilled water for 28 days. The standard drug and test drug were given from 15th days for successive 3 weeks (Divakaret *al.*,2010).

Experimental design:

Group 1 (C) serve as control group; group 2 (EG) received 0.75% ethylene glycol only; group 3(EG+B.O)received freshly prepared bitter orange

juice (3 ml of 1:2 diluted juice, twice daily, for each rabbit by oral gavages); group 4 (EG+C) received citraforte (300 mg/kg body weight) twice daily; group 5 (EG+B.O+C) received both freshly prepared bitter orange juice and citraforte with the same doses once daily for successive 3 weeks.

Assessment of antiurolithiatic activity:

Serum analysis

Blood was collected after the experimental period from the retro orbital puncture under ether anesthesia and separation of serum was done by centrifugation at 10,000 rpm for 10 min and analyzed for urea (Lequanget *al.*, 1987), creatinine (Bowers, 1980), uric acid (Hishamet *al.*, 2013) and the blood urea nitrogen was calculated. Serum total protein (Yatzidis, 1987) and albumin (Doumaset *al.*, 1997) were estimated. Serum phosphate (Fossatiet *al.*, 1988) and calcium (Parentoniet *al.*, 2001). Antioxidant markers including: (SOD) (Kakkaret *al.*, 1984), catalase (CAT) (Aebi, 1984), Glutathione (GSH) (Sedlak and Lindsay, 1986), as well as malondialdehyde (MDA) (Okhawaet *al.*, 1979) were performed.

The animals were sacrificed by cervical decapitation and the abdomen was cut open to remove either kidney from each animal. Isolated kidneys were cleaned off extraneous tissue. Then it was preserved in 10% neutral formalin and undergone to histopathological procedures. The obtained kidney slides were stained by (H&E), examined under light microscope.

Statistical analysis

The collected data were analyzed using the SPSS (Statistical Package for Social Sciences) version 15 for Windows® (SPSS Inc, Chicago, IL, USA). Qualitative data was presented as number. Normally distributed data was presented as mean \pm SD. $P < 0.05$ was considered to be statistically significant.

RESULTS:

The obtained results showed a significant increase ($P \leq 0.05$) in the serum levels of total proteins, albumin and phosphorus in EG group compared to the control group. EG+B.O, EG+C and EG+B.O+C groups showed significant decrease ($P \leq 0.05$) in total proteins, albumin and phosphorus comparing with the EG group. EG+B.O+C group revealed an insignificant difference compared to control group. However, the serum calcium levels revealed a significant decrease ($P \leq 0.05$) in EG group compared to the control

group. EG+B.O, EG+C and both showed significant increase ($P \leq 0.05$) comparing with EG group. Rabbits group (EG+B.O+C) revealed an insignificant difference in level of serum calcium compared to control group (Table 1).

The obtained results in a significant increase ($P \leq 0.05$) in the serum levels of uric acid, creatinine and urea in EG group 2 compared to the control group. EG+B.O, (EG+C) and EG+B.O+C groups showed significant decrease ($P \leq 0.05$) in the level of serum uric acid, creatinine and urea comparing with the EG group. EG+B.O+C group revealed an insignificant difference in level of serum uric acid, creatinine and urea compared to control group (Table 2). Additionally, BUN showed a significant increase ($P \leq 0.05$) in EG group compared to control. The groups (EG+B.O, EG+C and EG+B.O+C) showed significant decrease ($P \leq 0.05$) in the level of serum calcium comparing with (EG) group as shown in Figure (1).

There was a significant decrease ($P \leq 0.05$) in the serum levels of CAT, SOD and GSH in EG group compared to control. EG+B.O, EG+C and EG+B.O+C groups showed significant increase ($P \leq 0.05$) comparing with EG group. EG+B.O+C group revealed an insignificant difference in level of antioxidant enzymes compared to control group. Meanwhile, serum level of MDA showed a significant increase ($P \leq 0.05$) in EG group as compared to control. EG+B.O, EG+C and EG+B.O+C showed significant decrease ($P \leq 0.05$) in MDA comparing with EG group. EG+B.O+C group revealed an insignificant difference in level of serum MDA compared to control group (Table 3).

Sections of renal tissues revealed a normal histological structure of kidney in the control group. EG group showed a typical pathological lesions associated with formation of Ca oxalate crystals including: cystic dilatation of renal tubules, homogenous eosinophilic material replaced the necrotic nephron, focal haemorrhagic area and minute crystals of calcium oxalate between the sloughed epithelial tissues. Kidney sections of EG+B.O group showed a mild renal congestion, degenerative changes in the tubular epithelium, beside shrinkage or lobulation of some glomeruli and very fine Ca oxalate salts. Moreover, kidney sections from EG+C group showed a moderate renal congestion with less degenerative changes in the tubular epithelium and presences of leucocytic infiltrations. Kidney sections of EG+B.O+C group revealed an improvement of all the pathological lesions compared with other treated group and prove a recurrence to the normal histological picture of kidney as in control group (Figure 3).

Table(1): Effect of *Citrus aurantium* on serum parameters in urolithiaticrabbits:

Groups	Total protein (g/dl)	Albumin (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
C	6.28 ^b ± 0.82	3.95 ^b ± 0.24	12.25 ^a ± 0.78	5.84 ^b ± 0.53
EG	12.08 ^a ± 1.9	6.37 ^a ± 1.44	6.79 ^d ± 0.66	9.09 ^a ± 0.81
EG +B.O	7.64 ^b ± 0.69	4.67 ^b ± 0.76	8.72 ^c ± 0.93	8.03 ^a ± 0.44
EG+C	7.73 ^b ± 1.04	4.47 ^b ± 0.85	10.08 ^b ± 1.18	6.47 ^b ± 0.92
EG+B.O+C	6.87 ^b ± 1.22	4.22 ^b ± 0.28	11.76 ^a ± 0.71	6.18 ^b ± 0.88

- All data having different letters are significantly different at $p \leq 0.05$. (Letters "a" represented the highest values, followed by letter "b" and "c").

Table(2): Serum renal tests in urolithiaticrabbits (n=8):

Groups	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
C	1.58 ^c ± 0.39	0.96 ^c ± 0.11	22.67 ^d ± 1.59
EG	6.796 ^a ± 1.08	2.84 ^a ± 0.21	62.82 ^a ± 8.81
EG +B.O	4.32 ^b ± 0.69	1.49 ^b ± 0.40	38.95 ^b ± 3.76
EG+C	3.58 ^b ± 0.70	1.52 ^b ± 0.33	32.64 ^{bc} ± 4.79
EG+B.O+C	2.26 ^c ± 0.61	1.28 ^{bc} ± 0.29	28.37 ^{cd} ± 2.05

- All data having different letters are significantly different at $p \leq 0.05$. (Letters "a" represented the highest values, followed by letter "b" and "c").

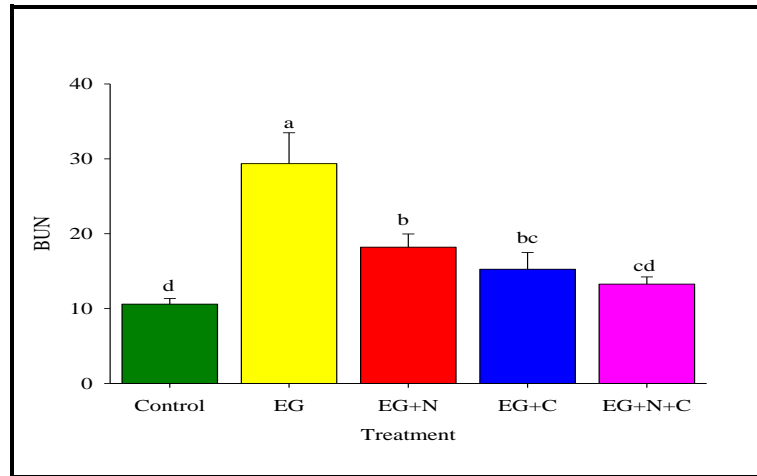


Figure (1): Effects of *Citrus aurantium* and/or Citraforte on BUN levels of urolithiatic male rabbits in all studied groups.

Table(3): Serum antioxidant markers in urolithiatic rabbits:

Groups	CAT (U/L)	SOD (U/ml)	GSH (mmol/L)	MDA (nmol/ml)
C	299 a ± 40.88	5.12 ab ± 0.79	0.99 a ± 0.12	6.41 c ± 0.60
EG	117.5 c ± 10.27	2.05 d ± 0.16	0.35 c ± 0.08	13.08 a ± 1.98
EG +B.O	249.5 b ± 33.42	4.39 bc ± 0.50	0.63b ± 0.19	7.26 bc ± 0.94
EG+C	212.38b ± 25.92	3.93 c ± 0.32	0.52 bc ± 0.12	8.43 b ± 1.06
EG+B.O+C	305.5 a ± 45.34	5.49 a ± 0.63	0.95a ± 0.16	6.10 c ± 0.58

- All data having different letters are significantly different at $p \leq 0.05$. (Letters "a" represented the highest values, followed by letter "b" and "c").

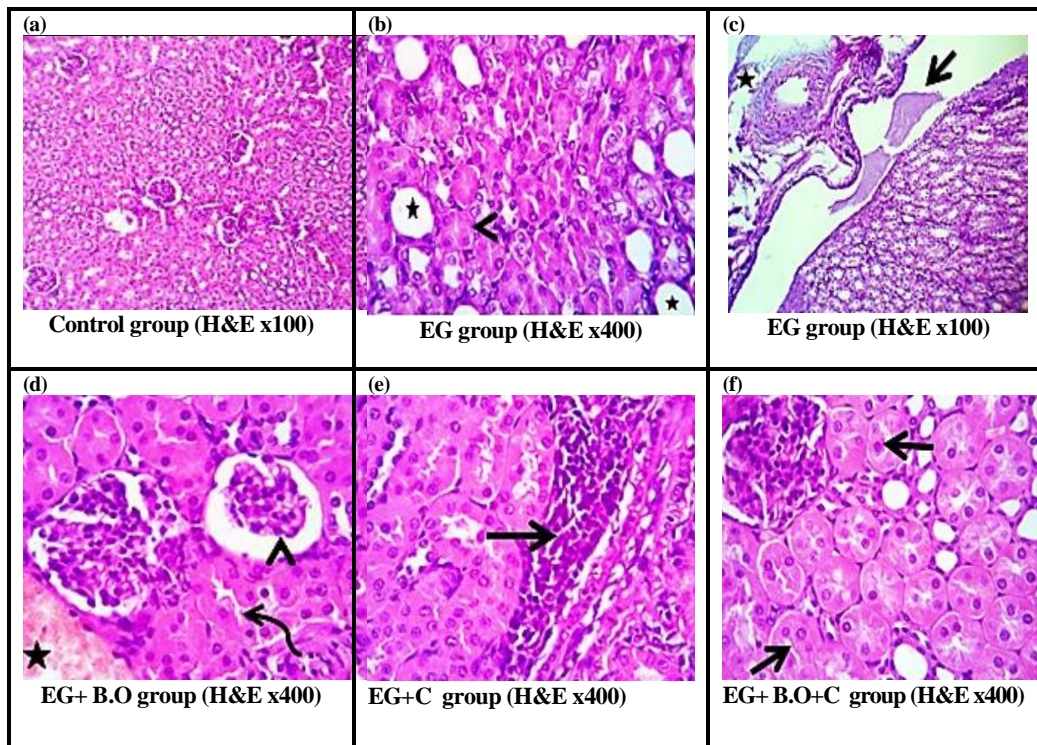


Figure (2): Photomicrograph of kidney sections showing: (a) normal histomorphological structures of kidney with preserved nephron structures, stroma and renal pelvis. (b, c) EG group showing cystically dilated renal tubules (star) with degenerative changes of tubular epithelium, homogenous eosinophilic material (arrow) with focally sloughed epithelium (arrow heads) with presence of calcium oxalate crystal on the necrotic epithelial cells. (d) a mild congestion (stars), degenerative changes (curved arrow) in tubular epithelium, shrinkage or lobulation of some glomeruli (arrow head) and perivascular lymphocytic aggregation (arrow) with a minute calcium oxalate crystals formation and mild edematous and inflammatory reaction. (e) A moderate congested renal blood vessels (star) with degenerative changes in some renal tubular epithelium and presence of leukocytic infiltrations (arrows). (f) A mild congested renal blood vessels (star) with degenerative changes in some renal tubular epithelium with absence of calcium oxalate crystals (arrows).

DISCUSSION:

The understanding of the pathophysiology of renal lithiasis has great importance in the development of effective and safe anti-nephrolithiatic agents (Prathibhakumari and Prasad, 2018). The present study was aimed at evaluating the urolithiatic effects of *Citrus aurantium* (bitter orange) juice as daily intake for dissolving renal stone and prevention of further stone formation parallel to a standard urolithiatic agent.

The urolithiatic action of EG is due to a combination of the severe metabolic acidosis caused by glycolic acid and the precipitation of calcium oxalate crystals resulting in impaired organ function, especially in the kidneys (Jacobsen, 2005). Calcium-oxalate crystals are the most common kidney stones. The mechanisms related to the development of kidney stones are not completely understood (Aggarwal *et al.*, 2013). The cause of kidney stones is multifactorial. The incidence of nephrolithiasis is affecting males and females in both developed and developing countries. This growing trend is believed to be associated with changes in lifestyle modifications such as lack of physical activity and dietary habits (Sofia and Walter, 2016).

The oxalic acid precipitates in the kidney in the form of calcium oxalate crystals and is believed to cause physical damage to the renal tubules, primarily in proximal tubule epithelium (Corley *et al.*, 2005). Our results were in agreement with Pomara *et al.* (2008) who stated that renal failure secondary to ethylene glycol depends on the recognition of the changes of tubular damage in association with calcium oxalate crystals deposition within the tubular epithelial cells and the widespread necrosis of the tubular epithelium in the proximal tubules.

Our results were in agreement with Prabhu *et al.* (2016) who stated the serum calcium levels of rats treated with ethylene glycol + ammonium chloride groups were significantly decreased (1.77 ± 0.01) than the rats treated with cysteine and/or citrus flavonoid. A possible explanation for this result is that the formation of Ca oxalate consumes free calcium (Ca) (Li *et al.*, 2015).

Also, Jacobsen (2005) who stated that, the urolithiatic action of EG is due to a combination of the severe metabolic acidosis caused by glycolic acid and the precipitation of calcium oxalate crystals resulting in impaired organ function, especially in the kidneys. Moreover, Preethi *et al.* (2010) who mentioned that, the decreased glomerular filtration rate in urolithiatic animals due to obstruction of urine flow by the stones in the urinary system. This causes impairment of renal function resulting in decreased excretion of waste products, such as uric acid.

The obtained results are of the same opinion of Preethi *et al.* (2010) revealed ethylene glycol + ammonium chloride induces urolithiasis increased

serum creatinine level. Since, citrus flavonoid decreased the serum creatinine levels significantly compared to the urolithiatic group, this suggests the anti-urolithiatic activity of citrus flavonoid (Prabhu *et al.*, 2016).

The present results are agree with Ramesh and John (2010) who stated that, it is evident that urolithiasis increases serum BUN. Moreover, Prabhu *et al.* (2016) concluded that, citrus flavonoid decreased the serum BUN significantly compared to the ethylene glycol in combination with ammonium chloride treated group, reflecting its anti-urolithiatic activity.

The obtained results are concord with Khan (2014) revealed crystals formation can induce the production of reactive oxygen species (ROS) in kidney tissue. The production of ROS can lead to renal epithelial injury, which increases the areas available for crystal attachment and eventual retention within the kidney.

The present results were in agreement with Li *et al.* (2015) stated that, SOD was decreased and MDA was increased in EG-treated rats when compared with control rats. After preventive treatment with bitter orange, SOD activity was restored, and MDA levels declined when compared with those in EG treated rats

The obtained results of histopathological examination of kidney sections for control rabbits revealed normal architectures of renal tissues without any pathological changes. Similarly, Saha and Verma (2013) showed no calcifications. The glomeruli, proximal and distal convoluted tubules as well as blood vessels were normal without any inflammatory changes.

Our results was in agree with Pomara *et al.*, (2008) stated that renal failure secondary to ethylene glycol depends on the recognitions of the changes of tubular damage in association with calcium oxalate crystals deposition within the tubular epithelial cells and the widespread necrosis of the tubular epithelium in the proximal tubules. Ebisuno *et al.* (1997) demonstrated that crystal-cell interaction resulted in movement of crystals from the tubular lumen into the cells as the results in kidney tissue of crystal adhesion and/or endocytosis. In addition, McMartin and Wallace (2005) reported that calcium oxalate crystals cause damages rat kidney mitochondria and induces the mitochondrial permeability transition. Hence, interference with mitochondrial energy production is a major cause of necrotic cell death.

Thus, the present results refer to the possibility of protection against kidney stones formation by prophylactic and therapeutic use of bitter orange juice daily.

Conclusively, Co-administration of bitter orange (*Citrus aurantium*) juice with Citra forte initiated a synergistic action for curing urolithiasis induced by ethylene glycol in male rabbits and prevention of newly formed stones.

The supplementation of *Citrus aurantium* (bitter orange) juice treated EG-induced urolithiasis in male rabbits as it reduced the growth of urinary stones. The mechanism underlying this effect might be due to its acidic properties, antioxidant, diuretic, and reduction in stone-forming constituents by balancing ions.

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الإمكانات العلاجية لحمضيات الأورانتسيوم على التحصي الكلوي المستحث بواسطة الإيثيلين جليكول في ذكور أرانب

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مرض حصى الكلى هو اضطراب في المسالك البولية يصيب حوالي 12% من سكان العالم ويعتبر ثالث أكثر أمراض المسالك البولية. تتميز حصى الكلى بشكل بلوري بأحجام مختلفة في الكليتين. وقد ارتبطت حصوات الكلى بزيادة معدلات الفشل الكلوي بشكل عام. بلورات أكسالات الكالسيوم هي المكون الأكثر شيوعاً للحصوات ويُعتقد أن تحصي البولي يسبب تجمع البلورات ونمو الجزيئات الغير القابلة للذوبان والتي تؤثر بدورها مع مرور الوقت وتجمعها علي وظائف الكلي. إن أسباب التحصي الكلوي متعددة العوامل تحصي الكلية في الذكور والإناث و يعتقد أن هذا الاتجاه المتزايد مرتبط بالتغيرات في تعديلات نمط الحياة مثل نقص النشاط البدني والعادات الغذائية. ويتم استخدام العديد من الأدوية ، بما في ذلك عقار الثيازيد و السيترات القلوية لمنع تكرار تكون بلورات الكالسيوم او كسالات ، ولكن هذه الادوية ليست واعدة بما يكفي بسبب فعاليتها المحدودة وقلة مقاومتها لتكون ومنع تكون الحصوات الجديدة. ونظراً لعيوب التقنيات الجراحية والاختيار المحدود في العلاج الدوائي ، فإن استكشاف العلاجات الدوائية الجديدة لإدارة حصوات الكلى أمر جدير بالاهتمام. ويأتي دور النباتات الغذائية كمكملات للوقاية من حصوات الكلى أو تقليلها بالإضافة إلى مكوناتها الكيميائية المسؤولة عن إذابة الحصوات. ومع ذلك ، فإن الأدلة الدوائية على النباتات الغذائية في الوقاية من حصوات الكلى لم تثبت بعد بشكل جيد.

في ضوء هذه الحقائق فقد أجريت هذه الدراسة لتقييم التأثيرات الوقائية لعصير حمضيات الأورانتسيوم (البرتقال المر) ضد التحصي الكلوي المستحث بواسطة الإيثيلين جليكول في ذكور الأرانب البالغة. بالإضافة إلى تحديد التغيرات النسيجية في الكلى لدى الأرانب المعالجة.

في هذه الدراسة تم اختيار اربعون من ذكور الجرذان البالغة كنموذج لحيوانات التجارب وتم تقسيمها عشوائيا الي خمسة مجموعات بحيث كل مجموعة تحتوى على ثمانية أرانب. وقد تلقت جميع المجموعة التي تم اختبارها باستثناء المجموعة الاولى الضابطة مادة الإيثيلين جليكول بمعدل 0.75 % في ماء الشرب لمدة 28 يوماً من أجل إحداث التحصي الكلوي. وقد تم إعطاء عقار السيترا فورت بجرعة (300 mg) لكل كجم من وزن الجسم وكذلك عصير حمضيات الأورانتسيوم الطازج بجرعة 3

ملم مرتين يوميا من اليوم الخامس عشر في التجربة ولمدة 3 أسابيع متتالية. وقد تم التصميم التجريبي على النحو التالي: المجموعة الاولى الضابطة، والثانية: اخدت الإيثيلين جليكول والثالثة: اخدت الإيثيلين جليكول مع التجريع بعقار السيترافورت، و الخامسة: اخدت الإيثيلين جليكول مع التجريع بعصير حمضيات الأورانتيوم، والرابعة: اخدت الإيثيلين جليكول مع الأورانتيوم وعقار السيترافورت. وفي نهاية التجربة تم ذبح جميع الأرانب وجمع عينات الدم لإعداد المصل لتنفيذ مختلف المعايير البيوكيميائية كذلك جمع عينات من الكلى لإجراء فحص الأنسجة.

وقد أظهرت النتائج التالي: ارتفاع ملحوظ لمستويات حمض اليوريك والكرياتينين واليوريا ومعدل يوريا النتروجين بالدم في مجموعة الإيثيلين جلايكول مقارنة بالمجموعة الضابطة. اما المجموعات الاخرى المعالجة فقد أظهرت انخفاضا معنويا لحمض اليوريك والكرياتينين واليوريا ومعدل يوريا النتروجين بالدم مقارنة بالمجموعة الايثيلين جلايكول. كما ان المجموعة المعالجة بعصير الأورانتيوم لم تبرز فرقا معنويا مقارنة بالمجموعة المعالجة بعقار السيترافورت. كما ان المجموعة المعالجة بكلاهما لم تظهر اختلاف معنوي بالمقارنة بالمجموعة الضابطة. أظهرت المجموعة المعالجة بعصير الأورانتيوم، و المجموعة الرابعة والخامسة اوضحت ارتفاعا في مستوى الكالسيوم في الدم وانخفاضا معنويا في مستويات الفوسفور مقارنة بالمجموعة الثانية. ولم تظهر المجموعة الاخرى المعالجة اي ارتفاع في معدلات البروتين الكلي والاليومين والجلوبيولين في مقارنة بالمجموعة الثانية. أظهرت انسجة الكلى من الأرانب المعطاة الإيثيلين جلايكول في المجموعة 2 تأثيرات مرضية مرتبطة بتكوين حصوات الكالسيوم ووكسالات والتي ادت الي بعض الظواهر النسيجية المرضية مثل: التوسيع الكيسي في الأنابيب الكلوية، النيفرونات النخرة، كذلك ظهور بلورات دقيقة من أكسالات الكالسيوم بين الأنسجة الكلوية. وكانت المجموعة المعالجة بعصير الأورانتيوم والمجموعة المعالجة بعقار السيترافورت قد ابرزت تحسنا في كل التغيرات النسيجية الباثولوجية في الكلى بالمقارنة بالمجموعة الثانية. اما المجموعة المعالجة بكلاهما اظهرت تحسنا ملحوظا بالانسجة الكلوية و تجدد الخلايا وعودة الانسجة لطبيعتها كما في المجموعة الضابطة.

التوصية: نستخلص من النتائج التي تم الحصول عليها في هذه الدراسة أن العلاج الوقائي بعصير حمضيات الأورانتيوم مع دواء سيترافورت ضد التحصي الكلوي المستحث بالإيثيلين جليكول قد اثبت مقدرته في تفكيك بلورات أكسالات الكالسيوم. بالإضافة إلى ذلك، كانت فعالة في تحسين وظائف النسيج كلوي وتقليل الاجهاد التاكسدي.