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Effect of Using Khella Baladi *(Ammi Visnaga)* Seeds on Kidney Stones in Experimental Rats

تأثير استخدام بذور الخلة البلدي على حصوات الكلى في فئران التجارب

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Abstract

Kidney stones disease is an increasing urological disorder of human healt .The study aims to show the effect of khella baladi on kidney stones in experimental rats, Sixty adulte male wistar albino rats weighing (140 ±10g) , which was divided into (6) groups each group (10) rats. Group (1): negative control group(-ve),Group (2): normal group fed on the basal diet plus 3% khella baladi , Group (3): normal group fed on the basal diet plus 6% khella baladi ,while thirty rats induced with calcium oxalates at a dose 1.5 mol/L for six weeks to get rats induced with kidney stones and divided in to sub groups, Sub group (4): rats induced kidney stone as a positive control group(+ve),Sub group (5): rats induced kidney stone fed on basal diet plus 3% khella baladi ,Sub group (6):rats induced kidney stone fed on basal diet plus 6% khella baladi , The results revealed that Khella Baladi contain moisture,ash,protein, fat, fiber ,and total carbohydrates were 4.5%, 9.5%, 37.53%, 12%, 30.84 and 5.63% , respectively. Moreover, khella baladi extract contains 13 phenolic compounds .In addition,treated with 3%and 6% khella baladi showed that decreased in urea, urea nitrogen ,uric acid, AST, ALT, ALP and GGT activities in group (3) with 6%.Also rats induced with Kidney stone and treated with 3% and 6% khella baladi indicate that a decreased in urea, urea nitrogen ,uric acid, AST, ALT, ALP and GGT activities. So this study recommended to use khella baladi Seeds in diets for it is many benefits.

Keywords

Kidney stones - Khella balladi seeds - Antioxidants – Kidney and Liver functions.

Introduction

Kidney an important organ in the human body. They are responsible for getting rid of toxins and waste products through urine. The kidneys produce hormones, which stimulate red blood cells production and regulate blood pressure as well as control calcium metabolism **(Bahmani *et al.*, 2016).** It also plays a vital role in excreting waste products and toxins, such as urea, creatinine, and uric acid. Moreover regulate extracellular fluid volume and serum osmolality. The functional unit of the kidney is the nephron, which consists of the glomerulus, proximal and distal tubules, and collecting duct. Assessing renal function is crucial in treating patients with kidney disease or pathologies affecting renal function **(Damiati, 2019).**

It's a major filter organ for the blood and the key organ responsible for maintaining total body water balance and circulatory pressure, receive a rich blood supply by which they monitor and modify the functional status of multiple organ systems. Besides clearing metabolic waste products, toxins and drugs from our body, the kidneys also clear circulating cytokines and bacterial toxins, contributing to homeostasis of the immune system. The removal of cytokines from the blood can limit inflammation ***(*Kurts *et al.*, 2013).**

Kidney stones is one of the most common diseases in urology, the majority of kidney stone cases are calcium oxalate stones. (Kusmartsev et al., 2016 and Zeng et al., 2017) Its prevalence has increased over the past few decades. Kidney stone is a disease caused by a combination of factors, including genes, environment, metabolism. The high morbidity and recurrence rates of kidney stone make it a burden on the medical and healthcare **(Ziemba and Matlaga, 2018).**There are four major types of stone are deposit in kidneys which are- calcium (75 to 85%), struvite (2 to 15%), uric acid (6 to10%) and stones of cystine (1 to 2%). The distribution and frequency of these stones is depending upon the geographical location of living being and population studied. Rarely, the long term used of drugs causes the kidneys stones which are about 1% **(Stamatelou *et al.*, 2003).**

Diet composition is considered to play a crucial role in urinary stone formation. There is strong evidence that an inadequate fluid intake is the major dietary risk factor for urolithiasis. While the benefit of high fluid intake has been confirmed, the effect of different beverages, such as tap water, mineral water, fruit juices, soft drinks, tea and coffee, are debated. Other nutritional factors,including dietary protein, carbohydrates, oxalate, calcium and sodium chloride can also modulate the urinary risk profile and contribute to the risk of kidney stone formation. The assessment of nutritional risk factors is an essential component in the specific dietary

therapy of kidney stone patients. An appropriate dietary intervention can contribute to the effective prevention of recurrent stones and reduce the burden of invasive surgical procedures for the treatment of urinary stone disease **(Roswitha, 2021).**

Herbs and medicinal plants have been used for centuries as source of a wide variety of biologically active compounds. The plant crude material or its pure compounds are extensively used to treat diverse ailments **(Swamy and Akhtar, 2019).**

Many of these herbal materials show medicinal activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral activities. Furthermore, these herbs can play the main role in drug synthesis and development. These materials show a significant role in different biological applications such as cancer therapy, cardiovascular disease treatment, neural disease treatment and skin regeneration ***(*Pohl *et al.*, 2016) and (Iid *et al.*, 2020).**

One of the herbal materials is *Ammi* species belong to the family *Umbellifereae (Apiciea)* and *Ammi visnaga* is a natural substance long used in herbal medicine and it contains khellin compound that promotes widening of the blood vessels. It has been used to treat conditions ranging from the menstrual cramps to atherosderosis and also some people take ammi visnaga orally and others use it topically to treat certain skin conditions **(Cathy, 2020).** It has a slight aromatic odour and a very bitter taste. The fruits of *Ammi visnaga* have been used in folk medicine for many years ago to relief kidney stones.In addition used in the treatment of coronary diseases and bronchial asthma, reduces blood pressure and has anti-inflammatory **(Beltagy *et al.*, 2015).** It is well known for its numerous therapeutic properties due to its high amount of biological active compounds Phytochemical analysis of *Ammi visnaga* extract using HPLC revealed the presence of numerous phenolic compounds including coumarin, apignin, kaempferol, caffeic acid, rutin, quercetin, visnagin and ferulic acid .In the same context, listed different bioactive compounds of *Ammi visnaga* such as flavonoids, coumarins and furocoumarins, isobensofurans, sesquiterpenes, phthalides, and miscellaneous.

These molecules possess different pharmacological activities including antibacterial, anticancer, antidiabetic, and antihyperlipidemic activities **(Ahmed *et al.*, 2021) and (Kamal *et al.*, 2022).**The study aims to show the using khella baladi *(Ammi Visnaga)* seeds and it's effect on kidney stones in experimental rats.

Materials and methods

Materials

Plant materials

One kilogram of Khella baladi *(Ammi visnaga)* seeds was obtained from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt).

Chemicals

Calcium oxalates was purchased from the CAYMAN Chemical Company, Michigan, USA.

Kits were used to determine, urea, Urea nitrogen, creatinine, uric acid, aspartate aminotransferases (AST),alanine aminotransferases (ALT), alkaline phosphatase (ALP), and gamma glutamyl transaminase (GGT) were obtained from Sigma Aldrich (St. Louis, MO, USA)

Experimental rats

Sixty Adult male Wistar albino rats, weighting (140 ±10g), were obtained from Animal House Colony, National Research Centre, Dokki, Egypt.

Methods

Prepration of khella baladi *(Ammi visnaga)* seeds

Dirt and dust were removed from khella seeds then milled with a (Molunix,AL-Araby Company,Banha,Egypt)electrical to pass through a sieve, It was stored in dark glass jars and kept at less than 30°C till

Chemical methods

Determination of the gross chemical composition of khella baladi seeds

Moisture, ash, protein, crude fat and crude fiber were determined according to the method outlined by **(A.O.A.C., 2010).**

Carbohydrate content

The total carbohydrate content of the studied Khella Baladi sample was calculated by difference 100 - (other nutrient composition) according to the method described in **(A.O.A.C., 2010).**

Caloric value

The total caloric value was calculated according to the methods of **(Seleet, 2010**)

Total Calories = (protein x 4) + (fat x 9) + (carbohydrate x 4)

Determination of phenols content

HPLC analysis was carried out using an Agilent 1260 series was determined using the method of **(kujala *et al.*, 2000).**

Experimental design

Sixty adult male Wistar albino rats,weighing (140±10g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt; the rats were kept in suitable plastic cages and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to ethical committee of Faculty of Science, Al-Azhar University, Assuit, Egypt; however, this study was approved by the same ethical committee AZHAR 14/2023. After the rats being acclimatized with materials and methods experimental room conditions, they were divided randomly into six groups (10 rats each) as follows:

Group (1): rats fed on basil diet as a negative control group (-ve).

Group (2): Comprise of normal rats those was subjected to feed on khella baladi seeds 3 % for six weeks.

Group (3): Comprise of normal rats those was subjected to feed khella baladi seeds 6 % for six weeks

Group (4): Comprise of induced rat with kidney stones buy calcium oxalates at dose 1.5 mol/L for six week and were not treated with any treatment ,and act as a positive control group(+ve).

Groups (5): Comprise of rats induced kidney stone and fed on khella baladi seeds 3% .

Groups (6):Comprise of rats induced kidney stone and fed on khella baladi seeds 6% .

Blood sampling

At the end of the study period, rats were fasted overnight and following diethyl ether anesthesia, about 0.5 ml of blood sample was collected into heparinized vacutainer tube immediately for the hematological investigations; while non-heparinized blood specimens (3-7 ml) from each rat) were drawn from the retro-orbital plexus using sterile glass capillary (single draw vacutainer needle) into open vacutainer collecting tubes. The non-heparinized blood specimens were left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -80°C until biochemical measurements could be carried out as soon as possible.

Biochemical determination

Body weight gain

At the beginning and the end of the experimental study, each rat was weighted; and the change in body weight (body gain) was calculated according to **(Ashry *et al.*, 2021)**

kidney functions

Serum urea, urea nitrogen, creatinine and uric acid were determined according to the method described by **(Chaney *et al.*, 1962 ;Husdan and Rupoport, 1968, &Trinder, 1969**) ; respectively.

Liver functions

Serum alanine aspartate aminotransferases (AST),alanine aminotransferases (ALT), alkaline phosphatase (ALP), and gamma glutamyl transaminase (GGT)were determined according to (**Schumann & Klauke, 2003; Moss and Henderson, 1999, IFCC, 1983&Trinder, 1969)**; respectively.

Statistical Analysis

The obtained data were statistically analyzed by SPSS computer software. Expressed as mean _SD. Effects of difference treatments analyzed by one –way (ANOVA) followed by Duncan's multiple range tests . Differences were considered significant at $P<0.05$ according to **Snedecor and Cochran (1986).**

Results and discussion

Gross chemical composition and caloric values of khella baladi seeds on dry weight basis

The data in **Table (1)** showed that moisture, ash, proteins, crude fat, crude fiber and total carbohydrates in khella seeds were, 4.5% , 9.5%, 37.53%, 12% ,30.8%, and 5.63%.while caloeic value was 280.64 (K.cal/100g).These results are in agreement with **(El-Absy *et al.*,2018)** they reported that ash and crude fiber were 10.35%,30.35%.While these results are disagreement with **(Ahlam *et al.*,2018)** they reported that ash was 37.8%. Variations in moisture contents, ash, protein, crude fiber, crude fat and carbohydrate due to several factors can these compositions such as climate and growing and postharvest management and processing conditions.

Table (1): Gross chemical composition and caloric values of khella baladi seeds on dry weight basis (mg / 100g)

Sample %	Moisture	Ash	Protein	Crude fat	Crude fiber	Total carbohydrates	Caloric value (K.cal/ 100g)
Khella Baladi Seeds	4.5	9.5	37.53	12	30.8	5.63	280.64

-Mean of three replicates

Phenolic constituents of the khella baladi seeds

The data in **Table (2)** reveled that 13 phenolic compounds were identified in Khella baladi seeds *(Ammi visnaga)* using HPLC analysis. The compounds identified were found to include high contents of chlorogenic acid, ellagic acid and rutin. The lowest values were in daidzein and cinnamic acid. These results are in agreement with **(Nekhla *et al.*,2023) and** (**Zineb *et al.*,2024*)** they found that the most phenolic components seen in large concentrations, chlorogenic acid, isorhamnetin_3-O-rutinoside, p-coumaric acid, and caffeic acid. These due to most intriguing bioactive substances are flavonoids, which have a broad spectrum of biological activities that include immunostimulant, antibacterial, anticancer, antidiabetic, and antioxidative actions.

Table (2): Phenolic constituents of the khella baladi seeds (mg/g)

Parameters	(mg/g)
Gallic acid	341.42
Chlorogenic acid	3254.70
Catechin	N.D
Methyl gallate	128.15
Coffeic acid	302.50
Syringic acid	N.D
Rutin	1407.11
Ellagic acid	2494.73
Coumaric acid	246.05
Vanillin	74.02
Ferulic acid	N.D
Naringenin	701.24
Rosmarinic acid	352.31
Daidzein	7.29
Querectin	264.88
Cinnamic acid	14.59

*N.D. Not detected

Body weight gain

Effect of khella baladi seeds on body weight gain in experimental rats
The data represent in **Table(3)** revealed the effect of khella baladi seeds on the experimental rats. Group (4) rats induced kidney stones showed a significant decrease in body weight; while groups (2) and (3) rats with treated with 3% and 6% khella baladi showed a significant increase in body weight when compared with the control group(-ve).Also groups (5) and (6) infected kidney stones, treated with 3% and 6% Khella baladi showed a significant increase in body weight when compared with the positive control(+ve).These results are in agreement with**(Sherif *et al.*, 2021**) they reported that groups treated with Khella baladi 1.5% and 4.5% showed an increase in body weight as compared to control group. The improvement of body weight may be the cause of khella baladi was rich in phenolic compound, antioxidation activities (**Amin *et al.*, 2015) .**

Tabel (3): Effect of khella baladi on body weight gain in experimental rats (%)

Parameter / Groups	Body weight gain %
Group (1) Negative Control(-ve)	44.1 ± 2.4^{A}
Group (2) Khella Baladi Seeds (3%)	44.8 ± 1.88^{A}
Group (3) Khella Baladi Seeds (6%)	45.4 ± 1.25^{A}
Group (4) 1.5 mol/L(+ve)	21.4 ± 2.25^{B}
Group (5) 1.5 mol/L+Khella Baladi Seeds (3%)	35.6 ± 3.4^{C}
Group (6) 1.5 mol/L+Khella Baladi Seeds (6%)	37.8 ± 4.77^{C}

The same column, means with different superscript letters are significantly different at ($p \leq 0.05$)

Kidney functions

Effect of khella baladi seeds on urea, urea nitrogen, creatinine and uric acid on kidney functions in experimental rats

Data in **Table (4)** revealed that group (4) infected with kidney stones showed a significant increase at ($p \leq 0.05$)in urea, urea nitrogen, creatinine and uric acid when compared to the negative control group(-ve).While groups (2)and (3) rats treated with 3% and 6% khella baladi seeds showed a non-significant changes in the same parameter. Besides groups (5) and (6) infected kidney stones, treated with 3% and 6% Khella baladi showed decreased in urea, urea nitrogen, creatinine and uric acid when compared to the infected group . These results are in agreement with **(Emad *et al.*, 2023)** they reported khella baladi the lowest value was found in the nephrotoxic group of rats fed on 4% powder with a significant difference ($P \leq 0.05$). These results disagreement with (**Sabri and Rasmia, 2022)** they reported that the evidence of nephrotoxic effect as creatinine and acid phosphatase were increased after the treatment with *Ammi visnaga* seed aqueous extract.

Table (4) Effect of khella baladi on urea, urea nitrogen, creatinine and uric acid on kidney functions in experimental rats

Parameters / Groups	Urea (mg/dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group (1) Negative Control(-ve)	38.02±1.36^{A}	19.2 ±0.68^{C}	0.853 ±0.013^{A}	3.3125 ±0.432^{D}
Group (2) Khella Baladi Seeds (3%)	35.12 ±1.37^{A}	17.56 ±0.68^{C}	0.841 ±0.06^{A}	3.31 ±0.505^{D}
Group (3) Khella Baladi Seeds (6%)	34.35 ±1.79^{A}	17.17 ±0.89^{C}	0.77 ±0.098^{A}	2.978 ±0.206^{D}
Group (4) 1.5 mol/L(+v)	54.05 ±1.28^{B}	27.02 ±0.64^{B}	1.145 ±0.050^{B}	4.456 ±0.165^{C}
Group (5) 1.5mol/L+Khella Baladi Seeds (3%)	29.96 ±3.84^{C}	14.86±1.92^{A}	0.839±0.014^{C}	2.622 ±0.175^{B}
Group (6) 1.5mol/L+Khella Baladi Seeds (6%)	26.87 ±1.76^{C}	13.43 ±0.88^{A}	0.802±0.036^{C}	2.088 ±0.202^{B}

The same column, means with different superscript letters are significantly different at ($p \leq 0.05$)

Liver functions

Effect of Khella Baladi on AST ,ALT,ALP and GGT activities in experimental rats

Data in Table (5) obtained that group (4) infected with kidney stones showed a significant increase at ($p \leq 0.05$) in AST, ALT, ALP and GGT activities when compared with control group(-ve),while the treated group with 3% and 6 % khella baladi were non-significant changes in serum AST, ALT, ALP and GGT activities.Also rats infected with kidney stones treated with 6% khella baladi showed a significant at ($p \leq 0.05$) decreased in serum AST, ALT, ALP and GGT activities . These results are in agreement with **(Wannamethee,2010)** Treatment with *Ammi visnaga* seeds lowered the level of AST and ALT. (**Osama *et al.*, 2019*)*** They found that phenolic compounds in khella baladi have reduce the increase in serum levels of AST and ALT.

Table(5):Effect of Khella Baladi on AST, ALT, ALP and GGT activities in experimental rats

Parameters / Groups	ALAT (U/L)	ASAT(U/L)	GGT (U/L)	ALP (U/L)
Group (1) Negative Control(-ve)	49.37 ±6.85^{A}	113 ±3.11^{B}	6.025 ±0.47^{D}	237.5 ±30.425^{C}
Group (2) Khella Baladi Seeds(3%)	47.76 ±2.75^{A}	112.14 ±1.76^{B}	4.56 ±0.32^{D}	228.8 ±22.01^{C}
Group (3) Khella Baladi Seeds (6%)	39.38 ±4.10^{A}	108.2 ±5.83^{B}	4.14 ±0.56^{D}	209 ±19.85^{C}
Group (4) 1.5 mol/L(+v)	127 ±14.52^{B}	216.6 ±26.33^{C}	13.2 ±1.08^{C}	281 ±5.75^{B}
Group (5) 1.5mol/L+Khella Baladi Seeds (3%)	53.74 ±4.43^{C}	83.56 ±5.89^{D}	5 ±0.74^{B}	133.6 ±6.04^{A}
Group (6) 1.5mol/L+Khella Baladi Seeds(6%)	50.98 ±3.05^{C}	60.02 ±4.09^{D}	2.6 ±0.33^{B}	111.6 ±4.07^{A}

The same column, means with different superscript letters are significantly different at ($p \leq 0.05$)

Conclusion

In conclusion, Khella baladi induced a marked amelioration in serum creatinine, blood urea, urea nitrogen and uric acid levels, induced a marked amelioration, serum ALT, AST, ALP and GGT activities,and induced a marked amelioration this study recommended to use Khella seeds in diets for it is many benefits.

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تأثير استخدام بذور الخلة البلدي على حصوات الكلى في فئران التجارب

المستخلص:

مرض حصوات الكلى هو اضطراب بولي متزايد على صحة الإنسان تهدف الدراسة إلى دراسة بذور الخلة البلدي وتأثيرها على حصوات الكلى في فئران التجارب على ستين ذكراً بالغاً من فئران الالبينو، والتي تم تقسيمها إلى (٦) مجموعات كل مجموعة (١٠) الفئران. مجموعة (١) مجموعة الضابطة السالبية، مجموعة (٢) مجموعة صحية تتغذى على العليقة الأساسية مضافاً إليها ٣% مسحوق الخلة البلدي، مجموعة (٣) مجموعة صحية تتغذى على العليقة الأساسية و٦% مسحوق الخلة البلدي، والثلاثون فأر المصابون بمادة اوكسالات الكالسيوم بجرعة *1.5*مول /لتر لمدة ستة أسابيع للحصول علي فئران مصابة وتقسيمها الي مجموعات فرعية ، المجموعة الفرعية (٤) فئران مصابة بحصوات الكلي كمجموعة تحكيم الموجبة، مجموعة (٥) فئران مصابة بحصوات الكلي و تتغذى على النظام الغذائي الأساسي ومسحوق الخلة البلدي ٣% ، مجموعة (٦) فئران مصابة بحصوات الكلي و تتغذى على النظام الغذائي الأساسي ومسحوق الخلة البلدي ٦%. وأظهرت النتائج أن الخلة البلدي تحتوي على رطوبة ورماد. والبروتين والدهون وآلياف وإجمالي الكربوهيدرات ٤.٥%، ٩.٥%، ٣٧.٥٣%، ١٢%، 30.84% ، ٥.٦٣% على التوالي. علاوة على ذلك، تحتوي بذور الخلة البلدي ١٣ مركبًا فينوليًا. بالإضافة إلي ذلك أظهرت المجموعات المعالجة بنسبة ٣%و٦% الخلة البلدي انخفاضا في أنشطة اليوريا واليوريا نيتروجين وحمض اليوريك ALP، AST،ALT، GGT في المجموعة (٣) كما تشير الفئران المحدثة بحصوات الكلي والمعالجة ب ٣% و٦% من الخلة البلدي الي انخفاضا في أنشطة اليوريا واليوريا نيتروجين وحمض اليوريك ALP، AST،ALT، GGT. لذا أوصت هذه الدراسة باستخدام بذور الخلة البلدي في الأنظمة الغذائية لما لها من فوائد عديدة.

الكلمات المفتاحية:

حصوات الكلى – بذور الخلة البلدي – مضادات الأكسدة–وظائف الكلي –وظائف الكبد