

Early Nephroprotective Effect of *Alhagi graecorum Boiss* in RabbitsAhmed H. Ismael *MBChB, PhD*

Department of Clinical pharmacy / College of Pharmacy / Karbala University.

Abstract**Keywords:** Nephrocalcinosis, Manna Tree, Oxalic Acid, Hydrochlorothiazide, Blood Urea, Serum Creatinine.**(Received :May 2014, Accept : Jun 2014)****Abstract:**

Nephrocalcinosis is the deposition of calcium in the form of phosphate or oxalate within the renal tissue, which may lead to impaire the renal function. These crystals may aggregate and provide a nidus for the formation of renal stone. This study aims to evaluate the potential effect of *Alhagi graecorum Boiss* (Manna tree) in prevention of induced nephrocalcinosis in rabbit model. A twenty-one local domestic rabbits were used in the study. Oxalic acid was given orally to induce nephrocalcinosis in a dose of 333 mg /kg. The animals were allocated into three groups (seven in each); a negative control group received D.W, a positive control group treated with 25 mg/kg Hydrochlorothiazide, and a third group that treated with the aqueous extract of Manna tree roots in a dose of 1 gm/kg. Animals were treated two hours before induction of nephrocalcinosis by using one of the above-mentioned agents for each group. Renal function was assessed by estimating blood urea nitrogen (BUN) and serum creatinine, on three occasions: 1st day before induction, 3rd and 5th day after induction. These results were compared with that of the control group to determine how much the nephroprotective effect of the tested agent. Parameters of second and third groups that were treated with Hydrochlorothiazide and Manna tree respectively, showed a highly significant reduction ($p \leq 0.001$) in the levels of BUN and serum creatinine due to their diuretic effect in comparison with the negative control group. From the above one can conclude that *Alhagi graecorum Boiss* possess nephroprotective effect when used in a dose of 1 gm/kg against induced nephrocalcinosis in rabbits.

التأثير الكلوي الواقي المبكر لنبات العاقول في الأرانب

أحمد حقي إسماعيل (بكالوريوس طب وجراحة عامة، دكتوراه في الفارماكولوجي والتداوي)

فرع الصيدلة السريرية، كلية الصيدلة، جامعة كربلاء.

مفتاح الكلمات: التكلس الكلوي، العاقول، حامض الاوكزاليك، الهيدروكلورثيازيد، اليوريا نيتروجين، الكرياتينين.

الخلاصة

التكلس الكلوي هو ترسب الكالسيوم بشكل فوسفات أو اوكزالات في النسيج الكلوي مما قد يؤدي إلى فشل وظيفة الكلية. هذه البلورات قد تتجمع مشكلة نواة لتكوين حصى الكلية. تهدف هذه الدراسة تقييم التأثير الكامن في نبات العاقول في منع التكلس الكلوي المحدث في الأرانب. استخدمت في هذه الدراسة واحد وعشرون أرنباً محلياً. تم إعطاء حامض الاوكزاليك بجرعة 333 ملغم/كغم عن طريق الفم لإحداث التكلس الكلوي. توزعت الحيوانات على ثلاث مجموعات (سبعة في كل منها): مجموعة سيطرة سالبة استلمت ماء مقطر، مجموعة سيطرة موجبة عولجت بالهيدروكلورثيازيد (25 ملغم/كغم)، ومجموعة ثالثة عولجت باستخدام المستخلص المائي لجذور العاقول بجرعة (1غم/كغم). تم إعطاء العلاجات لحيوانات المجاميع المختلفة قبل ساعتين من إحداث التكلس الكلوي. تم تقييم وظيفة الكلية من خلال قياس مستوى اليوريا نيتروجين في الدم ومستوى الكرياتينين في مصل الدم، لثلاث مرات: ابتداء من اليوم الأول قبل بداية الدراسة واليوم الثالث والخامس بعد إحداث التكلس الكلوي. تمت مقارنة النتائج مع مجموعة السيطرة لتحديد مدى التأثير الكلوي الواقي للمواد المختبرة. نتائج المجموعتين الثانية والثالثة والتي تم معالجتها باستعمال الهيدروكلورثيازيد والعاقول أظهرت انخفاضاً ملحوظاً ($p \leq 0.001$) لمستويات كل من اليوريا نيتروجين

Introduction

Nephrocalcinosis is a state of deposition of calcium in the form of phosphate or oxalate in the tissue of the kidney, a process that can impair the kidney function. Nephrocalcinosis usually applies to a generalized increase in renal calcium content rather than the localized increase seen in calcified renal infarction and renal tuberculosis (1). Calcium stones grow on the papillae. Most break loose and cause colic, but they may remain in place so that multiple papillary calcifications are found by x-ray, a condition termed nephrocalcinosis (2). The term nephrocalcinosis was first used to describe the presence of radiologically demonstrable calcium deposits in kidney tissue as distinct from calcium deposits in the renal pelvis or calyces (nephrolithiasis) (3), this calcification within the paranchyma of the kidney rarely cause symptoms however and usually not amenable to traditional therapies appropriate for urinary stone disease (4). Renal calculi develop from crystals that form on the calyx and aggregate to form a calculus. Nephrocalcinosis is usually associated with urolithiasis (5). Most patients with nephrolithiasis however, do not have obvious nephrocalcinosis (4).

Papillary nephrocalcinosis is common in hereditary distal renal tubular acidosis (RTA) and in other types of severe hypercalciuria. In medullary sponge kidney disease, calcification may occur in dilated distal collecting ducts (2). The great majority of idiopathic stone- formers, however, do not have nephrocalcinosis. Nephrocalcinosis may be associated with renal stone in renal tubular acidosis and in primary hyperparathyroidism, were as both tissue deposit and stone consist predominantly from calcium phosphate, and in primary hyperoxaluria, were calcium oxalate is the principle salt found in the tissue and in the stones. This association leads to suggestion that calcium deposit in the kidney may act as a nidus for the growth of kidney stone (6).

Thiazide diuretics reduce the excretion of calcium and oxalate in the urine and reduce the rate of stone formation (8).

Alhagi graecorum Boiss (Manna tree) is well known in India, Iran and Arabia where it is used as a general tonic, anthelmintic and to treat constipation, jaundice and arthritis, where as roots are used as aphrodisiac. In other countries, the plant is known to be: diuretic, blood purifier, with antimicrobial activity, used for dysentery, upper respiratory system problems, wounds, hemorrhoids & uterus problems (7).

Materials & Methods

Animals

Twenty one local domestic rabbits of both sexes weighing 750-1250 grams were used in this study. They were supplied by the animal house of veterinary collage. They were fed standard oxoid pellets, food and water were given *ad libitum*. Each animal was kept in a separated cage, which was provided with a wide wire – mesh floor. The animals were exposed to dark and light 12hr: 12hr.

Animals grouping:

The animals were allocated into three groups (seven animals in each group). Nephrocalcinosis was induced in each group of animals by using oxalic acid in a dose of 333 mg /kg. Lower doses of oxalic acid need a longer period of time to induce nephrocalcinosis (8). The tested agents were given at 9 am, followed by oxalic acid which was given orally by gastric tube after two hours at 11 am.

The effect of the tested agents was studied after five days in corresponding to biochemical analysis of renal function of the kidney of the treated animals.

The tested agents were given to the animals in the following schedules:

group one: The negative control group received 3 ml of distilled water orally 2 hours before induction of nephrocalcinosis by oxalic acid.

group two: The positive control group that were given Hydrochlorothiazide 25 mg/kg of body weight orally in a single daily dose started two hours before giving oxalic acid and continue for five days post induction.

group three: The animals were given the aqueous extract of *Alhagi graecorum Boiss* (Manna tree) roots 1 gm/kg of body weight orally once daily started 2 hours before giving oxalic acid and continue for five days post induction.

Agents used in the study:

A- Oxalic acid:

Acid oxalique, (COOH)₂. 2H₂O, M.W. = 126. 07 g/mol, The British drug houses LTD, BDH Laboratory chemical group, England.

B- Thiazides (Hydrochlorothiazide):

Each tablet contains Hydrochlorothiazide 25mg, The United Pharmaceutical Manufacturing Co. Ltd., Amman, Jordan

C- Alhagi graecorum Boiss (Manna tree):

The aqueous extract of the powdered dried roots was used. The plant was obtained from the local market and approved by the Iraqi medicinal plant center.

D- Chemicals used for biochemical analysis:

1. Urea-Kit S (BioMerieux-France):

It was used for enzymatic determination of BUN.

2. Creatinine-kit (Biolabo- France):

It was used for colorimetric determination of serum creatinine.

Methodology:

1-Method of nephrocalcinosis induction:

Nephrocalcinosis was induced by oxalic acid in a dose of 333 mg/kg body weight. It was a single oral dose administrated by gastric tube (8).

2-Method of aqueous extraction:

The medicinal plants were identified by the Natural council of herbs in Iraq. Aqueous extracts or infusions are dilute solutions containing readily soluble constituent of crude drugs.

Aqueous extracts are usually prepared by diluting one volume of the plant (well grinded) to ten volumes of water at 80 C⁰ in a stopper flask, shaking well and then is allowed to stand for ten minutes, cooled and filtered. For dispensing purposes, infusion should be used within 12 hours of their preparation (9&10).

3-Method of blood sampling:

Blood samples were aspirated for biochemical analysis of renal function at 3 occasions:

- 1- Before induction of Nephrocalcinosis to determine the normal values of BUN, serum creatinine for the tested animals.
- 2- At the third day of the experiment after induction of Nephrocalcinosis.
- 3- At the last day of the experiment after induction of Nephrocalcinosis.

Blood samples were obtained from the heart; 3-5 ml of blood could be aspirated in each occasion.

At most care they were taken to avoid hemolysis, the blood left stand for 30 minutes to be coagulated, and then centrifuged at 2500 rpm for 15 minutes.

The separated serum was aspirated by automatic pipette, and then transferred to an Epindroff tube, stored at -20C⁰ to be ready afterward for biochemical examination.

Methods of biochemical analysis:

Spectrophotometer (sp 300) OPTIMA[®] (Japan) was used for colorimetric determination of serum BUN and serum creatinine estimation.

Method of statistical analysis:

Statistical analysis was done by using statistical package for social science (SPSS) version 19. Data description was simplified as mean and standard deviation (S.D.). Analysis of variance (ANOVA) was made to compare among all groups. Paired sample t-test was done to find the difference between any treated groups with control group. P value was considered significant when it is less or equal to 0.05 (11&12).

Results

The parameters used to monitor renal functions include BUN and serum creatinine levels estimation.

The obtained results in table (1) revealed the normal values of blood urea and serum creatinine levels before administration of any drug to the rabbit.

Administration of a large dose of oxalic acid 333 mg/kg orally to induce nephrocalcinosis in rabbit caused a highly significant elevation in blood urea and serum creatinine measured at the third and fifth day after nephrocalcinosis induction.

Concerning the negative control group (group one), the results obtained as recorded in the third and fifth day 8.2 ± 0.05 versus 9.5 ± 0.4 mmol/L for BUN (Tables 2 and 4), and 96.5 ± 0.6 to 103 ± 1.2 mmol/L for serum creatinine (Tables 3 and 5). The results were more impressive when compared with the normal values before induction of nephrocalcinosis. (Table 1).

Concerning the positive control group (group two), pretreatment of the animals with Hydrochlorothiazide 25 mg/kg was given 2 hours before administration of oxalic acid and continued on the same dose for five successive days showed a highly significant change. These results were more obvious after 5 days with values of 6.4 ± 0.07 versus 9.5 ± 0.4 mmol/L for BUN (Tables 2 and 4), and 84.9 ± 0.4 versus 103 ± 1.2 mmol/L for serum creatinine (Tables 3 and 5).

Administration of aqueous extract of Manna tree at a dose of 1 gm/kg (group 3) orally followed by a large dose oxalic acid showed significant changes which was more impressive in the fifth day with values of 6.2 ± 0.16 versus 9.5 ± 0.4 mmol/L for BUN (Tables 2 and 4), and 79.9 ± 0.8 versus 103 ± 1.2 mmol/L for serum creatinine (Tables 3 and 5).

Discussion

Nephrocalcinosis is a state of deposition of calcium in the form of phosphate or oxalate in the tissue of the kidney, a process that can impair the kidney function.

The fragments of calcium phosphate or calcium oxalate may break freely from the kidney to provide nuclei for the formation of different types and sizes of stones accompanied by many symptoms (13).

For purposes of getting precise estimation of blood urea and creatinine, a pilot study was done for standardization of these value two groups with and without distilled water and there was no effect at all as negative control on the tested parameters.

Nephrocalcinosis was induced in this study by using a single large dose (333 mg /kg body weight) of oxalic acid given orally to the animals to produce this state in the tested groups at the first day of the study (8).

Oxalic acid is a highly oxidizing and strong calcium chelator. It is one of the strongest organic acids (14).

The oxalic acid dose had been selected after several trials by (8) to produce nephrocalcinosis in rabbits this effect was compatible to that work produced by (15) who used a single oral dose of oxalic acid 200 mg/day given to rats.

Five days is the period of the current study. Oxalic acid and the tested agents were given orally by using a gastric tube to avoid aspiration of these agents.

In this study manna tree was used in order to determine its potential effect in prevention or attenuation of nephrocalcinosis that was induced by oxalic acid. The animals were kept on a standard oxid diet, water *ad libitum* and good housing conditions.

The parameters that were used to monitor the renal functions: BUN and serum creatinine level estimation. The normal values of the parameters used for the tested animals were measured for each animal before treatment.

Animals of group 1 received 3 ml of distilled water orally 2 hours before induction of renal calcinosis by oxalic acid and continued for five successive days. This group produced a highly significant elevation ($p \leq 0.001$) in the levels of BUN and serum creatinine. Similar results of elevation in blood urea had been obtained by (15) in rats, and (8) in rabbits after nephrocalcinosis.

Although serum creatinine is the most useful blood analyte in reflecting deterioration of renal function, in this model of nephrocalcinosis, rapid elevation of serum creatinine from 78.8 ± 1.25 mmol/L before induction to 96.5 ± 0.6 mmol/L 3 days after induction and then to 103 ± 1.2 mmol/L 5 days after induction of nephrocalcinosis in the control group. It does not reflect the impairment in renal excretory function alone but also probably to the degree of rhabdomyolysis that occurred because of muscle damage that can release large amount of creatine as the precursor of creatinine (16).

In-group 2, Hydrochlorothiazide was given at a dose of 25 mg/kg of body weight orally started two hours before induction, in order to allow more time for absorption, and repeated as a single daily dose for five successive days post-induction resulted in a highly significant reduction ($p \leq 0.001$) in the levels of BUN and serum creatinine.

Using Thiazide is effective in preventing the progressive effect of nephrocalcinosis and stone formation, and the degree of renal calcification has been correlated with the progression of chronic renal failure (17).

Therefore, the nephroprotective effect of Hydrochlorothiazide may be due to the diuretic and hypocalciuric action. Similar nephroprotective effects had been reported by (18) study.

In-group 3, *Alhagi graecorum Boiss* (Manna tree) was used for its mentioned diuretic effect (19&20). It was tested as an aqueous extract of the plants roots, at a dose of 1 gm /kg given orally started two hours before induction, in order to allow time for absorption, and repeated as a single daily dose for 5 successive days post-induction in order to help in preventing the gradual accumulation of calcium oxalate crystals within the renal tissue. These results showed a highly significant reduction ($p \leq 0.001$) in the levels of BUN, serum creatinine. The results were more impressive after five days.

Really, there is no available data about the effect of Manna tree on renal stone but this study showed that the diuretic effect of the Manna tree roots extract was found to attenuate the renal damage and provide promising results in nephroprotection.

The nephroprotective effects in decreasing BUN, creatinine of Manna tree are similar to the results that was produced by using both barley and celery in (21) study.

In induced nephrocalcinosis, oxalic acid is one of the most highly oxidizing organic compounds and it acts as a strong chelator of cation especially calcium. These properties results in limiting the possibilities for its catabolism and energy production but also make oxalate toxic for most forms of life especially in mammals (22).

In conclusion, manna tree possessed nephroprotective activity at the tested doses in this model of nephrocalcinosis by restoring the normal renal functions and enhancing the bio-defensiveness of the kidney against the damaging effect produced by oxalic acid administration.

Finally, there is a possibility to use this plant in the management of patients with renal calcinosis after confirmation of their effect in clinical trials.

References

1. Monk R.D. and Bushinsky D.A. (2003): Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Feehally J, eds. *Comprehensive Clinical Nephrology*. 2nd Ed. Mosby: Pp. 731-4.
2. Kasper D.L., Braunwald E., Fauci A. S., Hauser S. L., Longo D. L., and Jameson J. L. (2005), "Harrison's Principles of Internal Medicine", 16th Edition, McGraw-Hill. : Pp. 1710-1714.
3. Hamburger C.J., Crosnier J., and Grunfeld J.P. (1979): "Nephrology"; Pp. 1019-1122:p.1099.
4. Smith L.H. (1996): "Urolithiasis ". In Schrier R.W. and Gotts Chalk C. W.: *disease of the kidney*, W.B. Saunders Company, Vol. 1:Pp. 785-813.
5. Behrman, Kliegman, and Jenson (2000): "Nelson text book of pediatrics", 16th Ed. Vol.2; Pp. 1655-1658.
6. Colin Tidy (2009): *Nephrocalcinosis*, Egton Medical Information Systems (EMIS), England. (21): p.701.
7. Al-Yahaya, M.A., Moussa, J.S., Al-Meshal, I.A. , Al-Badar, A.A., and Tariq, M., (1985) *Phytochemical and pharmacological studies for the treatment of fever*, 4th South Asian /Western Pacific Regional Meeting of Pharmacologists. Penang, Malaysia.
8. Al- Jawad F.H. and Al-Ebady Z.A. (2004) "Some drugs and medicinal plants that decrease or prevent nephrocalcinosis in rabbits." *Medicinal congress of Al-Nahrain, College of medicine*.
9. David L. Hoffman (1993): *The Herbalist*, 2nd version, Hopkins technology, Pp.96-112.
10. *Indian Herbal Pharmacopoeia*, Vol. 1, (1998): A joint publication of Indian drug manufacture's association and regional research laboratory, Tanushree Enterprise, Worli Naka, Mumbai, India.

11. Daniel W.W. (1987): "Biostatistics: A Foundation for Analysis in the Health Sciences". 3rd Ed., Canada: John Wiley & Sons: Pp. 136-142.
12. Niazi A. D. (2004): "Statistical Analysis in Medical Research". 2nd Ed., Dept. of Community Medicine, Iraq: Pp. 38-54.
13. Drach G.W. (1998): Urinary lithiasis, WB sanders Comp; Pp. 2085-100.
14. Lane B.G., (1994): "Oxalate, germin, and the extracellular matrix of higher plants." FASEB J. 8: Pp. 294-301.
15. Ahmad A.F. (1989): "Evaluation of the effect of succinic acid administration on the urinary excretion of oxalate in urolithiasis." M.Sc. Thesis. Al-Mosel University.
16. Al- Jawad F.H. and Khalaf R.M. (2007) "Nephroprotective effect of some vasodilators and vitamins in experimental model of acute renal failure" Thi-Qar medical J., volume 1, No. 1: Pp.65-74
17. Konard M. and Swebers (2003): "Recent advances in molecular genetics of hereditary magnesium losing disorder ". J. Am. Soc. Nephrol; 14(1):249-260.
18. Al-Ebady Z.A (2005). "A study of the effect of some drugs and medicinal plants in prevention or attenuation of induced nephrocalcinosis in rabbits." M.Sc. Thesis. Al-Nahrain University.
19. Batanouny K.H., (1999): "Wild Medicinal Plants in Egypt". (With contribution of: E. Aboutabl, M. Shabana & F. Soliman). With support of the Swiss Development Co-operation (SDC). Academy of Scientific Research and Technology, Egypt. The World Conservation Union (IUCN), Switzerland. Pp. 120-122.
20. Attia H.A, Soad M.N, Samar M.M, Naser A.A, and Soha S.E (2010): "Evaluation of the diuretic effect of Conyza dioscorides and Alhagi" , International J. of Pharmacy and Pharmaceutical Sciences, Vol 2, Suppl 3, 162-165.
21. Al- Jawad F.H., Al-Ebady Z.A., and Al-Razzuqi R.A. (2011) "Role of Barley in the improvement of Nephrocalcinosis in experimental rabbit model." International J. of nutrition and Pharmacology, vol.1, issue 1, 69-73.
22. Sivagnanam T. and Saeed R.K. (1998): "Oxalate and calcium oxalate crystals are injurious to renal epithelial cells. Results of invivo and invitro study ", Vol. 11, J. Nephrol.: Pp. 66-69.

Table (1): The BUN and serum creatinine levels of the tested groups measured before induction of nephrocalcinosis by oxalic acid:

Analytes	Mean levels (mmol/L) ± S.D.
BUN	5± 0.7
serum creatinine	78.8± 1.25

Table (2): The BUN levels of the tested groups measured at the 3rd day after induction of nephrocalcinosis by oxalic acid:

Group	Agent	No. of animals	Dose	BUN level(mmol/L)± S.D.
1	Oxalic acid(control group)	7	333 mg / kg	8.2±0.05
2	Hydrochlorothiazide	7	25 mg/kg	7.34±0.05**
3	Manna tree	7	1 gm/kg	7±0.2**

** = Highly significant difference ($P \leq 0.001$) as compared with the control group.

Normal value: 5± 0.7 mmol/L

Table (3): The serum creatinine levels of the tested groups measured at the 3rd day after induction of nephrocalcinosis by oxalic acid:

Group	Agent	No. of animals	Dose	Serum creatinine level(mmol/L)± S.D.
1	Oxalic acid(control group)	7	333 mg / kg	96.5±0.6
2	Hydrochlorothiazide	7	25 mg/kg	90.9±0.4**
3	Manna tree	7	1 gm/kg	88.7±0.3**

** = Highly significant difference ($P \leq 0.001$) as compared with the control group.

Normal value: 78.8± 1.25mmol/L

Table (4): The BUN levels of the tested groups measured at the 5th day after induction of nephrocalcinosis by oxalic acid:

Group	Agent	No. of animals	Dose	BUN level(mmol/L)± S.D.
1	Oxalic acid(control group)	7	333 mg / kg	9.5±0.4
2	Hydrochlorothiazide	7	25 mg/kg	6.4±0.07**
3	Manna tree	7	1 gm/kg	6.2±0.16**

**= Highly significant difference ($P \leq 0.001$) as compared with the control group.

Normal value: 5± 0.7 mmol/L

Table (5): The serum creatinine levels of the tested groups measured at the 5th day after induction of nephrocalcinosis by oxalic acid:

Group	Agent	No. of animals	Dose	Serum creatinine level(mmol/L)± S.D.
1	Oxalic acid(control group)	7	333 mg / kg	103±1.2
2	Hydrochlorothiazide	7	25 mg/kg	84.9±0.4**
3	Manna tree	7	1 gm/kg	79.9±0.8**

**= Highly significant difference ($P \leq 0.001$) as compared with the control group.

Normal value: 78.8± 1.25mmol/L.