Valley International Journals
Open Access Journal

International Journal Of Medical Science And Clinical Inventions
Volume 2 issue 07 2015 page no. 1167-1178 ISSN: 2348-991X
Available Online At: http://valleyinternational.net/index.php/our-jou/ijmsci**Plant Formulation Extract**

Prevents Sodium Oxalate Induced Histopathological Changes In The Kidney

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Abstract

The present investigation was carried out to evaluate the effect of plant formulation(Ziziphus spina-christi leaves , Alhagi maurorum roots, Zea mays silk, Hordeum vulgare grains and Pimpinella anisum seeds) extract on kidney of Sodium oxalate induced urolithiasis in rats. The aqueous extract of plant formulatiom /standard drug cystone were administrated simultaneously at a dose of 100, 200 or 400 mg/kg body weight/day, along with sodium oxalate (70 mg/kg body weight/day) w/v) for 30 days. Significant changes were observed in body weight and

kidney weight of sodium oxalate treated rats. Histopathological results showed disrupted renal parenchyma showing loss of structural arrangement of renal tubules, early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures were observed in the renal tissue of urolithiatic rats .Cystic dilation of renal tubules with completing distraction of other due to necrosis with thickening of Bowman's capsules . Hydropic degeneration of proximal and distal renal tubules leading narrowing it's Lumenic in sodium oxalate treated animals. Administration of plant formula extract or cystone along with sodium oxalate showed significant protective effect in body weight , kidney weight, with few stray areas of calcification in glomeruli and normal tubular structures, increased cellularity between tubules is clearly visible . Moreover, plant formula extract shows higher renoprotective than cystone .In conclusion, Aqueous extract of the formulation of these plants has proved to be an effective drug in prevention of nephrolithiasis.

Introduction

Mankind has been afflicted by urinary stones (Urolithiasis) since centuries, and it is proven to be an important cause of renal failure. Not only in humans but animals and birds also suffer from the urinary stone problem (chandirika *et al*., 2013). Urinary stones or kidney stone formed when the normal balance of water, salt, minerals and other things found in the urine changes. On the one hand kidney must play an important role in water

maurorum , *Ziziphus spina-christi* , *Zea mays*, *and Hordeum vulgare are* frequently chosen and world widely are used as an old folk therapeutic agent. However, until date there is no experimental basis is available to prove clinical evidence on this formulation, hence the present study was designed to evaluate the antilithiatic activity of aqueous extracts of the plant formulation (seeds of *Pimpinella anisum*, leaves of *Zizyphus vulgaris* , grains of *Hordeum vulgare* , silk of *Zea mays* and roots of *Alhagi graecorum*) .

Materials & Methods

Plant Materials

Ziziphus spina-christi leaves and *Alhagi maurorum* roots were collected from the gardens of University of Baghdad . *Zea mays* silk, *Hordeum vulgare* grains and *Pimpinella anisum* seeds were purchased from local markets. The plants were identified and authenticated at the herbarium of Biology Department, University of Baghdad. The part of the plants were washed with distilled water and shade-dried at room temperature , and then homogenized to a fine powder using electric grinder, and then stored in airtight bottles.

Preparation of Plant

Reflux instrument was used in the extraction. Aqueous extract of the plant sample also prepared as follows. To about 1g of the powdered sample, added l00 ml of distilled water and kept in a conservation, but at the same time, minerals with low solubility need to be excreted. The kidney filters waste products from the blood and adds them to the urine that the kidneys produce. When waste materials in the urine do not dissolve completely, crystals and kidney stone are likely to form (Prasobh and Revikumar, 2011). Though the cause of stone formation is hard to determine some factors include a genetic predisposition, age , metabolic disorders such as diabetes, myeloproliferative disease like leukemia, or hypocalcaemia (abnormally high amounts of blood calcium), diet imbalance, a poor intake of water and bacterial infections such as *Escherichia coli*, *Klebsiella*, *Staphylococcus*, or *Mycoplasma*. Stones are often more commonly found in males than females due to a longer urethra (Rathod *et al.*, 2013).

The medical management of urolithiasis, today, includes lithotripsy and surgical procedures which show some significant side effects such as renal damage, hypertension or renal impairment (Tombolini *et al*., 2000). A number of plant drugs have been used in Iraq and elsewhere which claim efficient cure of urinary stones . Herbal formulations have been widely used since very old times. In recent years, there has been an increased interest towards the herbal formulations due to the drift towards the natural sources and a healthy life style (Vyas *et al*., 2012), since, these systems are believed to be free from side effects and affordable. Due to the effectiveness in treating various ailments, *Pimpinella anisum* , *Alhagi*

Sodium oxalate was used to assess the antiurolithiatic activity in rats. Before the start of experiment , five mice were used for two weeks to test the effectiveness of the sodium oxalate substance to composition of stone . After two weeks of acclimation, animals were divided into six groups containing five animals in each. Group I served as control and received regular rat food and drinking water *ad libitum*. Sodium oxalate (70 mg/kg body weight/day) w/v) (Ibrahim and El-Khateeb , 2013) in drinking water was fed to Groups II, III, IV, V, and VI for induction of renal calculi for 30 days . Group III received standard antiurolithiatic drug, cystone (750 mg/kg body weight), from 15th day till 30th day. Group IV received aqueous extract of plant formulation (100mg/kg body weight) treated once daily by oral route from 15th day till 30th day. Group V received aqueous extract of formulation plant (200mg/kg body weight) treated once daily by oral route from 15th day till 30th day. Group VI received aqueous extract of formulation plant (400mg/kg body weight) treated once daily by oral route from 15th day till 30th day.

Experimental Protocols

Group I - Control (Treated with normal diet for 30 days).

Group II- Urolithic control [Treated with sodium oxalate (70 mg /kg/d) in drinking water for 30 days].

water bath at 60 ^{0}C for 4 hrs. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried using a rotary evaporator at a temperature below 60º C. The final dried samples were stored in labeled sterile bottles in the refrigerator , and were referred to as aqueous extract (Adeloye *et al*., 2007; Chandirika *et al*., 2013). We used 50 g of the powdered sample from each plant. The concentrations of the plants extract tested for their inhibitory potency were 100 , 200 and 400 mg/Kg/day.

Experimental Animals

Male rats (23-25 g) purchased from Central Health Laboratory-Baghdad. They were maintained in the animal house of Center of Biotechnology Research- University of Nahrain, Baghdad for experimental purpose. All the animals were acclimatized for two weeks under standard husbandry conditions, i.e.; room temperature of 25 ± 1°C; relative humidity 45-55% and a 12:12h light/ dark cycle. Rats were fed with standard laboratory food and had free access to drinking water (*ad libitum*) . Each experimental group had separate set of animals in standard cages and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for two weeks prior to experimental protocol to minimize if any of non-specific stress.

Experimental Design

histopathological examination. The histoarchitecture was obtained under microscope. (Junqueira & Carneiro, 2003)

Statistical Analysis:

The recorded data were statistically analyzed to obtain the level of significance using the Statistical Analysis System- SAS -computer package program(2012). The means were separated following least significance deference (LSD) test.

Results

There was no significant difference in initial body weight among the groups but at the end of the treatment the sodium oxalate treatment caused a significant loss in the body weight. The simultaneous administration of cystone or plant formula extract showed significant effect by restoring the body weight of treated rats ($p < 0.05$) Cystone and plant formula at a dose of 200 mg/kg was most effective and gave 28,8 and 27.91 g , respectively (Table .1). The isolated kidneys were weighed and compared between the groups. The weight of kidney 0.956 g in sodium oxalate

Group III - Cystone (std.) + urolithic control (Treated once daily by oral route from 15th day till 30th day).

Group IV - Urolithic control + aqueous extract of plant formulation (100mg/kg, treated once daily by oral route from 15th day till 30th day).

Group V - Urolithic control + aqueous extract of plant formulation (200mg/kg, treated once daily by oral route from 15th day till 30th day).

Group VI - Urolithic control + aqueous extract of plant formulation (400mg/kg, treated once daily by oral route from 15th day till 30th day).

Histopathology

At the end of 30th day of treatment, the abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and weighed on a balance, then preserved in 10% neutral formalin. The right kidneys were used for histological examination**.** processed in series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin for

Table 1 . Effect of plant formula extract on body weight (g) in rats.

Treatments	Day of treatment			
	First	S.D	30th	S.D
I	25.00	2.59	29.20	2.55
II	24.10	2.84	20.10	1.94
III	25.82	3.09	28.80	2.69
IV	23.15	2.17	25.76	2.03
V	25.99	2.86	27.91	

VI	25.18	2.33	2.64		
			24.19	1.78	
L.S.D. Value	N.S	-----		6.819 *	-----

N.S= Not-significant

* (P≤0.05).

(70 mg/kg body weight/day)treated group was significantly higher($p < 0.05$) than the Plant formula extract at dose of 200 mg/kg (V group) which gave 0.510 g. The kidneys in other treated groups had lowered weight as compared to sodium oxalate alone treated group but not reach significance (Table .2).

Table 2. Effect of plant formula extract on weight of rat kidney

Group	Mean weight of rat kidney(g)	S.D
I	0.477	0.008
II	0.956	0.026
III	0.498	0.007
IV	0.618	0.017
V	0.510	0.008
VI	0.621	0.014
L.S.D Value	0.328 *	---
* (P≤0.05).		

The longitudinal section of the kidneys that appears kidney cut into two halves(Figures 1 a,b).The section of normal rats kidney (Fig.1 a) showing normal cortex, tubules, capsule and epithelial lining. The capsule covers the surface of the kidney under heath , the capsule are the outer cortex and the inner medulla. The medulla surrounds the renal sinus. Whereas the infected rats kidney (Fig.1 b)showing deposition of microcrystals, marked dilation of tubules and degenerated of epithelial lining. Renal stone in renal pelvic contracted granular surface. And enlargement in medullary part.

Figure 1. longitudinal section of kidney. a- normal b- infected .

In histopathological observations gross examination of rats kidney from control group showed a normal cortical structure of the kidney including no sodium oxalate depositions with normal glomeruli, distended tubules, , proximal and distal convoluted tubules without any inflammatory changes (Fig. 2). On the other hand, (In sodium oxalate group) disrupted renal parenchyma showing loss of structural

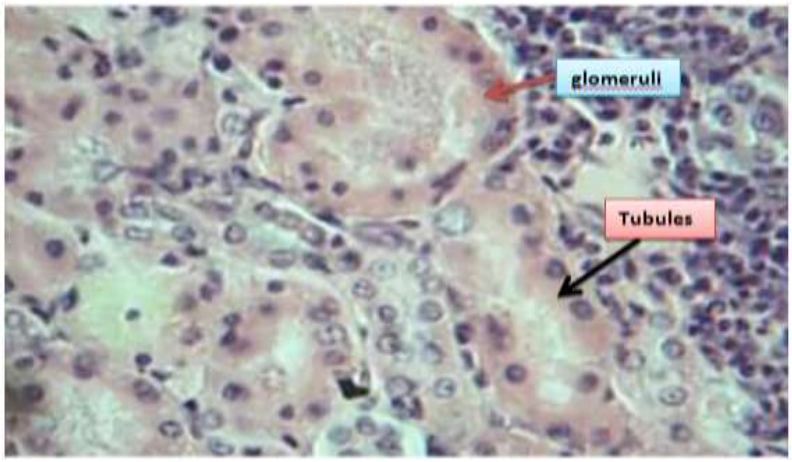

Figure 2 .Light microscopic architecture and calcification in the kidney section of control(I group) . Paraffin section of kidney, hematoxylin and eosin (H&E): 400X.

arrangement of renal tubules, early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures were observed in the renal tissue of urolithiatic rats . Cystic dilation of renal tubules with completing distraction of other due to necrosis with thickening of Bowman's capsules (Fig.3a). hydropic degeneration of proximal and distal renal tubules leading narrowing it's Lumenic(Fig .3 b).

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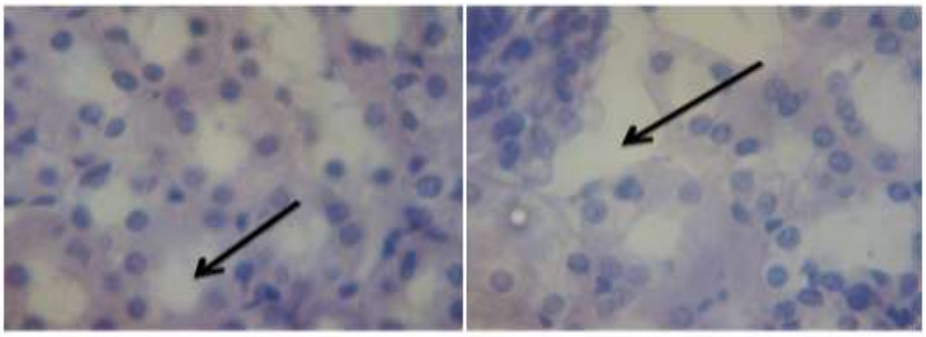
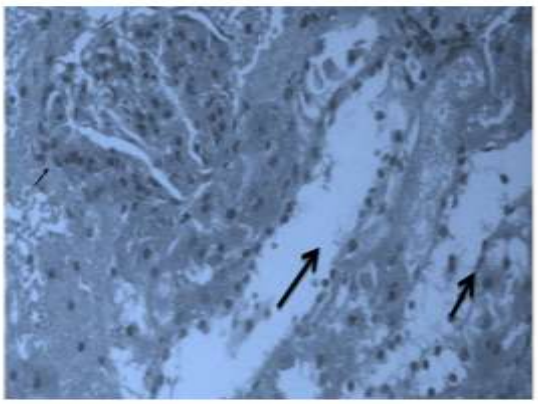


(b)

Figure 3. .Light microscopic architecture and calcification in the kidney section of urolithic(Sodium oxalate, II group) . Paraffin section of kidney, hematoxylin and eosin (H&E): 100X .

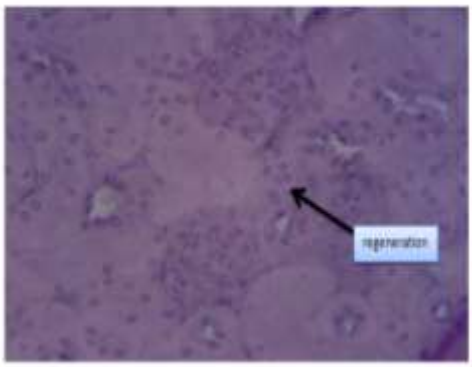
Standard (Cystine750 mg/kg) recovered distended tubules, sclerotic glomeruli, and increased cellularity between tubules(Fig.4 a,b).



1. (B)

Figure 4. .Light microscopic architecture and calcification in the kidney section of prophylactic treatment with Cystone drug (III group) . Paraffin section of kidney, hematoxylin and eosin (H&E): 400X .

The renal tissue of sodium oxalate along with plant formula at the dose of 100 mg/Kg shows regeneration of epithelial cells lining the renal tubules with perivascular cuffings (Fig.5a). regeneration of epithelial cells lining the proximal and distal renal tubules with repairing of glumeulus and Bowman's Capsules(Fig.5b), and regeneration of epithelial cell lining the renal tubules(Fig.5 c).



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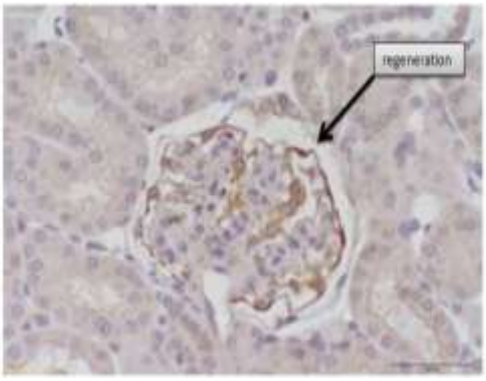

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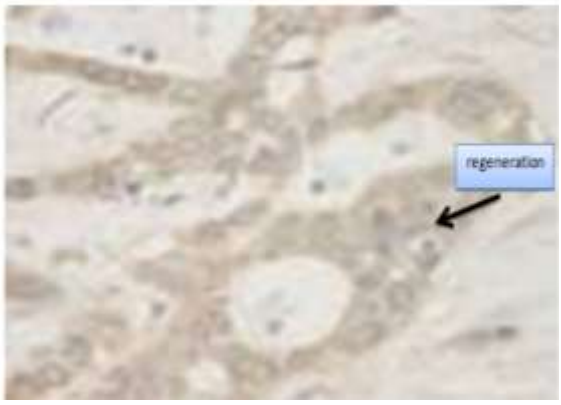

(C)

and normal tubular structures, increased cellularity between tubules is clearly visible (Fig.6).

The renal tissue of sodium oxalate along with plant formula at the dose of 400 mg/Kg shows recovered distended tubules, sclerotic glomeruli, and increased cellularity between tubules (Fig . 7).

Figure 5 .Light microscopic architecture and calcification in the kidney section of prophylactic treatment with plant formula at the dose of 100 mg/kg (IV group) . Paraffin section of kidney, hematoxylin and eosin (H&E): (A 400X), (B,400X), (C,100X).

The renal tissue of sodium oxalate along with plant formula at the dose of 200 mg/Kg shows only few stray areas of calcification in glomeruli

resembles that of humans (Richardson *et al*., 1961). On histological examination, sodium oxalate treated group showed crystals in majority of tubules (Fig.3).These observations support the presence of renal calculi in renal medulla region as observed in human urolithiasis. Treatment with the plant formula and Cystone drug showed very few crystals in the focal region of kidney (Figs.4 - 7), indicating the ability of plant formula in dissolving the preformed calculi. The type of stones formed in human subjects can be predicted from the pH of the fasting Urine(King, 1967). If the pH is acidic 5.0 or below, the stones likely to form are of uric acid type, if 5.0-6.5 calcium oxalate type and if alkaline (7.2 or above) indicates magnesium ammonium phosphate type (Gindi *et al*., 2013). In this study treatment with plant formula (pH=5.08) and Cystone (pH=6.55) may be reversed the pH to normal , this might be responsible for dissolving the complexes of calcium and oxalate, which contributes to their significant antiurolithiatic activity. In the treatment of kidney stones, plants are used as either to dissolve the stones or to aid their passing to guard against further retention. Oxalate and oxidative stress act in a synergy to enhance the risk of urinary stones (Gindi *et al*., 2013). Also histopathological results showed disrupted renal parenchyma, degenerative changes in glomeruli and focal calcification in glomerulo tubular structures in sodium oxalate treated animals(Fig.1b). The weight of each kidney of untreated animals was significantly

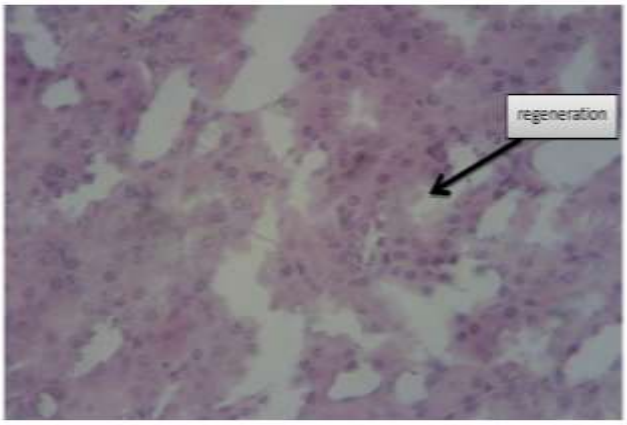

Figure 6 .Light microscopic architecture and calcification in the kidney section of prophylactic treatment with plant formula at the dose of 200 mg/kg (V group) . Paraffin section of kidney, hematoxylin and eosin (H&E): 400X .

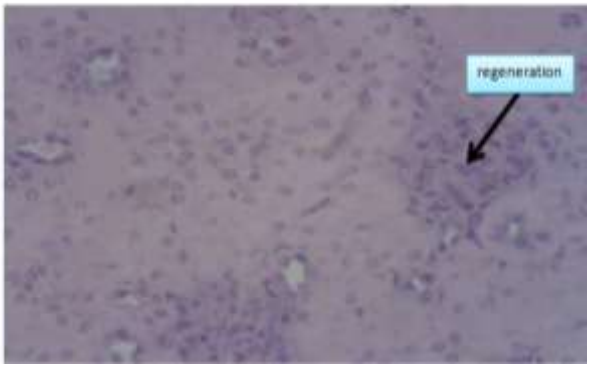

Figure 7 .Light microscopic architecture and calcification in the kidney section of prophylactic treatment with plant formula at the dose of 400 mg/kg (VI group) . Paraffin section of kidney, hematoxylin and eosin (H&E): 400X.

Discussion

Rat is the most frequent used to induce CaOx deposition into kidneys , mimic the etiology of the formation of stones in humans (Saha and Verma, 2011), and because their urinary system

increase supported the results of stone deposition in kidney . Thus, the observed antiurolithiatic activity of test formulation may be attributed to collective effect of these drugs. They suggested that the mechanism involved in observed activity profile may be, a) improving the renal tissue anti-oxidant status and cell membrane integrity. b) inhibition of crystal nucleation, aggregation and growth. c) by increasing urine volume, pH and anti-calcifying activity. And d) regulation of oxalate metabolism. Evidence suggests that in many calcium oxalate stone formers the earliest changes may be calcium salt deposition in the medullary interstitium, in marked hyperoxaluric states, primary hyperoxaluria directs calcium oxalate crystal adhesion to renal epithelial cells (Atmani *et al*., 2004). Sodium oxalate intake leads to increase in levels of promoters like calcium, oxalate, uric acid, and inorganic phosphate and decrease level of inhibitors like magnesium and citrate as observed in disease control groups(Kachchhi *et a*l. ,2012), and concluded that due to the obstruction to the urine outflow by stones and due to severe oxalate induced nephrotoxicity, waste nitrogenous substances accumulates resulting in decreased excretion of urea nitrogen in urine and creatinine clearance. In conclusion, Aqueous extract of the formulation of these plants has proved to be an effective drug in prevention of nephrolithiasis.

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