Effects of hydrophilic extract of Allium Jesdianum on prevention and treatment of ethylene glycol induced renal stone in male wistar rats

Razieh Vahdani[1], Sadrollah Mehrabi[*2], Janmohammad Malekzadeh[2], Haibatallah Sadeghi[2], Ramin Jannesar[2], Farhad Mehrabi[3]

[1]Medicinal plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
[2]Student committee research center, Yasuj University of Medical Sciences, Yasuj, Iran .
[3]Student committee research center, Shiraz University of medical sciences, Shiraz, Iran.
*Corresponding author: sadrollahm@yahoo.com

Abstract: Allium Jesdianum is an endemic plant of Zagross Mountains. It is used for the treatment of renal stones by native resident. The aim of this study is to determine the effects of hydrophilic extract of Allium Jesdianum on ethylene glycol-induced renal stone in male Wistar rats. **Materials and Methods**: In this study 44 male Wistar rats were randomly divided in four groups and studied during 30 days. Two groups of negative and healthy control received usual water and 1% ethylene glycol in water respectively. Low dose and high dose preventive groups, in addition to 1% ethylene glycol, daily gavaged with 1g/kg and 2g/kg of extract respectively. All rats were hold in metabolic cages individually in days 0, 15 and 30 and 24-hour urine samples were collected and checked for volume, oxalate, citrate, calcium, phosphor and uric acid.In 30th day, after taking serum sample, they were killed and their kidneys were sent for pathological evaluation which was examined for presence and volume of calcium oxalate crystals. **Results**: Number of calcium oxalate crystals in negative control groups (18.7 ±26.1), preventive groups with low dose (5.3±8.2) and high dose (80.6±82.8) in comparison to healthy control group increased and this difference between preventive group with high dose and healthy control group was significant ($p < .05$).In 30th day urinary oxalate concentration in preventive and negative control groups were more than healthy control group that was statistically significant ($p<0.05$). **Discussions:** This study showed that hydrophilic extract of Allium Jesdianum has some efficacy for prevention of calcium oxalate stones in rat as an animal model, but its effect on urinary and serum parameters efficient in renal stones is not significant.

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Key words: Allium Jesdianum, calcium oxalate, ethylene glycol, Nephrolithiasis, rat

Introduction

Prevalence of urinary stones is about 1-15 percent (in average 3-5 %) in different populations. The urinary stones are formed most in men, in the third to fifth decade of life and cause acute complications such as flank and abdominal pain, hematuria, and urinary infection(1-2). xantogranulomatos pylonephritis and unilateral and bilateral renal failure could be mentioned among chronic complications (Menon et al., 2007). Stone former patients are treated by different ways, such as conservative methods (consuming liquids, acid and alkaline materials), surgical methods including relief of obstruction, percutaneous nephrolithotomy, transurethral lithotripsy and open surgery (Menon et al., 2007; Khan, 2000; Grases, 2009). In spite of significant progress in diagnosis and treatment of urinary stones, the rate of recurrence is high (about 50% during 5 years)that is due to inefficacy of current methods in changing pathophysiology and course of stone formation.Therefore by providing interventional methods,an important step can be taken in prevention and prolonged treatment of urinary stones(Menon et al.,2007;Moatar, 1997; Shafizadeh, 2002; Chou- Huang, 2008, Khaksarian, 2010). Nowadays due to side effects of chemical drugs, using herbal medicines has attracted the attention of contemporary researchers (Yoshihiro, 1999). The plants Allium Jesdianum belong to Liliace family and grow on the 1800-2600meters altitudes of Zagros Mountains. Native people of this region use the aerial parts of the plant for the treatment of abdominal pain, rheumatic pain and urinary stones. Also in recent studies the analgesic effect of Allium Jesdianum has been shown (Yoshihiro, 1999; Nair, 2006). Further there are some steroids in its bulb, which have shown cytotoxic and cytostatic effects against malignant tumor cells (Grases, 2009; Atmani, 2009). Although using this plant by native people as a treatment for renal stones, no scientific research has been conducted on its effects on renal stones. Therefore we have studied the effects of aerial parts of the plant extract on

preventing calcium oxalate renal stones induced by ethylene glycol in male Wistar rats.

Materials and Methods

This experimental study was performed in 2011 in Medicinal Plant Research Center, Yasuj University of Medical Sciences. Forty four weanling male Wistar rats with a weight range of 120-180gramswere randomly divided into four groups of eleven rats and studied during 30 days.

Group1 received daily just distilled water without receiving ethylene glycol (Healthy control), but group two received distilled water in addition to ethylene glycol1 % (V/V) in drinking water (Negative Control). In addition to 1% ethylene glycol in drinking water, Groups3and4 received1g/kg and 2g/kg daily Allium Jesdianum extract respectively. Rats were acclimatized in stainless steel metabolic cages for one week, and maintained under the temperature of 25±2° C and 12-hours dark-light cycle and 50 percent humidity during the study period. 1% ethylene glycol was used to induce calcium oxalate renal stones. The stone forming dosage of ethylene glycol was determined after observing that 1% ethylene glycol in drinking water induced calcium oxalate crystals in 80% of rat's kidneys.

Measurements

Urine Analysis: 24-hoururine samples of all rats were collected in metabolic cages individually in days 1, 15 and 30 of the study and urine volume, Oxalate, Citrate, Calcium, Phosphorous and Uric acid were determined in laboratory.

Hematologic and pathologic variables:After 30th day, rats were anesthetized with ether and after taking blood samples from their heart for measuring calcium, uric acid and creatinine, they were killed and their kidneys removed. Right kidneys were weighed and for defining the interstitial tissue water incubated in80˚c for 24hours and weighed again.

Calcium oxalate deposits: left kidneys were fixed within10% formalin and cut into 5µm sections in pathology laboratory using CUTIX microtom (Klinpath, Netherland). Hematoxiline and Eosin were used for tissue staining. Then in each section, ten microscopic fields with magnification of 40×10 were randomly selected in equal numbers in cortex and medulla, and numbers of calcium oxalate crystals (number of renal tubes containing these crystals) were counted.

Allium Jesdianum Extract: the plant-Allium Jesdianum – were collected in spring from the Yasuj mountains and coded in herbarium by Herbarium number2526. The aerial part of plant was washed in cold water, dried in shade and room temperature and then grinded and 500 grams of grinded dried plant was soaked in distilled water and filtered after 48hours.The filtered liquid was concentrated by rotary at50˚c and under vacuum condition. The resulted concentrate was incubated in50˚cto be completely dried.195 grams of dried extract were obtained from 500 grams of dry plant. The obtained extract was kept in refrigerator and desired concentrations were prepared in distilled water to be gavaged to rats.

Statistical calculation: The data were analyzed by SPSS software version 15 and groups' means were compared using one way Analysis of Variance with post hoc Scheffé's Test to compare groups in pairs.

Results

Measurement of urinary variables: urinary oxalate levels are shown in table 1, for the first, fifteen and thirteenth days of the study. The mean levels of urinary oxalate are increased at 15th and 30th days in all groups receiving ethylene glycol but not in the healthy control. The differences was statistically significant compared with healthy controls ($p<0.05$). In the day of 15th, the lowest rate of urinary oxalate was seen in group receiving high dose and were statistically difference with the two other groups (prevention with low dose and negative control; $p<0.05$). In the third measurement (day30th) the level of urinary oxalate in the prevention groups with low dose (0.75±0.07) and high dose (0.75±0.14) and negative control group (1.11±0.11) were more in comparison to healthy control group (0.42±0.04) ($p<0.05$). Also the levels of urinary oxalate in prevention groups (high and low dose) were significantly lower than negative control ($p<0.05$).

Table 1. Mean and standard deviation of urine oxalate levels at first, 15th and 30th days in studied groups

Time / group	number	Day 1	Day 15	Day 30
Healthy Control	11	0.48±0.04	0.6±0.26	0.42±0.04
Negative Control	11	0.44±0.08	1.1±0.21	1.11±0.11
low dose	11	0.44±0.06	1.04±0.18	0.75±0.07
high dose	11	0.47±0.06	0.68±0.11	0.75±0.14
P value		0.521	0.000	0.000

Study of other measured urinary parameters such as 24-hour volume, citrate, pH, calcium, phosphorous, creatinine and uric acid did not show statistically significant difference between the groups.

serum biochemical parameters: it was observed that the lowest level of calcium was in prevention group with high dose (8.91±0.33) and highest level of calcium was in negative control group (9.47±0.06) and difference between these two groups is statistically significant($p<0.05$).The lowest level of serum phosphorous is observed in the prevention groups with low dose(7.87±0.36) and high dose(7.36±0.75) and these two groups have a statistically significant difference with healthy control group(8.9±0.78) ($p<0.05$). Study of other measured serum parameters including uric acid and creatinine did not show any statistically significant difference between the groups.

Kidney weight (percentage of interstitial water): As it is observed in table 2 the highest initial weight of kidney belong to prevention group with high dose (1.4±0.4) which has statistically significant difference with healthy control group(1.03±0.13) ($p<0.05$).

Table 2. Mean and standard deviation weight of the kidneys in different groups(gram)

Group	number	Initial Weight(gr)	Dry weight
Healthy Control	11	1.03±0.13	0.37±0.08
Negative Control	11	1.15±0.16	0.37±0.06
Prevention With low dose	11	1.17±0.15	0.44±0.08
Prevention With high dose	11	1.4±0.4	0.4±0.06
P value		<0.05	>0.05

Levels of calcium oxalate crystal depositions in kidney: The highest number of crystal depositions were in prevention group with high dose (80.9±82.83) which has statistically significant difference with other groups including healthy control(zero) negative control (18.7±26.05) and prevention with low dose(5.3±8.26) ($p<0.05$).deposition of *calcium oxalate crystal* has been showed in fig 1.

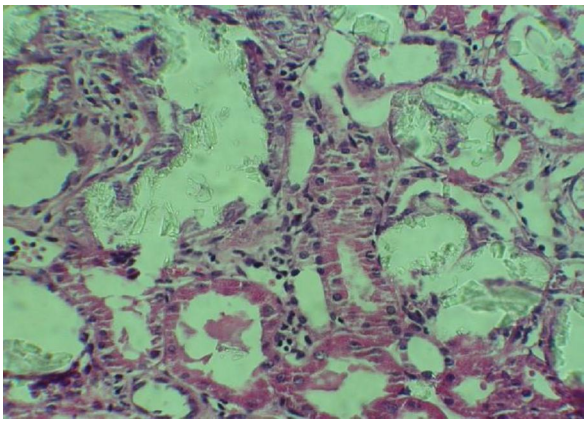

Fig 1. Calcium oxalate crystal depositions in tubules of rat's kidney

Discussion

Nowadays by advancement of technology and availability of different methods of lithotripsy such as Extracorporeal lithotripsy (ESWL), Transurethral lithotripsy (TUL) and Percutaneous nephrolithotomy (PCNL), great development has occurred in treatment of urinary tract stones, but still there is no effective and harmless pharmaceutical method which leads to perfect treatment or prevents urinary stone formation (Menon et al., 2007; Khan, 2000; Grases, 2009). Different herbal and industrial medicines have been marketed to reduce stone formation, which may be relatively effective on prevention and treatment of calcium and uric acid stones (Grases, 2009; Chou-Huang, 2008; Sevsen, 2008). Because many people of south part of Iran believe that AlliumJesdianum has efficacy for treatment of urinary stones and use it, the aim of this study was to study the effect of Allium Jesdianum extract on the calcium oxalate stones induced by ethylene glycol in male Wistar rats. In this study AlliumJesdianum increased urinary level of oxalate and also increased calcium oxalate crystal depositions in tubules of rat's kidney but it had no significant effects on other urinary parameters that are important in formation of renal stones. In a study conducted by Atmani F et al. in 2007, the effect of Cynodondactylon on renal stone was studied. In this study ethylene glycol was used as stone inducing substance. At the end of research, urine analysis was performed for oxalate, calcium, sodium and crystals.

Also histological study was carried out to determine the level of crystal depositions in kidney. Except urinary oxalate in prevention group, and calcium and sodium in treatment group, the extract had no effect on other urinary factors, but caused a reduction in level of the crystal depositions in kidney, which shows its preventive effect on renal stone formation (Atmani.2007). Also in a study conducted by Hajzadeh et al. in 2006, the effect of alcoholic extract of Nigella Sativa on ethylene glycol induced renal stone in rat model was studied. The findings of this research showed that the alcoholic extract of Nigella has preventive effect on calcium oxalate crystal depositions in kidney (Hajzadeh.2006). Our study is not in consistency with previous studies, and in the extract receiving groups, an increase in calcium oxalate crystal depositions in kidney is observed. Increase in number of crystals is dose dependent and in prevention group with high dose of extract, more crystals were observed. Also in this study the level of urinary oxalate in the extract receiving groups showed a reduction, which could be because of deposition of these crystals in kidney.In a study by Akanae et al. the protective effect of Orthosiphon grandiflorus on calcium oxalate stone formation was studied and ethylene glycol 5% in drinking water with vitamin D were used as stone inducing diet. The result of this study indicates that Orthosiphon grandiflorus has a significant inhibitory effect on crystal depositions in the calcium oxalate stone forming rat model (Akanae, 2010).

Also in a study by Moriyama et al. the inhibitory effect of Quercus Salicina extract on urinary oxidative stress and level of urinary calcium in rat ca-oxalate renal stone model was studied. The result of this study showed that the extract of Quercus Salicina has antioxidative effect and could prevent renal stone formation (Moriyama, 2009).The results of these two studies were not in consistency with the result of our study, because in these studies, the plants which were used had anti oxidative effect and were effective in prevention of urinary stones formation, while in our study the extract had no effect on reducing the level of stone formation. Also according to the results of our study, the extract of Allium Jesdianum had no effect on pH, ca, creatinine, uric acid and 24-hour volume. Although there isn't any study regarding efficacy of AJ extract in treatment and prevention of renal stones, but in contrast to above mentioned studies that show effect of Quercus Salicina and Cynodondactylon on urinary stones and in spite of domestic believe about effectiveness of AJ on renal stones its extract has no effect on the most urinary factors effective in formation of urinary stones such as urine volume, and urinary calcium, phosphor and uric acids that are the most important metabolic factors efficient in formation of urinary stones. Also it increases urinary concentration of oxalate that may be due to synergistic effect of ethylene glycol with extract of AJ. In a research by khaksarian et al. the analgesic effect of AlliumJesdianum extract was studied, this research showed that A.J consists of morphine in addition to quinolone and benzene and has analgesic effect (Khaksarian, 2008). In our research the analgesic effect of A. J wasn't studied, but the result of khaksarian research may support its efficacy on relieving pains of urinary stone passage.

Also in our study the kidney weight in A.J receiving rats was more than healthy control group, and this increment may be due to accumulation of interstitial water in kidney because of inflammatory damage of epithelial cells of nephrons which is created by crystals deposition in tubules of kidney. The results of this research showed that the A.J extract in low doze have some preventive effect on renal stone formation and the increment in co ax crystal depositions in preventive groups may be due to stone forming effect of A.J or its synergic effect with ethylene glycol on stone formation, which needs more researches.

Corresponding Author:
Sadrollah Mehrabi Professor of Endourology, Urology Department, Yasuj University of medical sciences, Yasuj, Iran
Email: sadrollahm@yahoo.com

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