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A designed model of urinary stone formation and the effect of *Lapis judaicus* on urinary parameters in rat

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Abstract

Urinary stones after urinary tract infections and prostate disorders are the third most common urinary tract diseases. In recent years various herbal medicines for preventing or treating renal stones have been marketed and may be helpful in prevention and treatment, but still effectiveness and safe drug therapy without surgical intervention is controversial. This study aimed to evaluate the effects of *L. judaicus* in the treatment of urinary stones. In order to form stone in an animal model, 1% Ethylene Glycol (EG) dissolved in drinking water was used. Forty rats were divided into eight groups. After 45 days of drug administration, to determine the effects of the drug on urinary and serum parameters, 24-hour urine and blood samples were obtained. Then animals were sacrificed and kidneys were sent to the pathology laboratory for histological examination. Results of our study showed that prescribing *L. judaicus* in the co-treatment group reduced serum BUN, elevated urinary citrate and urine pH, and reduced urinary parameters such as urine protein, calcium, oxalate, phosphorus, and creatinine, therefore *L. judaicus* is effective in inhibition of urinary stones formation. Histopathologic results showed a decline of urinary stones in *L. judaicus* groups in comparison with ethylene glycol group.

Keywords: Ethylene glycol, Urinary stone, Lapis judaicus, Urine

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1. Introduction

Urinary stones are one of the most common urologic disorders with increasing prevalence due to changes in lifestyle and diet (1). The main reason for urinary stones is still unclear. However, there are environmental and physiologic factors involved in the formation of stones (2). These factors include genetic predisposing factors, age, and gender. Lifetime-related factors include low consumption of liquids, a sodium-rich diet and high consumption of red meat and proteins, consumption of high amounts of oxalate and calcium in foods, and complementary vitamins (3-5).

Formation of urinary stones is a repeated occurrence and approximately half of patients will manifest the signs of disease in 10 years (6). Some conditions facilitate the formation of urinary stones. One of the important conditions is the presence of urinary crystalloids in urine. In the natural state, magnesium (Mg) in the kidneys reacts with oxalate and forms an Mg-oxalate complex. A decrease of this complex in urine would accelerate the formation of urinary stones (6, 7). Another important factor in the formation of stones and conformation of stones is the urine pH. A pH less than 7 and more than 7 lead to the formation of calcium oxalate and calcium phosphate stones, respective-

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ly. Consumption of a protein-rich diet, (more than 2 g/kg/day) results in elevated amounts of calcium and uric acid in urine and reduced pH of urine and therefore increases the possibility of calcium oxalate stones in urine (8, 9)

There are four main types of urinary stones, including calcium, uric acid, cysteine, and struvite stones. The incidence of these stone types is different. Prevalence of calcium oxalate and calcium phosphate is the most common and cysteine is less common (10, 11).

There are several treatment options for urinary stones, such as ureteroscopy, radiation, and medication. Choosing the method of treatment depends on the patient's clinical condition, size of the stone, severity of obstruction, and kidney function. There are some histories of using herbal drugs in the management of urinary stones and related complications (12-14).

L. judaicus is a granite stone with a formation of quartz strains. Extractions of *L. judaicus* are used as a drug for urinary stones in traditional medicine in some West Asian countries. *In vivo* studies demonstrated inhibitory effects of *L. judaicus* on calcium oxalate crystallization. Evidence for pharmacologic effects of *L. judaicus* is limited (15, 16). The present study aimed to evaluate the effects of *L. judaicus* on the rat urinary stones, urinary and biochemical factors, and histopathologic changes. Formation of urinary stones in rats was conducted using ethylene glycol (EG) with a concentration of 1%.

2. Material and methods

2.1. Study design

This experimental study was conducted using Sprague-Dawley rats. Eight sanitary rat cages were provided and five male rats were placed in each cage. Rats were kept in standard conditions, including 22-25 °C temperature, relative humidity of 50-60%, darkness and brightness periods of 12 hours, and standard nutrition and water sources. Forty male rats in the weight range of 190-210 grams were kept in cages for a week and then divided into the four main groups. The first group received only water for 45 days as a normal control group. The second group had three subgroups as the co-treatment group. Subgroup 1-A received 1% EG dissolved in water for 45 days. Subgroup 2-A received 1% EG dissolved in water and powder of *L. judaicus* with a concentration of 50 mg/kg via gavage for 45 days. Subgroup 3-A received 1% EG dissolved in water and powder of *L. judaicus* with a concentration of 100 mg/kg via gavage for 45 days. The third group had three subgroups as a treatment group. Subgroup 1-B received 1% EG dissolved in water for 45 days and received water 10 ml/kg from the 15th to the 45th day. Subgroup 2-B received 1% EG dissolved in water for 45 days and powder of *L. judaicus* with a concentration of 50 mg/kg via gavage from the 15th to the 45th day. Subgroup 3-B received 1% EG dissolved in water for 45 days and powder of *L. judaicus* with a concentration of 100 mg/kg via gavage from the 15th to 45th day. The fourth group received powder of *L. judaicus* with a concentration of 100 mg/kg via gavage for 45 days.

2.2. Urinary evaluations

After 45 days rats were transferred to a metabolic cage and kept for 24 h and, 24-h urine sample was collected and sent to a laboratory to measure urinary factors including protein, creatinine, uric acid, calcium, chlorine, magnesium, sodium, potassium, phosphorus, pH, oxalate and citrate levels.

After urinary collection, all rats were sacrificed by animal ethics guidelines through an anesthesia procedure, and blood samples and kidneys were collected.

2.3. Biochemical evaluations

Blood samples were collected from the hearts of rats and serum was provided from blood by centrifuging at 3500 rpm for 5 min. Serum samples were sent to a laboratory to measure BUN, creatinine, calcium, and phosphorus levels.

2.4. Histopathology evaluations

The kidneys of rats were brought out and transferred to the pathology department using 10% formalin to evaluate possible kidney damage and stone formation.

2.5. Statistical analysis

The data was analyzed by SPSS *v. 22* software. The one way ANOVA followed by Tuckey post-test was used to demonstrate differences between observed variables. Also, a $p<0.05$ was considered statistically significant.

Table 1. Urinary parameter in co-treatment groups

Groups name (n=5) Factors	Tap water	*L. judaicus* 100 mg/kg	1% EG	*L.judaicus* 50 mg/kg+1% EG	*L.judaicus* 100 mg/kg +1% EG
Protein mg/day	78.23±10.12	75.29±18.56	98.65±8.25*	51.23±11.26++	68.12±10.25++
Cr mg/day	21.25±2.35	19.25±4.11	31.13±3.68**	20.14±3.28++	24.13±2.22++
Uric acid mg/day	3.80±2.30	4.13±1.23	6.20±1.24*	6.12±1.58	6.16±2.25
Oxalate mg/day	1.11±0.22	1.13±0.21	2.95±0.26**	2.09±0.38+	2.34±0.54
Citrate mg/day	8.56±1.55	8.60±0.89	8.15±2.20	11.28±1.70+	9.24±1.33
Phosphorous mg/day	17.25±2.66	9.89±2.80*	21.23±1.16	14.25±3.26++	10.68±4.25++
Ca mg/day	9.63±1.11	6.25±2.35**	12.36±1.18**	6.11±3.41++	6.20±3.00++
Cl mg/day	72.27±7.20	67.13±8.70	70.41±8.11	69.65±6.56	68.99±6.89
Mg mg/day	4.60±0.96	3.85±0.44	4.62±0.66	4.66±0.99	4.30±0.45
Kmg/day	5.30±1.02	5.63±1.02	5.66±0.63	5.41±1.01	5.27±1.65
Na mg/day	140.23±8.25	141.23±4.26	150.40±6.99	147.65±6.77	142.36±4.56
pH	8.5±1.39	9.80±0.88	8.90±0.60	9.60±0.36+	10.20±0.28++

*Indicates significant difference ($p<0.05$) between *L .judaicus*100 mg/kg or 1% EG groups compared to tap water group. **Indicates significant difference ($p<0.01$) between *L .judaicus*100 mg/kg or 1% EG groups compared to tap water group. +Indicates significant difference ($p<0.05$) between *L. judaicus* 50 mg/kg + 1% EG or L.judaicus 100 mg/kg + EG 1% groups compared to 1% EG group. ++Indicate significant difference ($p<0.01$) between *L. judaicus* 50 mg/kg + 1% EG or *L. judaicus* 100 mg/kg + EG 1% groups compared to 1% EG group.

3. Results

3.1. Urinary parameter results

The results of urinary parameters of co-treatment and treatment groups are shown in Tables 1 and 2, respectively. Results demonstrated that the mean of urinary protein in rats that received *L. judaicus* (100 mg/Kg) in comparison with the tap water group was not significantly different. The mean of protein in co-treatment and treatment groups in rats that received 1% EG alone, in comparison with rats that received tap water, were significantly higher. In the co-treatment group, mean of urinary protein decreased by 48% and 30% in rats that received *L. judaicus* (50 and 100 mg/Kg) in comparison with the rat that recieved 1% EG,

Table 2. Urinary parameter in treatment groups

Groups name (n=5)Factors	Tap water	*L. judaicus* 100 mg/kg	1% EG	*L. judaicus* 50mg/ kg+ 1% EG	*L. judaicus* 100 mg/kg+1% EG
Protein mg/day	78.23±10.12	75.29±18.56	117.2±14.12**	139.00±22.32	71.50±26.11+
Cr mg/day	21.25±2.35	19.25±4.11	41.26 ± 6.98**	29.33±9.12+	29.14±5.66+
Uric acid mg/day	3.80±2.30	4.13±1.23	5.65 ± 2.35*	3.5±2.45	6.48±1.99
Oxalate mg/day	1.11±0.22	1.13±0.21	2.56± 0.36**	1.98±0.12+	2.22±0.24
Citrate mg/day	8.56±1.55	8.60±0.89	8.23 ± 1.16	09.84±1.06	9.11±0.88
Phosphorous mg/day	17.25±2.66	9.89±2.80**	24.12 ± 2.1**	16.15±2.33++	10.29±3.40++
Ca mg/day	9.63±1.11	6.25±2.35**	11.36 ± 1.23	9.38±3.11	3.35±1.21++
Cl mg/day	72.27±7.20	67.13±8.70	88.15 ± 9.12*	84.11±9.55	79.29±6.30
Mg mg/day	4.60±0.96	3.85±0.44	4.96 ± 0.78	4.45±1.10	4.92±0.69
Kmg/day	5.30±1.02	5.63±1.02	6.01 ± 1.20	5.56±1.30	5.19±0.96
Na mg/day	140.23±8.25	141.23±4.26	143.25 ± 9.65	141.19±9.55	139.28±8.25
pH	8.50±1.30	9.80±0.88	8.80 ± 1.20	8.90±1.10	8.70±0.69

*Indicates significant difference ($p<0.05$) between *L. judaicus*100 mg/kg or 1% EG groups compared to tap water group. **Indicates significant difference ($p<0.01$) between *L .judaicus*100 mg/kg or EG 1% groups compared to tap water group. +Indicates significant difference ($p<0.05$) between *L. judaicus* 50 mg/kg + 1% EG or *L. judaicus* 100 mg/kg + 1% EG groups compared to 1% EG group. ++Indicate significant difference ($p<0.01$) between *L. judaicus* 50 mg/kg + 1% EG or *L. judaicus* 100 mg/kg + 1% EG groups compared to EG 1% group.

Table 3. Biochemical results in co-treatment group

Groups name (n=5)Factors	Tap water	*L. judaicus* 100 mg/kg	EG 1%	*L. judaicus* 50mg/ kg + 1% EG	*L. judaicus* 100 mg/kg+1% EG
BUN mg/dl	23.40±2.01	20.87±2.13	28.75±2.13*	28.60±3.11	24.00±3.00+
Cr mg/dl	0.58±0.12	0.58±0.23	0.53±0.23	0.76±0.18	0.56 ±0.11
Ca mg/dl	9.78±1.11	8.42±2.32	8.38±0.98	9.92±1.25	9.50±1.69
Phosphate mg/dl	7.32±0.89	6.24±0.28*	6.50±0.56	6.84±0.65	7.06±0.45

*Indicates significant difference ($p<0.05$) between *L. judaicus*100 mg/kg or 1% EG groups compared to tap water group. +Indicate significant difference ($p<0.05$) between *L. judaicus* 50 mg/kg +1% EG or *L. judaicus* 100 mg/kg + 1% EG groups compared to EG 1% group.

respectively. The treatment dose of *L. judaicus* (100 mg/Kg) reduced the mean of urine protein by 39% in comparison with the EG group. The mean of creatinine in co-treatment and treatment groups in rats that received EG alone in comparison with rats that received tap water was significantly higher. In the co-treatment group, rats received *L. judaicus* (50 and 100 mg/Kg) the mean creatinine decreased by 35% and 22% in comparison with the EG group, respectively. In the treatment group, rats received *L. judaicus* (50 or 100 mg/Kg) the mean of creatinine decreased by 29% in comparison with the EG group. The mean of uric acid in the co-treatment group in rats that received 1% EG alone in comparison with rats that received tap water was significantly higher with a 63% increase. The mean of oxalate in the co-treatment and treatment rats that received 1% EG alone in comparison with rats that received tap water was significantly higher (more than 1.6 and 1.5 fold increase, respectively). In the co-treatment group, rats received *L. judaicus* (50 mg/Kg) the mean of citrate increased by 50% in comparison with the EG group. In the co-treatment group, the mean of phosphorus in rats that received *L. judaicus* (100 mg/Kg) decreased by 43% in comparison with rats that received water only. In the co-treatment group, mean of phosphorus decreased by 17% and 38% in rats that received *L. judaicus* (50 and 100 mg/Kg) in comparison with the rat received 1% EG, respectively. In the co-treatment group, the mean of calcium in rats that received *L. judaicus* (100 mg/Kg) decreased by 35% in comparison with rats that received water only. The mean of calcium in the co-treatment group in rats that received EG alone in comparison with rats that received tap water, was significantly increased (28%). In the co-treatment group, mean of calcium decreased by 51% and 49% in rats that received *L. judaicus* (50 and 100 mg/Kg) in comparison with the rat received EG, respectively. In the co-treatment group, mean of pH increased by 8% and 15% in rats that received *L. judaicus* (50 and 100 mg/Kg) in comparison with the rat received EG, respectively. The mean of magnesium, K and Na were not significantly different between the study groups.

3.2. Biochemical results

The results of serum biochemical of co-treatment and treatment groups are shown in Tables 3 and 4, respectively. The mean of serum BUN in co-treatment and treatment groups in rats that received EG alone in comparison with rats that received tap water were significantly higher (with 23% and 17% increase, respectively). The mean of serum BUN in the co-treatment group in rats that received *L. judaicus* (100 mg/Kg) and the treatment group which received *L. judaicus* (50 mg/Kg) in comparison with rats which received 1% EG had 16% decrease and 23% increase, re-

Table 4. Biochemical results in treatment group.

Groups name (n=5)Factors	Tap water	*L. judaicus* 100 mg/kg	EG 1%	*L. judaicus* 50mg/ kg + 1% EG	*L. judaicus* 100 mg/kg+1% EG
BUN mg/dl	24.40±1.98	21.29±2.19	28.50±2.15*	35.00±2.15++	28.40±1.89
Cr mg/dl	0.57±0.13	0.56±0.32	0.58±0.18	0.55±0.21	0.58±0.13
Ca mg/dl	9.63±1.11	8.26±2.19	9.83±1.13	9.74±1.56	8.40±2.01
Phosphate mg/dl	7.22±0.77	6.24±0.29*	7.13±0.36	7.90±0.41++	6.78±0.23

*Indicates significant difference ($p<0.05$) between *L .judaicus*100 mg/kg or 1% EG groups compared to tap water group. ++Indicate significant difference ($p<0.01$) between *L. judaicus* 50 mg/kg + 1% EG or *L. judaicus* 100 mg/kg +1% EG groups compared to1% EG group.

Table 5. Histopathology results in co-treatment group.

Group	Glomerule	Tubular atrophy	Intestric fibrosis	Intestric inflammation	Crystal
Water	normal	-	-	-	-
L. judaicus 100 mg/kg	normal	-	-	+	-
EG1%	normal	-	-	+	++
L. judaicus 50 mg/kg + EG1%	normal	-	-	+	+
L. judaicus 100 mg/kg + EG1%	normal	-	-	+	+

-: Negative, +: Low, ++: Moderate, +++: High

spectively. The mean of serum phosphate in co-treatment and treatment groups in rats that received 1% EG alone in comparison with rats that received tap water were not significantly different. The mean of serum phosphate in co-treatment and treatment groups in rats that received *L. judaicus* (100 mg/Kg) alone in comparison with rats that received tap water were significantly different (with 14% and 43% decrease, respectively). The mean of serum phosphate in the co-treatment and treatment groups in rats that received *L. judaicus* (50 mg/Kg) in comparison with rats which received 1% EG had 11% increase and 33% decrease, respectively. On the other hand, the mean serum phosphate in treatment group in rat that received *L. judaicus* (100 mg/Kg) in comparison with rats which received 1% EG had 57% decrease. The mean of serum creatinine and calcium were not significantly different between study groups.

3.3. Histopathology results

Evaluation of kidney damage and stone formation conducted on kidneys of rats. The results of these evaluations are shown in Tables 5 and 6.

4. Discussion

In experimental and in vivo studies, the formation of oxalate stones in animals is based on using ethylene glycol. There are three methods for induction of kidney stones in laboratory animals: administration of 0.75% EG in water for 28 days, administration 1% EG in water for 30-45 days, and administration 0.75% EG added to 2% ammonium chloride in water for 10 days. Most studies used 1% EG for induction of kidney stones and in the present study, we used this method (17-20).

Results of our study demonstrated that administration of *L. judaicus* 100 mg/kg and 50 mg/kg doses reduced the levels of BUN in co-treatment and treatment groups significantly, which is similar to other studies (21, 22), but for creatinine, *L. judaicus* had no significant effect on decreasing levels of creatinine. In a study conducted by Lulat et al., results showed that factors that decrease the levels of calcium could be effective in reducing the risk of stone formation in Wistar rats (23). Results of our study showed that 1% EG leads to elevation of calcium levels in rats and the administration of *L. judaicus* with both 100 mg/kg and 50 mg/ kg

Table 6. Histopathology results in treatment group

Group	Glomerule	Tubular atrophy	Intestric fibrosis	Intestric inflammation	Crystal
Water	normal	-	-	-	-
L. judaicus 100 mg/kg	normal	-	-	+	-
1% EG	normal	-	-	+	++
L. judaicus 50 mg/kg + 1%EG	normal	-	-	+	+
L. judaicus 100 mg/kg + 1%EG	normal	-	-	+	+

-: Negative, +: Low, ++: Moderate, +++: High

reduced levels of calcium by 50% and 49%, respectively. The mean of serum phosphate in the treatment group in rats that received *L. judaicus* (50 mg/Kg) in comparison with rats that received 1% EG had a 10% significant increase. Ethylene glycol caused the formation of crystals in the kidney, and *L. judaicus* with 50 mg/kg and 100 mg/kg doses significantly reduced these crystals.

5. Conclusion

TResults of our study showed that administration of *L. judaicus* in the co-treatment group reduced serum BUN, elevated urinary citrate and urine pH, and reduced urinary parameters such as urine protein, calcium, oxalate, phosphorus, and creatinine, therefore, *L. judaicus* is effective in inhibition of urinary stones formation. In the treatment group *L. judaicus* leads to a decrease of urinary parameters such as urine protein, calcium, oxalate, phosphorus, and creatinine and hence results in the treatment of urinary stones. Also, histopathologic results showed that the amount of urinary crystals was reduced in the treatment groups. It is suggested that further studies could be performed with a larger sample size and other treatment doses of *L. judaicus* to evaluate the effects of this compound on the prevention of formation and treatment of urinary stones.

Conflict of Interest

The authors declare no conflict of interest.

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