Effect of Thymoquinone on Ethylene Glycol-Induced Kidney Calculi in Rats

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Introduction: The aim of this study was to investigate the effects of thymoquinone, a major component of *Nigella Sativa* seeds on ethylene glycol-induced kidney calculi in rats.

Materials and Methods: Sixty male Wistar rats were randomly divided into 6 groups (intact control, ethylene glycol control, and 4 experimental groups) and treated for 28 days according to the protocol of the study. The rats in experimental groups received ethylene glycol and intraperitoneal injection of thymoquinone either from the first day of the study or the 15th day, with either doses of 5 mg/kg or 10 mg/kg. Blood and 24-hour urine samples were collected at baseline and on day 28. Urine oxalate and citrate and serum electrolytes were also measured. On day 29, all rats were decapitated and their kidney specimens were studied.

Results: On day 28, urine oxalate concentration significantly decreased in the experimental groups compared to the ethylene glycol group ($P < .001$). Also, serum calcium levels were significantly higher in the experimental groups ($P = .001$). Calcium oxalate deposits were smaller in the experimental groups than the ethylene glycol group. The mean number of deposits was lower in these groups, too ($P < .001$). Treatment with the lower dose of thymoquinone was associated with fewer deposits.

Conclusion: Thymoquinone significantly decreased the number and size of calcium oxalate deposits in the renal tubules. The dose and duration of treatment, however, does not have a linear relation with the outcomes. Further studies on thymoquinone as a preventive and therapeutic drug for kidney calculi are suggested.

Keywords: urinary calculi, rats, thymoquinone, calcium oxalate, ethylene glycol

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INTRODUCTION

Invasive procedures for the treatment of urinary calculi may cause serious complications and they also impose a great load of costs to the healthcare system.[1,2] In traditional medicine, there are noninvasive treatment options for urinary calculi that are worth to undergo scientific evaluation. *Nigella Sativa* L (NS) seeds (known as *black seed* in Iran) have been used for this purpose in folk medicine for centuries.[3,4] We have previously shown the preventive effect of ethanolic extract of NS seeds on calculus formation,[5] and other investigators have demonstrated its increasing effect on glutathione content in the kidney and also its anti-inflammatory, antioxidant, and analgesic activities.[6-8] As a

major component of NS seeds, thymoquinone was reported to reduce blood pressure and heart rate, prevent chromosomal aberrations, and act as anticonvulsant, antioxidant, and a scavenger of free radicals and superoxide anions.[9-13] Of other effects of thymoquinone are hepatoprotective activity, improvement of cisplatin-induced nephrotoxicity, inhibition of cyclooxygenase pathway and eicosaniod generation in leukocytes, and inhibition of 5-lipooxygenase products.[14-18]

In our previous study, we used ethylene glycol (EG) to induce kidney calculus formation in rats and found that NS can inhibit this effect. Metabolites of EG such as glycolaldehyde, glycolate, and oxalate can induce tissue damage, hyperoxaluria, and calcium oxalate (CaOx) calculi.[19,20] To scrutinize the exact mechanism of NS effect on the kidney, we decided to test its components on the kidneys of rats who were fed by EG. The aim of this study was to investigate the effects of thymoquinone as a major component of NS seeds on EG-induced CaOx kidney calculi in rats.

MATERIALS AND METHODS

We randomly divided 60 male Wistar rats into 6 groups of 10 (groups A to F). They weighted 190 ± 20 g. All groups of rats were kept at 25 ± 2°C with a standard diet and tap drinking water and were treated according to the protocol of the study for 28 days. Rats in group A (intact controls) received tap drinking water, and those in group B (EG controls) received 1% EG (Merck KGaA, Darmstadt, Germany) in drinking water for 28 days. Rats in groups C to F received thymoquinone (Aldrich, WI, USA) with different protocols as well as the same dosage of EG as those in group B; in groups C and D, thymoquinone, 5 mg/kg/d, was administered as intraperitoneal injection from day 1 and day 15 to the end of the experiment, respectively. Rats in groups E and F were treated by 10 mg/kg/d of thymoquinone from day 1 and day 15, respectively. The animal procedures were complied with the international guidelines and national laws, and the study was approved by Mashhad University of Medical Sciences.

Twenty-four-hour urine samples were collected on the first and 28th days of the study, while each rat was kept in a separate metabolic cage. Blood was also collected from the cavernous sinus on the same days. Serum levels of calcium, potassium, and magnesium were measured by enzymatic method, flame photometer, and xylidyl blue reaction, respectively. Urine oxalate and citrate were measured by enzymatic methods (Darman Kaw, Tehran, Iran) with an auto-analyzer. All rats survived until day 29 when they were decapitated by guillotine. Their kidneys were removed, weighed, and kept in formalin for histological studies. Five-μm sections were prepared from both kidneys, and the slides were stained with hematoxylin-eosin method. The slides were examined under light microscope and CaOx deposits were determined. Aggregations of CaOx deposits (tubules containing deposits) were counted in 10 microscope fields.

Data were expressed as mean ± standard deviation and were analyzed by the Kruskal-Wallis test to find the differences among all groups, and Mann-Whitney U test for comparison between each two groups. A P value less than .05 was considered significant.

RESULTS

No CaOx deposits or other pathological defects were found in different segments of the nephrons of the rats in group A (intact controls; Figure 1). On the other hand, many CaOx deposits were found in the proximal tubules, loops of Henle, distal tubules, collecting ducts, and calyxes of the rats in group B (EG controls; Figure 2). Aggregations were composed of 3 to 4 large polygonal crystals in different parts of the tubules. Renal tubular dilation with epithelial damage and leukocyte reaction were also observed on pathology examination (Figure 3). The mean number of CaOx deposits in 10 microscopic fields was 28.0 ± 3.2 in group B, which was significantly more than that in group A (P = .001; Figure 4).

In the kidney specimens of group C, a few thin and tiny crystals of CaOx were detected in different parts of the tubules (mean, 1.4 ± 0.9; P = .001, compared with group B). Tiny calcium oxalate crystals were also detected in different

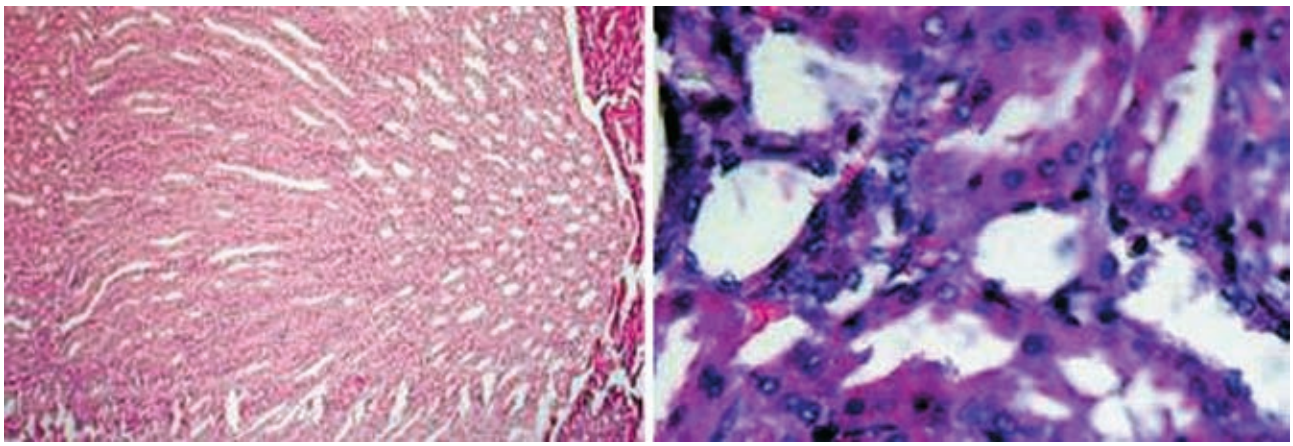

Figure 1. Left, Normal medullary and papillary tubules in the kidney of a Wistar rat (hematoxylin-eosin, × 20). **Right**, Normal tubular and collecting ducts (hematoxylin-eosin, × 40).

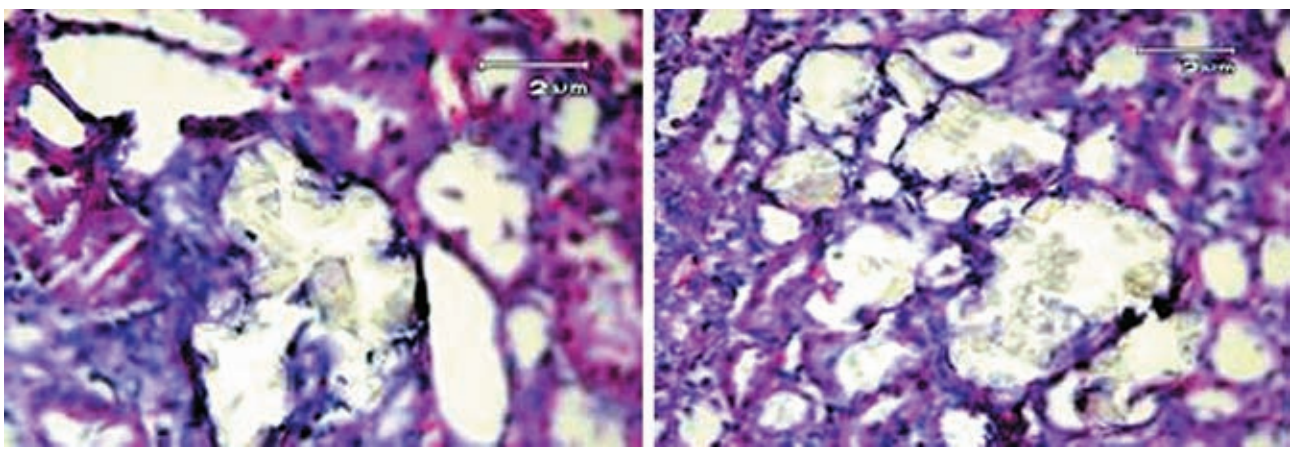

Figure 2. Left, Tubular calcium oxalate deposits (arrows) in a rat treated with ethylene glycol (hematoxylin-eosin, × 40). **Right**, Multiple tabular calculi (arrows) in the same rat (hematoxylin-eosin, × 40).

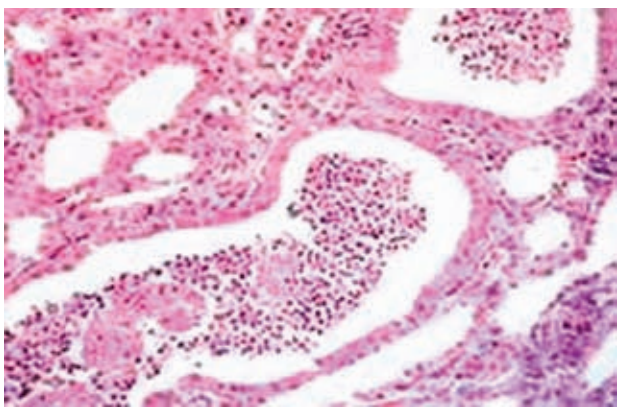

Figure 3. Renal tubular dilation with epithelial damage and leukocyte reaction, producing granular and leukocytic cast (hematoxylin-eosin, × 40).

segments of the nephrons in group D (with thymoquinone started on day 15). The rats in this group had less deposits than those in group B, too (mean, 2.2 ± 1.0; $P = .001$). Thymoquinone was administered with a higher dose in groups E and F. Crystals in different parts of nephrons in the kidney specimens of these groups were also thin, small, and fewer compared with those in group B (mean, 5.2 ± 1.6 and 14.6 ± 3.0, respectively; $P = .001$ and $P = .03$). The number of CaOx crystals in experimental groups C and D was not statistically different from that in group A. The number of the deposits in group D was significantly less than that in group F ($P = .008$); however, the difference between groups C and E was not significant.

Urine oxalate concentration of the rats was higher in group B than in group A on day 28 ($P < .001$). Also, its concentration was higher in group B than in any of the experimental groups (C to F) on day 28 (Figure 5). Urine samples of the rats in group C had a lower levels of oxalate than those

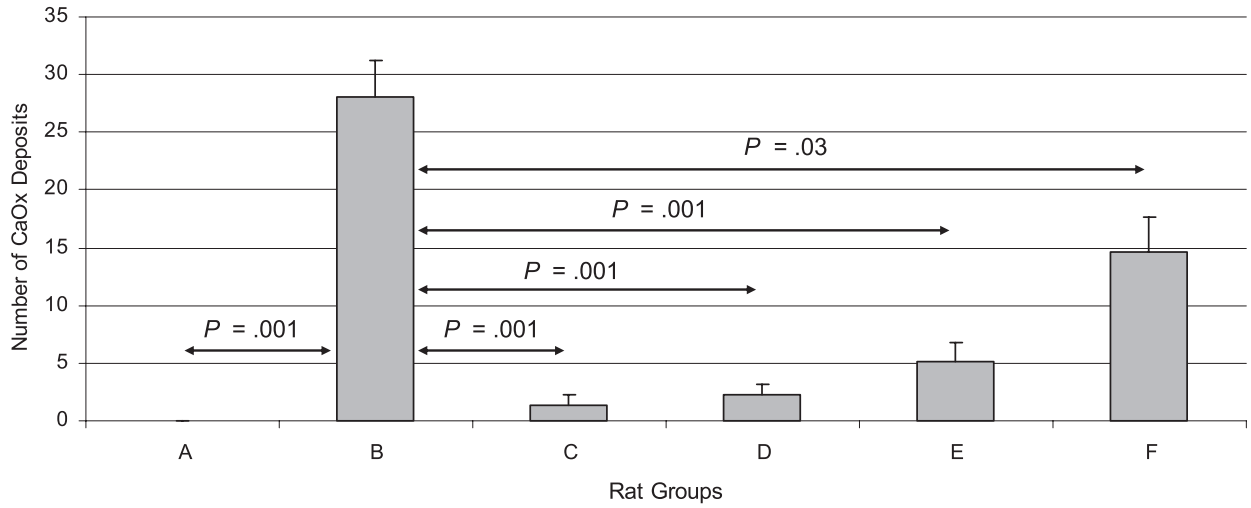

Figure 4. The number of calcium oxalate crystal deposits (in 10 microscopic fields) in the kidneys of the rats at the end of the experiment. Data are expressed as mean ± standard deviation. The Kruskal-Wallis test demonstrated a significant difference between the six groups ($P < .001$).

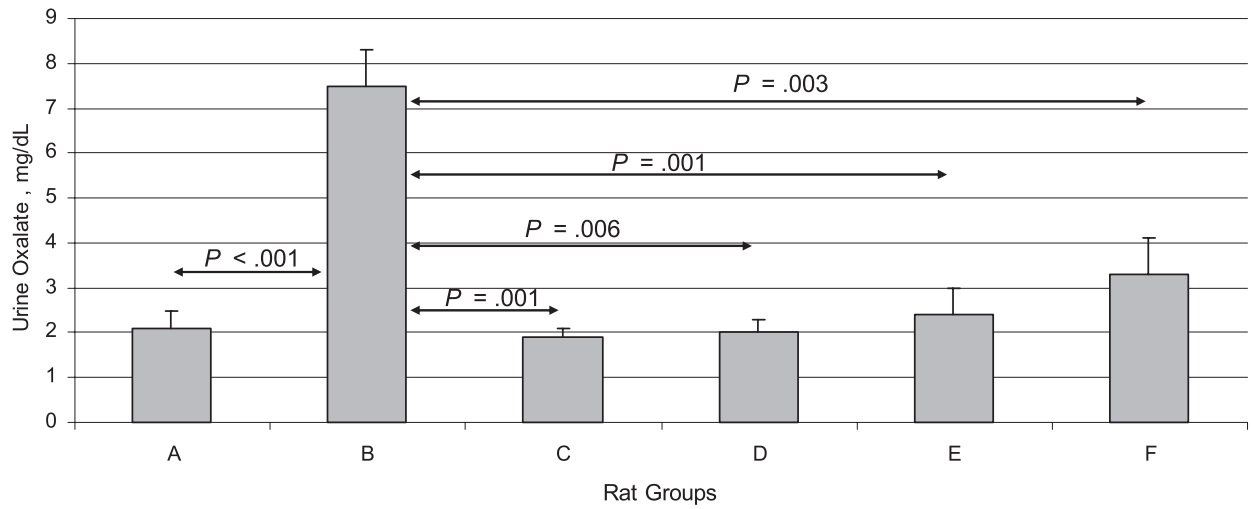

Figure 5. The 24-hour urine oxalate concentration in different groups of rats on day 28. The Kruskal-Wallis test showed a significant difference between the six groups ($P < .001$).

in group E ($P = .003$), but the difference between groups D and F was insignificant. Table 1 shows urine citrate concentrations at baseline and the end of the study. Urine citrate of the rats in group A was significantly higher than those in group B and the experimental groups except for group D. No difference was found between group B and the experimental groups in urine citrate concentration.

Serum potassium concentrations in group B and group A were not significantly different, but it was lower in all the experimental groups than in group B (Table 2). Concerning serum calcium levels, rats in group B had a lower concentration on day 28 than those in any of the other groups (Table 3). However, no significant difference was found between serum magnesium levels of the studied groups on day 28 ($P = .74$; Kruskal-Wallis test).

Finally, the extracted kidneys were weighed and compared between the groups. The kidneys in group B was 62% heavier than those in group A (mean, 2.81 ± 0.26 g versus 1.73 ± 0.07 g, respectively). The kidneys in the experimental groups, although had a lower weight, were not significantly different from those in group B. The

Table 1. Urine Citrate Concentrations Before and After the Study Period in Control and Experimental Groups*

Group	Urine Citrate, mg/dL		P†
	Baseline	Day 28	
A	31.75 ± 3.25	32.27 ± 3.35	…
B	29.37 ± 3.12	19.37 ± 2.52	.04
C	32.90 ± 3.51	12.90 ± 3.51	.01
D	29.34 ± 2.82	21.34 ± 2.22	.13
E	33.96 ± 3.83	13.96 ± 4.83	.01
F	32.18 ± 3.23	12.18 ± 1.93	.002

*Data are expressed as mean ± standard deviation. No difference was found between the groups on day 0 (baseline); however, the Kruskal-Wallis test showed a significant difference between the groups (P = .007) on day 28. The differences between the experimental groups (C to F) and group B were insignificant.
†P values are related to comparisons of the values on day 28 between group A and other groups (Mann-Whitney U test).

Table 2. Serum Potassium Concentrations Before and After the Study Period in Control and Experimental Groups*

Group	Serum Potassium, mg/dL		P†
	Baseline	Day 28	
A	4.94 ± 0.20	6.22 ± 0.62	.09
B	5.04 ± 0.18	4.94 ± 0.22	…
C	5.24 ± 0.25	0.80 ± 8.20	.008
D	5.04 ± 0.26	0.60 ± 8.40	.008
E	5.34 ± 0.29	0.47 ± 7.02	.008
F	5.26 ± 0.25	0.93 ± 6.30	.03

*Data are expressed as mean ± standard deviation. No difference was found between the groups on day 0 (baseline); however, the Kruskal-Wallis test showed a significant difference between the groups (P = .006) on day 28.
†P values are related to comparisons of the values on day 28 between group B and other groups (Mann-Whitney U test).

Table 3. Serum Calcium Concentrations Before and After the Study Period in Control and Experimental Groups*

Group	Serum Calcium, mg/dL		P†
	Baseline	Day 28	
A	9.16 ± 0.16	9.18 ± 0.18	.008
B	9.26 ± 0.22	8.28 ± 0.30	...
C	9.26 ± 0.16	11.66 ± 0.89	.008
D	9.04 ± 0.16	11.20 ± 0.31	.008
E	9.14 ± 0.20	10.08 ± 0.26	.008
F	9.04 ± 0.24	10.58 ± 0.52	.008

*Data are expressed as mean ± standard deviation. No difference was found between the groups on day 0 (baseline); however, the Kruskal-Wallis test showed a significant difference between the groups (P = .001) on day 28.
†P values are related to comparisons of the values on day 28 between group B and other groups (Mann-Whitney U test).

mean weight of the kidneys were 1.86 ± 0.08 g, 1.63 ± 0.09 g, 1.76 ± 0.09 g, and 1.94 ± 0.11 g in groups C, D, E, and F, respectively.

DISCUSSION

According to our results, thymoquinone has a preventive effect on CaOx calculi formation in the kidneys of rats. In a similar way, thymoquinone has a disruptive effect on CaOx crystals formed by EG, demonstrating a therapeutic effect on kidney calculi in rats (Figure 4). To our best knowledge, this is the first report on the effects of thymoquinone on kidney calculi. Thymoquinone, as a major component of NS seeds, has been reported to reduce blood pressure and heart rate, prevent chromosomal aberrations, and act as anticonvulsant, antioxidant, and a scavenger of free radicals and superoxide anions.[9-13] Of other effects of thymoquinone are hepatoprotective activity, improvement of cisplatin-induced nephrotoxicity, inhibition of cyclooxygenase pathway and eicosaniod generation in leukocytes, and inhibition of 5-lipooxygenase products.[14-18]

We found that with a dose of 5 mg/kg, thymoquinone had a potent preventive effect on calculus formation and a highly disruptive effect on CaOx kidney calculi. We also tried 10 mg/kg of thymoquinone and documented its potent preventive effect on kidney calculus formation and a moderate effect on disruption of the kidney calculi. These data suggest that lower doses of thymoquinone might be more effective on the treatment of CaOx kidney calculi. In a study on the effect of thymoquinone on lipid profile of rats, Bamosa and colleagues tested intraperitoneal injection of thymoquinone with varying doses of 0.4 mg/kg to 8 mg/kg. They found that there was no linear association of the dose and lowering effect of thymoquinone on serum lipids. The highest dose they used (8 mg/kg) had toxic effects.[21] This is in accordance with our findings. Unpublished data at our university have shown that oral thymoquinone with a dose of 0.4 mg/kg could disrupt CaOx calculi in rats' kidney.[22]

It has been reported that an ethyl-acetate phase remnant fraction and N-butanol fraction from aqueous-ethanolic extract of NS seeds had preventive effects on EG-induced kidney calculi.[22] Both polar and nonpolar components are present in ethyl-acetate phase remnant fraction and only nonpolar components are present in N-butanol phase of the extract. Thymoquinone is the major nonpolar compound in the extracts of NS seeds; it

was concluded by researchers that thymoquinone might be the main compound with preventive effect on kidney calculus formation in these studies.[22] These reports are in agreement with our data in the present study.

Calcium oxalate crystals in tubules may damage epithelial cells that leads to production of superoxide anions and free radicals and induction of hetrogenic crystal nucleation.[23-25] On the other hand, thymoquinone, as an active quinine, has antioxidant effect, scavenges free radicals and superoxide anions, and inhibits cyclooxygenase and 5-lipoxygenase pathways; therefore, it inhibits inflammatory products.[17,18] Consequently, it can be suggested that a part of its effects on prevention and disruption of CaOx calculi is due to these roles.[13,18,26] Thymoquinone has an antibacterial effect, and therefore, calculi with a bacterial origin such as struvite calculi may be prevented by thymoquinone.[27,28] Urine oxalate concentration was also decreased by thymoquinone which is in agreement with its preventive effects on the CaOx kidney calculi. However, thymoquinone had no significant effect on the weight of the kidney which in part may be due to very short period of the treatment.

CONCLUSION

According to our results, intraperitoneal injection of thymoquinone was effective for prevention and treatment of CaOx kidney calculi in rats. A dose of 5 mg/kg of thymoquinone significantly decreased the number and size of CaOx deposits in different segments of the renal tubules. A higher dose of thymoquinone had also preventive and therapeutic effects on CaOx kidney calculi, although the therapeutic effect of thymoquinone with a dose of 5 mg/kg was more potent. Further studies to determine the same effects on human beings are recommended.

CONFLICT OF INTEREST

None declared.

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