Ethanolic Extract of Nigella Sativa L Seeds on Ethylene Glycol-Induced Kidney Calculi in Rats

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Introduction: The aim of this study was to investigate the effects of the ethanolic extract of *Nigella sativa* L (NS) seeds on kidney calculi in rats.

Materials and Methods: Thirty-two Wistar rats were randomly divided into 4 groups: group A received tap drinking water for 30 days (intact control). Groups B, C, and D received 1% ethylen glycol for induction of calcium oxalate calculus formation. As the preventive, and treatment subjects, rats in groups C and D received ethanolic extract of NS, 250 mg/kg, in drinking water since day 0 and day 14, respectively. Urine was collected on days 0, 7, 14, and 30 of the study period. After 30 days, the kidneys were removed and prepared for histologic evaluation of calcium oxalate deposits. Urine calcium oxalate concentrations were determined by atomic absorption.

Results: The number of CaOx deposits was significantly greater in group B ($P = .001$). Calcium oxalate concentrations in the urine on days 14 and 30 increased significantly in group B and were higher than those in group C ($P = .006$ and $P = .002$, respectively). Urine oxalate concentration in group D decreased on day 30 and was lower than that in group B ($P = .04$).

Conclusion: Treatment of rats with ethanolic extract of NS reduced the number of calcium oxalate deposits in a group of rats that received ethanolic extract of NS. The NS could also lower the urine concentration of calcium oxalate. We suggest further studies on the therapeutic and preventive effects of the NS on kidney calculus formation in human.

Keywords: Nigella sativa, kidney calculus, ethylene glycol, calcium oxalate, rat

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INTRODUCTION

Urinary calculi are the third prevalent disorder in the urinary system.[1] Approximately, 80% of these calculi are composed of calcium oxalate (CaOx) and calcium phosphate.[2,3] Urinary calculi may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly cost-effective and may cause severe complications. Spontaneous passage of calculus is accompanied by severe renal colic which is not relieved by conventional analgesics, and therefore, narcotics are drugs of choice in many cases. The seeds of *Nigella sativa* L (NS) or *black seeds*, a member of the family of ranunculaceae, are used in traditional medicine all over the world. Black seeds have been reported to be analgesic, anti-inflammatory, anticonvulsant, antidiabetic, anticancer, and antioxidant and have been proposed to lower serum levels of cholesterol and triglycerides,

balance enzyme activities, increase glutathione in the kidney, and reconstruct kidney tissue after nephrotoxicity.[4-12]

Black seeds with honey have been mentioned to disintegrate the calculi in the kidney and bladder to small pieces and remove them.[13,14] However, there is no evidence for this traditional therapeutic usage. Therefore, we decided to investigate the effect of ethanolic extract of NS seeds on calcium oxalate calculi in a rat model.

MATERIALS AND METHODS

The animal procedure was conducted in conformity with institutional guidelines and national laws, and the study was approved by Mashhad University of Medical Sciences. Thirty-two male Wistar rats weighed 200 ± 10 g were housed at 25 ± 2°C on a standard diet and tap water. They were randomly divided into 4 groups and treated according to the experimental protocol for 30 days. Rats in group A received tap drinking water and served as the intact control group. Groups B, C, and D were considered as ethylene glycole control, preventive, and treatment groups and received 1% ethylene glycol (Merck, Darmstadt, Germany) in drinking water for 30 days.[15-17] Groups C and D were also treated with 250 mg/kg body weight of ethanolic extract of NS since the first and the 14th day through the end of the experiment, respectively.

The NS seeds were purchased from a local herb store in Mashhad, Iran. They were powdered and dried. Then, 100 g of the prepared powder was mixed with a sufficient volume of 96% ethanol and extracted with a soxhlet apparatus for 16 to 18 hours. After removing the solvent in vacuum, the extract was dried in an oven with the temperature of 50°C to 60°C. The dried extract weighed 33.3 g, and therefore, it was 33.3%. The extract was then kept in a refrigerator and was added daily to the drinking water of the rats. Ethanolic extract was dissolved in water by adding a few drops of toin 80. The 24-hour urine samples were collected on days 0, 7, 14, and 30, while each rat was kept in a metabolic cage. Urine oxalate was measured by atomic absorption.[18] Each sample was prepared and the yielding color was read by spectroscopy at 422.7 nm wave length. At the end of the experiment (day 31), all rats were killed by guillotine. The kidneys were removed, weighed, and kept in formalin for histological processing. Five-micrometer sections of both kidneys were prepared for each rat and slides were stained with hematoxylin-eosin. The slides were examined under light microscope and CaOx deposits were determined. Aggregations of CaOx deposits (tubules containing CaOx deposits) were counted in 10 microscopic fields and expressed as mean ± standard error for each group. Data were analyzed by nonparametric Kruskal-Wallis test and Mann-Whitney *U* test. *P* values of less than .05 were considered significant.

RESULTS

The Table outlines the urine levels of oxalate on the follow-up days in each group of rats. At the baseline there were no differences between the 4 groups in unrine oxalate levels. In comparison with the rats in other groups, those in group B (ethylene glycole controls) had a significantly higher urine oxalate concentration on days 14 ($P = .003$) and 30 ($P = .005$). Urine oxalate level in group B was higher compared to group C (preventive group) on days 14 ($P = .006$) and 30 ($P = .002$), while no significant difference was found between groups C and A on these days. Urine oxalate in the rats of group D (treatment group) was significantly lower than that in group B on day 30 ($P = .04$).

No CaOx deposits or other abnormalities were found in the nephron segments of group A (Figure 1).

Changes of Urine Oxalate Concentration in Rats*

Days	Urine Oxalate Concentration, mg/dL			
	Group A (Control)	Group B (Ethylene Glycol)	Group C (Treatment)	Group D (Preventive)
0	6.27 ± 1.13	4.93 ± 1.17	7.68 ± 0.63	7.17 ± 0.51
7	8.76 ± 0.60	9.31 ± 0.96	7.31 ± 1.11	8.63 ± 0.5
14	8.88 ± 0.44	13.47 ± 0.50	9.39 ± 1.25	12.68 ± 1.23
30	8.43 ± 1.00	15.57 ± 1.26	8.10 ± 0.70	10.64 ± 1.20

*Data are expressed as mean ± standard error.

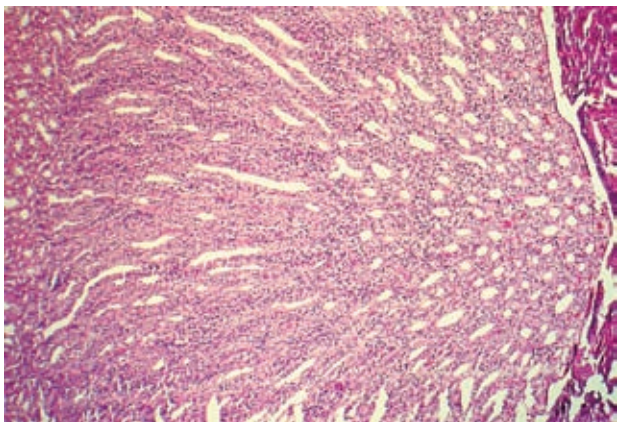

Figure 1. Normal medullary and papillary tubules are shown in a rat's kidney (hematoxylin-eosin, × 200).

On the other hand, many CaOx deposits were found in the proximal tubules, loops of Henle, distal tubules, collecting ducts, and even calyxes in group B (Figures 2 to 5). Deposits were composed of 3 to 4 large polygonal crystals in different segments of the renal tubules. The number of CaOx deposits in 10 microscopic fields in the kidney specimens of group B was 55.05 ± 9.88 which was significantly higher than that in group A ($P = .001$; Figure 6). In group C, the number of deposits was 19.75 ± 7.40 which was significantly lower than that in group B ($P = .02$; Figure 6). Calcium oxalate crystals in different parts of the renal tubules in the group C were clearly smaller in comparison with group B. In group D, oxalate crystals were deposited both at small and large sizes in the nephron segments. The number of oxalate deposits in this group was calculated to be 24.14 ± 9.08 which was 56% smaller when compared with group B; however, the difference was insignificant ($P = .07$; Figure 6).

At the end of the study, the weight of the kidneys was greater in group B compared with group A, but the difference was not significant. No significant differences were found between ethanolic extract-treated rats and those in group B.

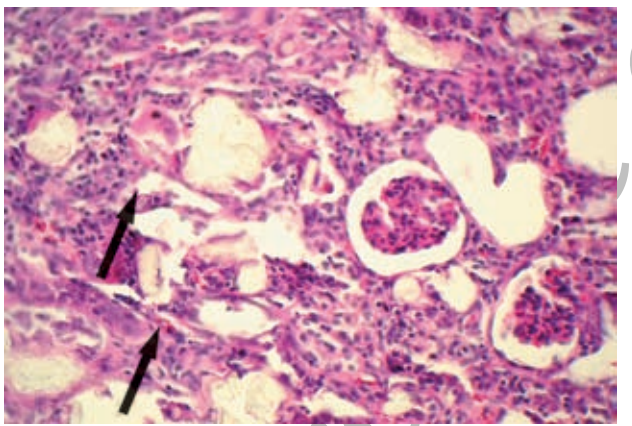

Figure 2. Multiple tubular calculi (arrows) in an ethylene glycol-treated rat (hematoxylin-eosin, × 400).

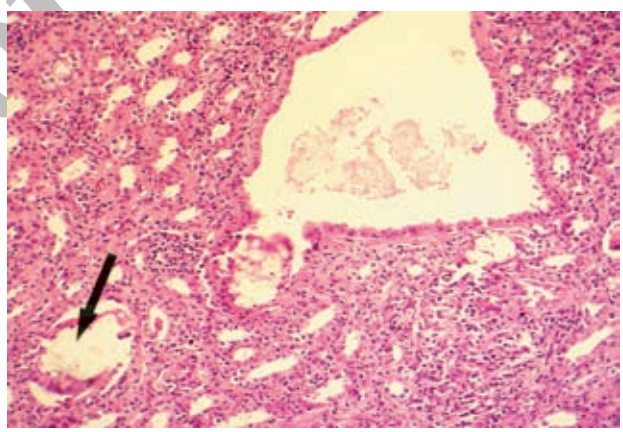

Figure 3. Tubular calculi (arrow) with secondary tubular dilatation (hematoxylin-eosin, × 200).

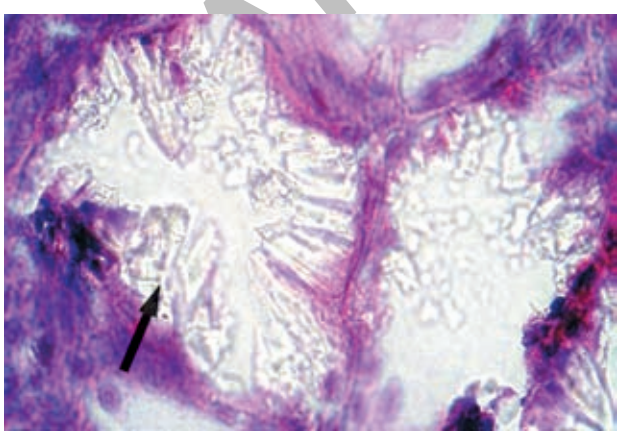

Figure 4. Calcium oxalate crystals (arrow) in a renal tubule (hematoxylin-eosin, × 1000).

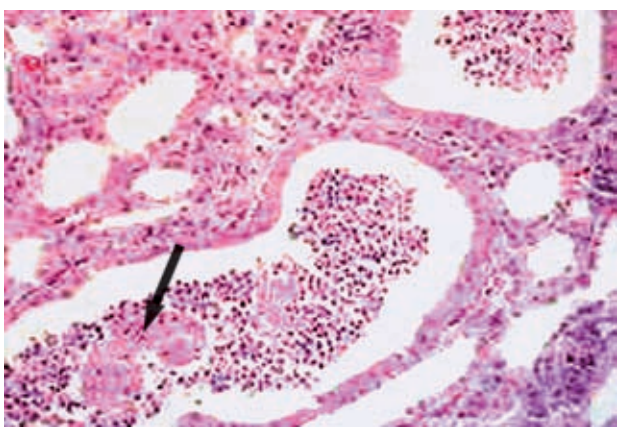

Figure 5. Secondary renal tubular dilatation with epithelial damage (arrow) and leukocyte reaction producing granular and leukocyte cast (hematoxylin-eosin, × 400).

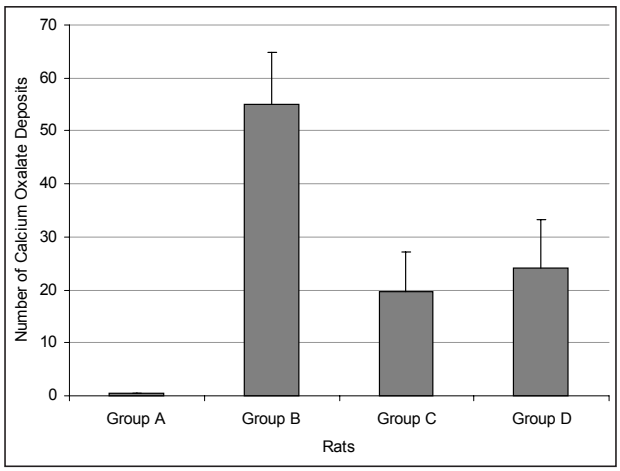

Figure 6. The number of calcium oxalate crystal deposits (per 10 microscopic fields) in the kidneys of the rats at the end of the experiment. Data are expressed as mean ± standard error. The Kruskal-Wallis test demonstrated a significant difference between the 4 groups (P = .001).

DISCUSSION

Our data demonstrated that ethanolic extract of NS seeds had a preventive effect on CaOx calculus formation in the kidney of rats. The ethanolic extract also decreased the number of CaOx calculi in the treated group by 57%, and therefore, demonstrates a therapeutic effect, albeit trivial, on the disruption of CaOx calculi formed in the kidney due to ethylene glycol consumption (Figure 6). The NS (black seeds) extract with the dose of 250 mg/kg had a significant preventive effect on the formation of CaOx kidney calculus (Figure 6). To our best knowledge, this is the first report on the effect of ethanolic extract of the NS on the prevention and treatment of CaOx kidney calculus.

Since the crude extract was used in this study, discussing about the exact mechanisms involved in the effect of the black seeds on CaOx calculi. Calcium oxalate crystals and high oxalate levels in nephrons can produce damages in the epithelial cells, and consequently, the cells may produce some products, as well as free radicals, inducing heterogenous crystal nucleation and cause aggregation of crystals.[19] Black seeds have glycoside flavonoids such as kaempferol, quercetin, and quercetin-3. Phytochemical analysis of black seeds of Khorasan province has demonstrated that the seeds contain tanin, flavonoids, and alkaloids which also constitute a portion of the ethanolic extract of the seeds.[20-23] Several studies have reported that flavonoides—especially qurecetin and kaempferol—have anti-inflammatory and antioxidant effects.[22-25] It can be speculated that of the role of the NS ethanolic extract in preventing formation of CaOx calculi and disruption of them, as seen in the present study, is in part due to the anti-inflammatory and antioxidant effects of the different compounds of the black seeds. These compounds may interfere with the process of epithelial cell damage induced by crystals or may exert inhibitory effect on inflammation.[26]

Aglichon and glyceride flavonoles which are present in black seeds have strong antioxidant and scavenging effects; thus, it may be suggested that the preventive and disruptive effects of black seeds on CaOx calculi are attributed to these mechanisms.[24] It has been reported that CaOx calculi such as struvite calculi may have a bacterial origin such as nanobacteria.[27] Black seeds also have antibacterial effects and therefore, may be effective in this mechanism of CaOx calculus formation.[28]

The weight of the kidneys increased in the group of rats which received only ethylene glycol (group B); this may be due to water retention or inflammation of the epithelium of nephrons. The ethanolic extract was not able to decrease significantly the weight of kidneys in experimental groups (C and D), which in part may be due to the very short period of treatment.

CONCLUSION

We could find that the ethanolic extract of NS seeds with a dose of 250 mg/kg significantly decreased the number and size of CaOx deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes. It also seems that the preventive effect of ethanolic extract is more effective than its treatment effect. Black seeds are commonly used in folk medicine; therefore, it may be suggested that ethanolic extract or other products of the NS seeds be used for prevention and perhaps treatment of CaOx calculi in human. Further studies on larger animal models and on human are warranted to draw final conclusions.

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CONFLICT OF INTEREST

None declared.

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