Original Research Article

Study of preventive effectiveness of *Nigella sativa* L. against rat kidney stones, generated by ethylene glycol

Abstract

Aims: The objective of this research is to study the preventive effectiveness of *Nigella sativa* L. against rat kidney stones, generated by Ethylene Glycol.

Methodology: In this study, 40 Wistar rats were categorized randomly into four groups of ten. During the research, drinking water labeled group A is mixed with mineral water through utilization of positive control procedures. Into the drinking water labeled group B and other research groups, 1 percent Ethylene Glycol is added through the administration of negative control. In preventive group C, *Nigella sativa* is added from the first day of the research period and in the treatment of group D. After the 15^{th} day of the research period, 750 mg/kg *Nigella sativa* powder is administered into the drinking water of rats per day. At the end of the research, kidney tissue samples of rats were stained with haematoxylin and Eosin through the utilization of an optical microscope. Furthermore, serum and urinary samples of rats were analyzed biochemically.

Results: The results indicated that the number of Calcium Oxalate crystals in group B increased in comparison with that of group A. Biochemical analysis of serum and urinary samples indicated a significant increase in the number of Calcium Oxalate crystals of group B in comparison with group A. Furthermore, the analyses depict a unanimous decrease of crystals in all the research groups (except in group C) in comparison with group B.

Conclusion: The findings of this research indicate that *Nigella sativa* L. does not have any preventive effectiveness against Calcium Oxalate accumulation.

Keywords: *Nigella sativa*, Kidney stone, Urinary stone, Calcium Oxalate, Ethylene Glycol

1. Introduction

Urinary stones are the third most prevalent malady of the urinary system. Prevalence of this malady is due to drastic changes in lifestyle and diet; however the exact clinical cause of this disease has not been recognized yet. Acquiring prevalence rate of 1-15 percent worldwide [1], this malady is caused relatively by a number of factors such as age, sex, hereditary characteristics and environmental factors such as meteoric characteristics, diet and the level of healthiness of the drinking water. Urinary stones generate a series of clinical malfunctions such as colic symptoms, renal failure, kidney hydronephrosis, urinary retention and infection, squamous cell carcinoma, nephrocalcinosis and urinary dilation. In today's medical world, various supportive therapies of urinary stones, such as drinking of water and analgesic administration, are administered. In the case of big urinary stones, which are not excreted and therefore, result in serious clinical consequences, revascularization and surgical therapies such as chemical dissolution, transurethral lithotripsy, extracorporeal shock wave litotripsy and surgery are utilized, all of which are accompanied by high monetary and clinical consequences [2].

80 percent of urinary stones consist of Calcium Oxalate and Calcium Phosphate. Oxalate ion is the most prevalent element in tea, coffee vegetables such as spinach. After its absorption through the small intestine, Oxalate is not metabolized in tissues and blood plasma. Therefore, it is accumulated in the urine flow. The presence of calcium in the small intestine is the key in Oxalate absorption. Magnesium and Sodium of the urine flow can generate complex compounds with Oxalate and result in serious consequences. Therefore, the level of Oxalate in urine flow is of utmost importance in predicting Calcium Oxalate formation in kidneys and urinary system [1]. High level Calcium, Oxalate, Phosphate, Urea and low level Nitrate, Magnesium and Citrate indicate a considerable amount of calcium oxalate density and therefore, result in crystal accumulation in the urinary flow. In addition to the aforementioned characteristics, high level Potassium and low level Magnesium is an indicator of stone

formation in kidneys and urinary system [2]. In recent studies, *Nigella sativa* is proved to possess therapeutic effects including analgesic [3,4], anti inflammation [3-6], antimicrobial [7,8], antiepileptic [9,10], hypolipidemic [11,12], hypoglycemic [13], antioxidant, pro-oxidant [14] and effectiveness against kidney stones formation [15]. Today, considering serious monetary and clinical consequences of surgery, implantation and chemical medicines, researchers rely heavily on administration of herbal and alternative medicines. In the present research, therapeutic effectiveness of *Nigella sativa* L. against rat kidney stone, generated by Ethylene Glycol is studied.

2. Materials and Methods

2.1. Fauna Treatment: 40 Wistar rats with the average weight of 188±19 g were categorized randomly into 4 groups (A-D) of ten. Rats were sustained under 25±2 Centigrade temperature and 12 hour long day and night cycles. Food and drinking water were provided unrestrictive for the rats. The amount of consumed water and the average weight of each rat were measured daily and the amount of *Nigella sativa* was administered in a way that can be considered compatible with the amount of water and average weight each rat acquires. The clinical treatment of each group was administered in a 28 day long research period.

2.2. Plant material: ***Nigella sativa*** was collected from the north Khorassan.

2.3. Preparation of extracts: The powdered seed (20 g) was boiled in 200 ml boiling water for 15 min. After that, the mixture was filtered and concentrated under reduced pressure at 35°C.

2.4. Groups:

2.4.1. Positive Control group (A): During the research, drinking water of one of the groups is mixed with 1 percent mineral water.

2.4.2. Negative Control Group (B): In the 28 day long research period, 1 percent Ethylene Glycol is added to the drinking water of one of the groups. During the research, no other therapeutic procedure is utilized. It is worth mentioning that administration of Ethylene Glycol in drinking water is considered to be a certified procedure for Calcium Oxalate formation [16-21].

2.4.3. Preventive Group of *Nigella sativa* L (C): Drinking water mixed with 1% ethylene glycol and 1 ml of *Nigella sativa* L. orally (alongside 750 mg/kg *Nigella sativa* L. powder) were administered from the first day of the research period.

2.4.4. Therapeutic Group of *Nigella sativa* L (D): In the first day, rats are given 1 percent Ethylene Glycol and from the 15th day of the research period, it was administered orally alongside *Nigella sativa* L, which is provided through Soxhlet Extractor.

2.5. Urine Collection: During the zero and 28 day of the research period, 24-hour urine collection of rats of each research group were administered individually in the metabolic cage. The day before the first day of the research period, each rat was weighed separately in the metabolic cage. After 24 hours, urine samples were collected and preserved in laboratory freezers. In order to measure the level of oxalate and Citrate, enzymatic methodology was utilized and Flame Photometer was administered in the measurement of the Potassium level of the serum samples.

2.6. Serum Sample Preparation: For serum sample preparation, blood samples of each group was utilized from the optic sinuses in the day zero of the research and in the 29th day, blood samples were extracted from the heart muscle after moderate Etherization. After their clinical clotting, two samples were centrifuged at 3000 rounds for 20 minutes. The third sample was separated by pipette and poured in another test tube. In order to measure the Potassium and Magnesium level of the samples, Flame Photometer and Xylidylblue Reagent were utilized respectively.

2.7. LD_{50} Value: LD50 values of *Nigella Sativa L.*, obtained by single doses, orally and intraperitoneally (I.P.) administered in rat, were 28.5 ml/kg body wt. p.o. [25.9-30.4] and 2.03 ml/kg body wt. i.p. [1.82-2.25].

2.8. Pathological Analyses: After clinical treatment of rats in the 29th day, they were etherized moderately so that their kidneys can be removed through their abdomen and weighed. Immediately, each pair of kidneys were placed in a test tube which contains 10 percent Formaldehyde solvent. Microscopic incisions with 5 micron thickness were administered on the kidneys of the rats and then, the incised tissues were stained with haematoxylin and Eosin through utilization of Olympus optical microscope. In each incision, 10 microscopic fields with the magnification of 10×40 were selected randomly and the number of Oxalate crystals was measured separately. Considering the number of crystals and fields in each slide and sample, the average number of Oxalate crystals is reported statistically.

2.9. Statistical Measurements: For data analysis, In Stat software application was utilized and for group comparison, ANOVA and Kramer Tukey tests were administered. $P< 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Results of Pathological Analyses:

3.1.1. Positive (A) and Negative (B) Control Groups: There was no Oxalate crystal or other renal malfunction in kidney tubes of group A (Figure No. 1, Positive Control). However, in group B, Calcium Oxalate sediments were present in the renal tubes to the extent that in the microscopic analyses of the kidneys, considerable accumulation of Calcium Oxalate crystals was detected in the urinary tubes and other sections of the system such as Proximal, Distal, Loop of Henle, Collecting duct and Nephrons (Figure No. 2, Negative Control). The number of Oxalate crystals was 28 in 10 microscopic fields, which acquires significant increase in comparison with group A ($P<0.001$).

3.1.2. Preventive (C) and Therapeutic (D) Groups of *Nigella sativa* L: In group C, the number of crystals of 10 microscopic fields was 23.8±4.609 and calcium oxalate crystals with different sizes to very large numbers were observed of tubules. The number of crystals was lower in comparison with the statistical reports of the negative control group but did not show significant differences with the negative control group. In group D, calcium oxalate crystals with different sizes, but in smaller numbers than in group C were observed in the urinary tubules. Based on the average (12.8 ± 3/732), the number of crystals in this group compared to the negative control group C decreased a lot but do not show significant differences with the negative control group (group B) and the other groups.

3.2. Results of Biochemical Analyses of Urine Samples

In the day zero, the level of Oxalate was statistically equal in all the research groups and there existed no statistical difference between them. Urinary oxalate concentration of negative control group on the last day increased and in comparison with a healthy control group showed no significant difference ($P<0.01$). The negative control group with treatment group showed no significant difference ($P<0.01$).

Regarding the citrate level of urine samples in different stages of the research, this level decreased in the negative groups, but did not acquire any statistical significance over the positive group. On the other hand, the positive group acquired statistical significance over the preventive and therapeutic groups of *Nigella sativa* L. ($P<0.001$). None of the groups acquired any statistical significance with the negative group.

3.3. Results of Biochemical Analyses of Serum Samples:

In day zero, the level of serum Potassium was statistically equal in all the research groups and there existed no statistical difference between them. In the last day, the level of Potassium decreased in negative group but depict no statistical significance over the positive control group. On the other hand, the negative control group acquired statistical significance over the preventive and therapeutic groups ($P<0.01$). None of the groups acquired statistical significance over the positive control group.

3.4. Kidney Weights:

The average weight of kidneys in negative control group was 2.81±0.26 gr, indicating that they were heavier than the ones in the positive control group (1.73±0.07 gr).

High mass Calcium, Oxalate, Phosphate, Urea excretions and low mass Nitrate, Magnesium, Citrate excretions in urine will increase the level of Calcium Oxalate crystals. Apart from the aforementioned facts, high level of Potassium and low level of Magnesium will facilitate crystal formation. Since levels of Oxalate, Citrate, Potassium and Magnesium are of key factors in etiological studies of kidney stones, it is indicated then that through administration of Ethylene Glycol, the level of urine Oxalate increased in Wistar rats and in the preventive, therapeutic group of *Nigella sativa* L., the clinical utilization of *Nigella sativa* decreases the level of Urine Oxalate.

Citrate is considered to be one of the most important elements in elimination of Calcium ions in urine through its synthesis with Calcium. Meager rate of urine Citrate will cause Oxalate accumulation in the urinary system [2]. Therefore, it is suggested that administration of Citrate Potassium in people inflicted with kidney stones will therapeutically impact their hypocitraturic condition through facilitation of Citrate absorption and the level of their urine excretion [22]. Oral administration of L-arginine will facilitate the level of Citrate absorption as well [23]. Furthermore, administration of Citrate- Magnesium- Potassium compounds will curb the possibility of kidney stone regression for 3 years up to 85 percent [24].

The findings of the present study depicts that administration of Ethylene Glycol will curb the average level of urine Citrate of the second group in comparison with the first group. Such an administration will cause hypocitraturic disorder and as a result, facilitate Oxalate crystal accumulation. In the present study, it is indicated that the level of Citrate is not significantly different in those groups which were administered by *Nigella sativa*. According to the results of the study, it is deduced that *Nigella sativa* does not exercise its therapeutic effectives on kidney stones and Oxalate crystals by facilitating the level of urine Citrate.

In another study, average Potassium level in research positive and negative groups were compared and it is deduced that administration of Citrate Potassium will therapeutically impact subjects with hypocitraturic condition through facilitation of Citrate absorption at the level of

urine excretion [25]. Furthermore, administration of Citrate-Magnesium-Potassium compounds will curb the possibility of kidney stone regression for 3 years up to 85 percent [26]. It is worth mentioning that this finding is not in correlation with the findings of the present study, since it is based upon supersaturated Potassium theorem which believes in clinical elimination of kidney stones through facilitation of urine Potassium level. Considering variations in Potassium level, it can be affirmed that administration of *Nigella sativa* L. do not acquire their clinical impacts on formation or elimination of kidney stones through increase or decrease of urine Potassium level of Wistar rats.

Ethylene Glycol causes sudden increase in weight of rat kidneys. Such an increase is due to water retention in renal tissues or it is the result of inflammatory condition of epithelial Nephron tissues, caused by high level Oxalate accumulation (Figure No. 2).

4. Conclusion

The findings of this research indicate that *Nigella sativa* L. does not have any preventive effectiveness against Calcium Oxalate accumulation. Pathological observations of the present study indicate that administration of *Nigella sativa* L. acquires therapeutic non-effectiveness against kidney stones. Of course, in order to verify such findings on therapeutic effectiveness of *Nigella sativa* L. more accurately, similar studies must be administered on human subjects.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws were applicable. All experiments have been examined and approved by the appropriate ethics committee.

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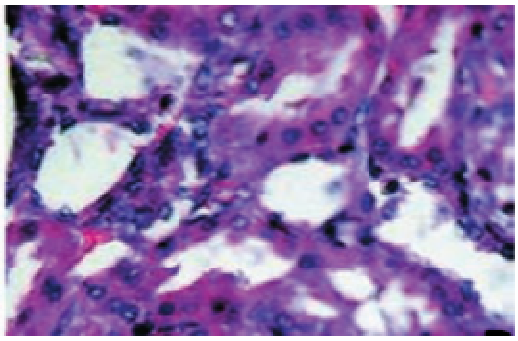

Figure 1. Nephrons and Renal Tubules, Magnified 10×40, H/E Stained

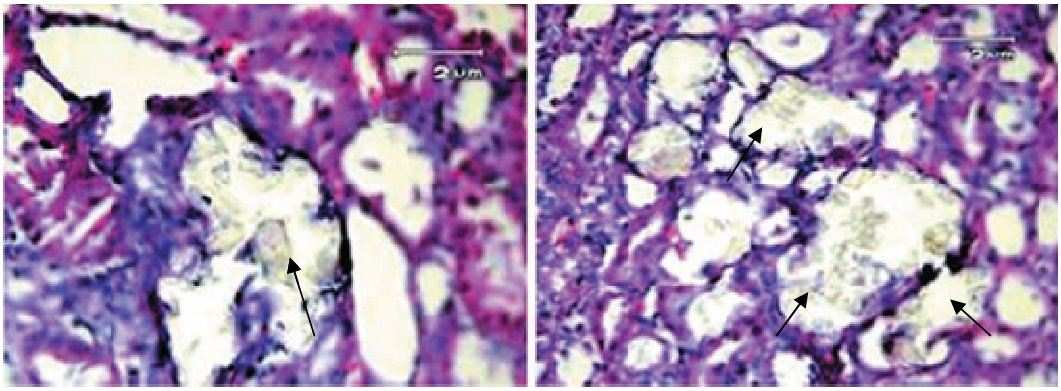

Figure 2.

Left: Urinary Tubes with Calcium Oxalate Crystals in the Ethylene Glycol Groups, Magnified 10×40, H/E Stained

Right: Urinary Tubes with Calcium Oxalate Crystals in the Ethylene Glycol Groups, Magnified 10×40, H/E Stained