Nephroprotective and Diuretic Effect of Nigella sativa L. Seeds Oil on Lithiasic Wistar Rats

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Abstract

Background and objective: The purpose of the current investigation was to study the influences, preventive and diuretic, of Nigella sativa L. seeds oil (N.S.O) on calcium oxalate (CaOx) urolithiasis induced in Wistar male rats.

Methodology: Seeds of Nigella sativa L. (N.S) were analysed for the evaluation of the concentration of oxalate and calcium. Nigella sativa L. seeds oil is obtained by hydrodistillation and HPTLC densitometric method was adopted to determine the amount of thymoquinone (TQ) present.

Thirty male Wistar rats were divided into 5 groups (N=6). Group I, negative control, drank tap water. The other groups were II Positive control, III, IV and V received a treatment model inducing calcium oxalate urolithiasis for 28 days, using an aqueous solution involve 0.75% (EG) ethylene glycol and 1.0 % (AC) chloride ammonium. Rats in group III received in addition, 750 mg/kg Cystone from the beginning to the end of calculi induction experimentation. However, rats in Groups IV and V received (N.S.O) at 5 ml/kg b.w by gavage on days 1st to 28th and 15th to 28th days, respectively.

On days 0, 7, 14, 21 and 28, body weights were measured and the 24-hour urine samples were accumulated and analysed for biochemical elements. On the 28th day, blood samples were collected for the estimation of serum parameters including creatinine, BUN and uric acid. All animals were sacrificed at the end of the experiment and the kidneys were detached for histopathological examination.

Results: Administration of (N.S.O) at 5 ml/kg body weight/dose/day for 28 days exerts a protective effect by reducing significantly (p <0.01) urinary and serum rates of calcium, phosphate and oxalate. This preventive diet could increase the volume of urine excreted.

Conclusion: The nephroprotectrice and diuretic activity demonstrated by Nigella sativa L. gives a scientific basis that approves their traditional use like a remedy against urolithiasis.

Keywords: Renal stones; Nigella sativa L.; Ethylene glycol; Calcium oxalate; Wistar rat.

List of Abbreviations: N.S.O: Nigella sativa L. Seeds oil ; CaOx: Calcium Oxalate; N.S: Nigella sativa L.; HPTLC: High performance thin layer chromatography; TQ: Thymoquinone; N: Number; EG: Ethylene Glycol; AC: Chloride Ammonium; BUN: Blood Urea Nitrogen; LD50: Lethal Dose 50; b.w: body weight; H & E: Haematoxyline and Eosin ; HPLC-UV: ;Caph: calcium phosphate; FR: glomerular filtration rate

Introduction

Urolithiasis has been known since ancient times and that appear inseparable from the history of Humanity (Daudon et al. 2008). However, over the 25 years past, significant changes have been felt in developing countries, so that today, stones became essentially kidney and calcium oxalate became the main component of a most calculations in most countries of the world (Daudon et al. 2004). This condition affects 13% of the male population and 6% of women, 50% of this population are exposed to recurrent lithiasic (Bihr and Meyers 2001). Thus, the non-surgical therapeutic indications are increasingly being used to prevent recurrence of urinary stones.

Several products from medicinal plants have been tested in urinary calculi therapy for centuries (Laroubi et al. 2007; Atmani et al. 2004). Historically, most therapies are derived from natural products, but there has been an abandonment of their use with the progress and before dominance of molecular approaches for the benefit of drug discovery (Harvey 1999). Among natural sources most used in chemothrapy or chemoprevention include Nigella sativa L. (Ranunculaceae) (Vihan and Panwar 1987). It is a picturesque plant planted in different places of the world. There seeds, also known as black cumin, are generally used in the Middle East, India and North Africa for medicinal purposes as a natural remedy against a number of diseases including bronchial asthma, rheumatism, hypertension, diabetes, inflammation, cough, headaches, eczema, fever and flu (Burits and Bucar 2000; Salem 2005).

Thymoquinone (TQ) is the most important component pharmacologically present in the essential oil of these seeds (Amm et al. 2011), with an important antioxidant power (Rifaioğlu et al. 2013) which allows it to keep many organs opposed to oxidative damage beget by various free radical (Yaman and Balikci 2010). Having regard to the low toxicity of its oil highlighted by high values LD50 = 28.6 ml/kg (Ghedira and Le Jeune 2010), this suggests a significant therapeutic range. Many in vitro and in vivo studies evaluating
its biological activity, including immune-potentiation, anti-tumor, anti-inflammatory, analgesic, anti-hypertensive, anti-diabetic stimulation, anti-ulcerogenic, antibacterial, antifungal have been reported (Kumar and Huat 2001; Al-Naggar et al. 2003).

The current investigation focuses on the evaluation of beneficial effects; preventive and diuretic of Nigella sativa L. seeds oil (NSSO) administrated by gavage to male Wistar rats on calcium oxalate stones induced by ethylene glycol (EG) and ammonium chloride (AC).

Materials and methods

Plant material

We have used Nigella sativa L. (voucher number MPS2012/22) seeds that were collected in 2012 from the region of Slatna (Bordj Bouarerridj, west of Algeria). The plant species is identified by Dr. A. Mokhebi and a sample was deposited at the herbarium of the Department of Biological Sciences of the University of Abdelhamid Benbadis Mostaganem, Algeria. Seeds were kept in the dark at 4°C.

Preparation of extract of Nigella sativa L seeds

Dried seeds of Nigella sativa L. 100 g were ground and extracted by hydrodistillation at atmospheric pressure (4 h) and the essential oil obtained was dried by Na₂SO₄ (Hanen et al. 2007) and stored at 4°C.

Analysis of Nigella sativa L seeds

Determination of the levels of calcium and oxalate:

The calcium level was determined by atomic absorption after mineralisation of N.S powder at 550 °C/12h. The ash obtained was dissolved in HNO₃ (Falade et al. 2005). The oxalate level was analysed by the technique reported by Roswitha. S (Roswitha et al. 2006).

Quantitative determination of thymoquinone by HPTLC

The amount of thymoquinone in our sample was determined by densitometric method HPTLC (Prawez et al. 2013).

Animals

Thirty male Wistar rats healthy adults of approximately the same age weighing between 120 and 130 g, were obtained from Algiers Pasteur Institute, Algeria. Rats were acclimated in polypropylene cages with aseptic conditions and fed with standard animal and water ad libitum, they were housed in a single temperature controlled 20-25 °C cage, at relative humidity of 50-55%, in 12h/12h dark/light cycle. All procedures were performed according with ethical guidelines for care and use of the animals laboratory, and they were allowed by the care of Experimental Animals Committee (MESRS/IVST/CEA/12/27). Rats were arbitrarily sectioned into five groups of six rats each.

Ethylene glycol and ammonium chloride induced urolithiasis Model

Kidney stones were induced by ethylene glycol and ammonium chloride in rats (Fan et al., 1999). Group I served as control group (negative control), received regular rat food and drinking water ad libitum. All the remaining groups (II - V) received 0.75 % ethylene glycol (EG) (E-Merk, Germany) and 1.0 % ammonium chloride (CA) (E-Merk, Germany) in drinking water for 28 days, of which for 3 days comprised of (EG + AC) to accelerate lithiasis followed by only (EG) for 25 days. Group III received in addition Cystone at 750 mg/kg b.w during the experimental test period (Mitra et al. 1998). Rats in Groups IV and V have received NSSO daily by gavage at dose equivalent to 5 ml/kg b.w (El-Abbar et al. 2003), from 1⁰ and 15⁰ to 28⁰ day, respectively.

Body Weight

Body weights were measured on days 0, 7, 14, 21 and 28. Evaluations of body weights (BWe) were determined using the formula (Bouanani et al. 2010):

\[
\text{BW}_e = (\text{BW}_{a0} - \text{BW}_{a1}) / \text{BW}_{a0}
\]

\(\text{BW}_e\): Body weight evaluation on days (0, 7, 14, 21 and 28)  
\(\text{BW}_{a0}\): Body weight on the first day (day 0)  
\(\text{BW}_{a1}\): Body weight on days (0, 7, 14, 21 and 28)

Collection and urinalysis:

Twenty-four hours urine samples were collected and measured on days 0, 7, 14, 21 and 28 using individual metabolic cages. At the same time, oxalate, phosphate, uric acid, calcium and magnesium were determined using commercially accessible test kits (Sigma-Aldrich, USA) (Rajagopal 1984; Sriboonlue et al. 1998).
Serum analysis

On day 28, sanguine fluid was accumulated from the retro-orbital sinus under anaesthetised condition and the serum was recovered by centrifugation at 10,000 g /10 minutes and analysed for uric acid, BUN and creatinine using an automated analyser (Model 705, Hitachi, Tokyo, Japan).

Renal Histology

At the experiment termination, the right and left kidneys were removed and used as a support for histological and biochemical examination respectively. The right renal (excretory organ) was put in formalin (10%), then in a range of ranked alcohol and xylene concentration, inserted in paraffin and cut to section of 5 micrometer in thickness, treated with Haematoxyline and Eosin (H & E) for histopathological microscope examination. Slides were analysed under optical microscope for calcium oxalate deposits (Hadjzadeh et al. 2007). The left renal (excretory organ) was chopped and 20% of mixed was prepared in Tris-HCl buffer (0.02 mol/L, pH=7.4). Left renal mixed was used to determine the rate of calcium (Chow et al. 1975), phosphate (Medeiros and Mustafa 1985) and oxalate (Hodgkinson and Williams 1972).

Statistical analysis

All results were expressed as mean ± standard deviation. Statistical analysis of data was performed by ANOVA, followed by Dunnett’s Comparison Test, (p <0.05) was considered significant. Statistical package for Social Sciences (SPSS 12.0) was used for this analysis.

Results

Seeds Analysis of Nigella sativa L.

The oil yield and concentration of calcium and oxalate existing in Nigella sativa L. seeds are presented in Table 1. All results are shown by an average of three measurements for each sample.

| Component | Nigella sativa |
|-----------|---------------|
| Yield^b   | 1.72 ± 0.08   |
| Calcium^a | 536 ± 18.93   |
| Oxalate^a | 0.031 ± 0.01  |

The values are expressed as mean±S.E.M of three determinations.

Table 2: Effect of Nigella Sativa seeds oil on the body weight of wistar rats (g).

| days | group I | group II | group III | group IV | group V |
|------|---------|----------|-----------|----------|---------|
| 0    | 126.33 ± 4.03 | 127.33 ± 3.50 | 128.33 ± 2.94 | 125.50 ± 3.93 | 124.66 ± 3.14 |
| 7    | 163.00 ± 5.83 | 118.17 ± 1.94 | 149.00 ± 5.44^a** | 124.50 ± 3.51^*** | 111.00 ± 3.03^a** |
| 14   | 186.00 ± 5.73 | 110.17 ± 2.99 | 169.83 ± 6.58^a** | 127.17 ± 4.44^b** | 102.33 ± 2.80^a** |
| 21   | 207.67 ± 5.00 | 100.83 ± 2.04^a** | 188.33 ± 5.82^a** | 145.67 ± 4.54^b** | 102.17 ± 3.49^a** |
| 28   | 234.17 ± 7.98 | 94.67 ± 2.87^a** | 204.33 ± 3.77^a** | 166.33 ± 4.03^a** | 109.33 ± 1.86^a** |

The values are given as mean±S.E.M. (n = 6 rats/group).

Table 3: Effect of Nigella Sativa seeds oil on urinary output in different groups of Wistar rats (ml).

| days | group I | group II | group III | group IV | group V |
|------|---------|----------|-----------|----------|---------|
| 0    | 08.00 ± 1.09 | 07.00 ± 0.89 | 07.83 ± 0.75 | 07.17 ± 0.75 | 06.83 ± 0.75 |
| 7    | 07.83 ± 1.17 | 12.33 ± 0.82^a** | 17.00 ± 0.89^b** | 14.50 ± 2.59^a** | 12.33 ± 0.51^a** |
| 14   | 09.00 ± 0.63 | 17.00 ± 0.55^a** | 19.16 ± 0.75^a** | 18.67 ± 2.16^a** | 18.00 ± 0.89^a** |
| 21   | 09.83 ± 0.75 | 18.50 ± 1.05^a** | 21.83 ± 0.75^a** | 24.33 ± 2.42^a** | 20.00 ± 2.28^a** |
| 28   | 09.33 ± 1.03 | 19.67 ± 0.81^a** | 23.33 ± 1.03^a** | 27.83 ± 2.31^a** | 21.83 ± 2.31^a** |

The values are given as mean±S.E.M. (n = 6 rats/group).

Table 4: Comparison Test, (p <0.05). aComparisons are made with group I; b Comparisons are made with group II.

Asterisks: significant * p < 0.05.; ** p < 0.01.; *** p < 0.001.
Figure 1: Chemical structure of thymoquinone (TQ)

Figure 2: HPTLC chromatogram of *Nigella sativa* seeds essential oil

Figure 3: Normal renal parenchyma in negative control rats; (H&E x40).

Figure 4: Damage of renal tubules and collecting system (blue arrowhead) of positive control rats; (H&E x40).
Figure 5: Tubules surrounded by inflammatory infiltration mainly lymphocytes (black arrow); (H&E x40).

Figure 6: Flattened epithelium with focal vacuolar degeneration; (H&E x40).

Figure 7: Normal renal histology with normal glomeruli and slight edema of tubular cells in rats treated with N. Sativa seeds oil; (H&E x40).

Figure 8: Almost recovery of renal parenchyma in rats treated with cystone; (H&E x40).
Table 4: Effect of Nigella Sativa seeds oil on urinary Magnesium, Uric acid, oxalate, calcium and phosphate levels in rats with induced urolithiasis (mg/24 h).

| Parameters | days | group I | group II | group III | group IV | group V |
|------------|------|---------|----------|-----------|----------|---------|
| Oxalate    | 0    | 05.21 ± 0.15 | 05.08 ± 0.08 | 05.03 ± 0.10 | 05.11 ± 0.09 | 05.06 ± 0.08 |
|            | 7    | 05.24 ± 0.06 | 06.75 ± 0.06 | 05.36 ± 0.06 | 05.49 ± 0.10 | 06.68 ± 0.09 |
|            | 14   | 05.26 ± 0.10 | 08.25 ± 0.07 | 05.62 ± 0.09 | 05.88 ± 0.08 | 08.16 ± 0.11 |
|            | 21   | 05.22 ± 0.03 | 10.17 ± 0.12 | 05.92 ± 0.08 | 06.42 ± 0.09 | 08.87 ± 0.06 |
|            | 28   | 05.23 ± 0.08 | 11.82 ± 0.07 | 06.30 ± 0.10 | 06.98 ± 0.07 | 09.27 ± 0.12 |
| Phosphate  | 0    | 05.87 ± 0.12 | 05.99 ± 0.06 | 05.87 ± 0.08 | 05.88 ± 0.09 | 05.91 ± 0.09 |
|            | 7    | 05.85 ± 0.16 | 06.38 ± 0.06 | 06.02 ± 0.07 | 05.96 ± 0.08 | 06.28 ± 0.03 |
|            | 14   | 05.93 ± 0.06 | 06.81 ± 0.07 | 05.93 ± 0.06 | 06.05 ± 0.11 | 06.81 ± 0.05 |
|            | 21   | 05.95 ± 0.08 | 07.37 ± 0.11 | 05.90 ± 0.11 | 06.07 ± 0.08 | 06.96 ± 0.06 |
|            | 28   | 05.87 ± 0.09 | 07.99 ± 0.09 | 05.92 ± 0.06 | 06.16 ± 0.10 | 07.00 ± 0.07 |
| Calcium    | 0    | 0.00 ± 0.03  | 0.56 ± 0.05  | 0.68 ± 0.03  | 0.61 ± 0.08  | 0.53 ± 0.05  |
|            | 7    | 0.00 ± 0.03  | 0.34 ± 0.07  | 0.55 ± 0.02  | 0.48 ± 0.06  | 0.32 ± 0.09  |
|            | 14   | 0.00 ± 0.03  | 0.24 ± 0.08  | 0.56 ± 0.07  | 0.41 ± 0.06  | 0.28 ± 0.05  |
|            | 21   | 0.00 ± 0.07  | 0.21 ± 0.04  | 0.60 ± 0.10  | 0.39 ± 0.07  | 0.24 ± 0.08  |
|            | 28   | 0.00 ± 0.08  | 0.13 ± 0.04  | 0.61 ± 0.11  | 0.34 ± 0.08  | 0.21 ± 0.04  |
| Uric acid  | 0    | 0.01 ± 0.03  | 0.34 ± 0.06  | 0.41 ± 0.07  | 0.40 ± 0.04  | 0.41 ± 0.04  |
|            | 7    | 0.01 ± 0.04  | 0.68 ± 0.08  | 0.49 ± 0.08  | 0.52 ± 0.04  | 0.63 ± 0.05  |
|            | 14   | 0.01 ± 0.02  | 0.16 ± 0.06  | 0.62 ± 0.10  | 0.65 ± 0.03  | 0.82 ± 0.06  |
|            | 21   | 0.01 ± 0.05  | 0.47 ± 0.06  | 0.72 ± 0.07  | 0.78 ± 0.06  | 0.26 ± 0.07  |
|            | 28   | 0.01 ± 0.04  | 0.79 ± 0.09  | 0.79 ± 0.13  | 0.94 ± 0.08  | 0.22 ± 0.07  |
| Magnesium  | 0    | 0.02 ± 0.08  | 0.74 ± 0.12  | 0.88 ± 0.06  | 0.85 ± 0.05  | 0.81 ± 0.10  |
|            | 7    | 0.02 ± 0.07  | 0.36 ± 0.07  | 0.76 ± 0.04  | 0.71 ± 0.07  | 0.48 ± 0.09  |
|            | 14   | 0.02 ± 0.07  | 0.06 ± 0.06  | 0.69 ± 0.05  | 0.51 ± 0.08  | 0.35 ± 0.05  |
|            | 21   | 0.02 ± 0.07  | 0.80 ± 0.07  | 0.52 ± 0.06  | 0.37 ± 0.04  | 0.23 ± 0.04  |
|            | 28   | 0.02 ± 0.08  | 0.56 ± 0.05  | 0.40 ± 0.06  | 0.23 ± 0.06  | 0.11 ± 0.04  |

The values are given as mean±S.E.M. (n = 6 rats/group).
a Comparisons are made with group I; b Comparisons are made with group II.
Asterisks: significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

Table 5: Effect of Nigella Sativa seeds oil on serum parameters (creatinine, uric acid, BUN) in experimental rats (mg/dl).

| Parameters | group I | group II | group III | group IV | group V |
|------------|---------|----------|-----------|----------|---------|
| Creatinine | 0.00 ± 0.04 | 0.10 ± 0.02 | 0.04 ± 0.04 | 0.09 ± 0.01 | 0.02 ± 0.02 |
| Uric acid  | 0.01 ± 0.05 | 0.81 ± 0.08 | 0.04 ± 0.04 | 0.13 ± 0.03 | 0.03 ± 0.03 |
| BUN        | 0.03 ± 0.06 | 0.65 ± 0.29 | 0.62 ± 0.11 | 0.64 ± 0.12 | 0.69 ± 0.09 |

The values are given as mean±S.E.M. (n = 6 rats/group).
a Comparisons are made with group I; b Comparisons are made with group II.
Asterisks: significant; * p < 0.05; ** p < 0.01; *** p < 0.001.
The method of HPTLC revealed a significant decrease in serum oxalate levels present in rats of different groups. However, these animals (group I) have excreted a volume of 9.33 ± 1.0 ml/day on the last test day (Table 3) and which has elevated significantly (p <0.01) to 19.67 ± 0.81 ml/day in the group (II) where rats are rendered lithiasic with a diet of EG + AC.

### Biochemical analysis of urine

Table 4 shows the details of 24h-urine excreted and its oxalate, calcium, phosphate, magnesium, and uric acid content in different rats of different groups. Rats in group II, which received for four weeks ethylene glycol 0.75 % regime were marked by a significant hyperoxaluria (P <0.01) compared to rats in control group (-). Similarly, phosphate and uric acid levels present in 24h-urine excreted increased significantly (P <0.01) while urinary calcium and magnesium have dropped as compared to group I. However, for a curative and preventive treatment with NSSO, oxalate, phosphate, and uric acid urinary levels excreted were significantly reduced (P< 0.01). These results are comparable to those recorded in rats of group treated with Cystone (group III).

### Serum analysis

Creatinine, uric acid and BUN serum rates are functioning kidney parameters. From this we evaluated the effect of NSSO on rats made lithiasic with EG and AC. Serum analysis on 28th day showed significant increase in these parameters (P <0.01) in rats with urolithiasis (Table 5). Group II compared with control (-) group I while both groups preventive IV and curative V treated with NSSO reduced exhibited significant (P <0.01) decrease in serum BUN, uric acid and creatinine. Similarly, the results of group III are comparable to those of Groups IV and V.

### Biochemical analysis of renal homogenate

In group II, phosphate, calcium and oxalate rates deposited in renal tissue were notably elevated (P <0.01) in comparison to group I (Table 6). However, cure diet with 750 mg/kg Cystone and 5 ml/kg Nigella essential oil in curative as in preventive system reduced significantly (P <0.01) increased rates of phosphate, calcium and oxalate stored in renal tissue.

### Histopathology

Histopathological microscopic analysis of renal sections also reinforces the above results. Optical microscope has revealed to us the deposition of calcium oxalate crystals (CaOx) and consequently the resulting damage on the glomeruli and tubules with interstitial inflammation compared to renal texture of control rats (-) (Fig 3 and 4). Similarly, tubules exhibited accumulation of mature lymphocytes (Fig. 5) and the epithelium is flattened with focal vacuolar degeneration and necrosis of the epithelial lining of tubules, irregular crystals were present within the tubules, along the nephron and papillary level (Fig. 6). All these histological changes were restored with Cystone (Fig. 8) and with applying preventative regime with NSSO, these groups have shown a relatively normal renal histology with normal glomeruli and tubules compared to Group I, but with a slight edema of the tubular cells (Fig. 7) and a number of evidence of small-sized crystals in the tubules but lower than group II.
Discussion

In Algeria as in many other countries, many herbs have proven useful in traditional medicine for the prophylaxis and treatment of many diseases including urinary lithiasis. *Nigella sativa* L. (NS) is one of these herbs with a rich historical and religious background. There are seeds are the source of active substances (Goreja 2003). The most challenging aspect is to provide base trace elements of plant material and obtain sufficient concentration that the supplement be ingested without consuming large amounts of plant tissue. As for minerals, *N. sativa* L. seeds, which we reported in (Table 1), appeared to involve useful concentration of calcium (536 mg/Kg); this amounts being comparable to that obtained with Tunisian (572 mg/Kg) and Iranian (564mg/Kg) varieties (Salma et al. 2007).

Many people from Indian subcontinent and Mediterranean region use *N. sativa* seeds oil constantly as protective and curative natural treatment. Many analyses to quantify *N. sativa* seeds composition approved that are very rich in volatile and fixed oils, proteins, amino acids, carbohydrates (Abdel-Aal and Atta 1993; Salem 2001; Takruri and Dameh 1998). Many of the pharmacological activities of this miraculous herb were attributed to thymoquinone (TQ). This one is the essential effective component of black cumin volatile oil. Recently, the presence of TQ in *N. sativa* L. seeds was confirmed using highly advanced methods; HPLC-UV (Goreja 2003) and HPTLC (Velho-Pereira et al. 2011). HPTLC is among the most recently employed methods described and used to quantify the most pharmacologically active component (TQ) (Prawez et al. 2013). The total volatile constituents extracted show the presence of a low percentage of thymoquinone (2.70 ± 0.05 %). These results are quite similar to those obtained by Farid for Algerian *N. sativa* L. (Farid et al. 2013) and Nickavar to that from Iran (Nickavar et al. 2003).

Since the urinary system of humans is similar to that of rats, they were chosen to induce urolithiasis (Liu et al. 2007), thus, recent studies have shown that the amount of calculations deposition in female rats was less significant than in male (Karadi et al. 2006). This model was approved to trigger urolithiasis kind of CaOx kind by calcium oxalate crystallisation and the high oxalate rate in the nphron damage the epithelial cells. However, crystals aggregation can begin after heterogeneous crystal nucleation (Scheid et al. 2004, Thamilselvan et al. 2003). Therefore, we investigated the effect of *Nigella sativa* L. seeds oil against EG and AC generates CaOx urinary calculi formation in Wistar male rats. Urinary stone formation takes place because of the changes in urine chemical composition, as hypercalciuria and hyperoxaluria that lead to concentrated urine, which subsequently finish up in calculi formation (Pak and Resnick 2000). Evidences in previous studies have shown that, in response to 14 days period of (0.75 % v/v) ethylene glycol and (1.0 %) of ammonium chloride administration, young albino rats form urinary stones composed mainly of CaOx. Generally, in mammals and by enzymatic medium, ethylene glycol is decomposed in vivo into four organic acids; glycolaldehyde, glycic acid, glycoxalixide acid and oxalic acid beginning to hyperoxaluria which is the essential factor of urolithiasis formation, composed mainly of oxalate (Leth and Gregersen 2005). The enzyme disturbances are the cause factors for the idiopathic hyperoxaluria; while, the defective intestinal absorption of oxalate plays an vital role in enteric hyperoxaluria and leads to an increase in urinary oxalate concentration (Williams and Wanzilak 1989). In the present study, chronic administration of 0.75% ethylene glycol and 1.0% ammonium chloride dissolved in drinking water to Wistar male rats resulted in hyperoxaluria. However, oxalate and calcium excretion in urine has significantly increased. It is accepted that high level in oxalate is more markedly risk parameter in the induction of kidney stones than hypercalciuria, so levels of urinary oxalate are comparatively more important than calcium (Divakar et al. 2010). Oxalate has a crucial role in calculi formation and has about 15 fold higher effect than urinary calcium (Borghesi et al. 1996).

Urinary calcium levels increases are promoter factor of nucleation and precipitation of CaOx or apatite (Caph) and later crystals aggregation (Leemann et al. 1991). Deficient renal tubular reabsorption system may be caused by the elevation in calcium urinary excretion and its deposition in kidney (Hautmann and Lehmann 1980) or an increase in absorption from the intestine as in patients with kidney stones calcium are reported to have a calcium hyperabsorption (Pak et al. 1974). However, *Nigella sativa* L. seeds oil decreased urinary levels of oxalate and calcium and even their retention in kidneys.

During the whole treatment period, body weight of control rats (+), treated with Cystone and who have undergone preventive regime with *Nigella sativa* L. seeds oil has increased. However, a decrease down of body weight gain was detected in group IV rats, in comparison with Groups I and III rats. The action of *Nigella sativa* L. on lipid metabolism can explain these acts and influences. Oral and intraperitoneal administration of *Nigella sativa* L. fixed oil give the same results as previous (Zaoui et al. 2002; Alsaif 2008). Rats in group II showed a significant elevation in urinary phosphate was observed in. The urinary excretion growth of phosphate and oxalate seems to give an area adapted for stone formation by composing Caph crystals, which increase epithalial CaOx retention (Karadi et al. 2006; Divakar et al. 2010). Treatment with *N. sativa* L. seeds oil restores urinary phosphate rate, thereby decreasing the risk of calculi formation. Increase in urinary uric acid elimination was also watched in urolithiasic rats. Literature reports that the high rate of uric acid excretion has been affirmed in urolithiasic rats. However, calcium oxalate solubility can react with uric acid and lowers the inhibitory activity of glycosaminoglycans (Selvam et al. 2001). Magnesium is one such well known inhibitor in stones formers. Magnesium can reduce the supersaturation of calcium oxalate and it can also decrease CaOx nucleation and crystallization (Selvam et al. 2001; Soundararajan et al. 2006). Urinary magnesium was significantly decreased in urolithiasic rats. Treatment with *N. sativa* L. seeds oil has restored magnesium excretion which has decreased the development of CaOx crystals in urolithiasic rats.

In urolithiasis, the glomerular filtration rate (GFR) reduces, due to the blockage to the excretion of urine by calculi in renal (excretory organ); Thus products, which contains nitrogen materials in particularly creatinine, uric acid and urea are stocked in blood (Ghodkar 1994). Moreover, lipid peroxidation and diminution in rates of antioxidant potential have been reported in the renal of rats enriched with diet inducing stones. For this, oxalate has been described to cause lipid peroxidation and kidney deterioration by operating with polyunsaturated fatty acids in cells tissue (Karadi et al. 2006; Ghodasara et al. 2010). In stones induced rats, high serum levels of creatinine, uric acid and BUN indicate marked renal damage (Anil and Niraj 2015). Treatment with NSSO showed significant reduction of serum rates of these parameters and prevented lipid peroxidation (Badary et al. 2003).

However, in traditional medicine, NSSO is used as a diuretic (Sathish et al. 2010), hence, it accelerates the process of dissolving the preformed stones by curing and preventing the formation of new stones in the urinary system (Anil et al. 2012). Moreover, the histopathological examination of renal tissues confirmed the biochemical results reported, where treatment with *N. sativa* L. seeds oil prevented the degenerative changes in renal tissues induced by EG (Sayed-Ahmed and Nagi 2007). The markedly
hight serum rates of BUN, uric acid and creatinine in urolithiasic rats were parameters indicative of marked necrosis of kidney epithelium and dilatation of proximal tubules with interstitial inflammation. In addition, in stones induced rats, there was damage in the last part of nephron and collecting system. High rates of urinary oxalate and even its deposition in renal (excretory organ) can cause the peroxidative deterioration of the kidney epithelium (Rathod et al., 2012), however, preventive and curative treatment with NSSO prevented lipid peroxidation induced by oxalate and caused regeneration of renal epithelium. Earlier studies described a large antioxidant and anti-inflammatory power of NSSO (Ragheb et al. 2009; Nagi and Mansour 2000; Mansour and Tornhamre 2004). This is why oil extract from Nigella may protect against CaOx crystal retention in kidneys and protecting hyperoxaluria engendered peroxidative deterioration to kidney tubular membranes.

Conclusion

In conclusion, the present data agree with the popular use of the oil isolated from Nigella sativa L. seeds for the treatment of urolithiasis. Thymoquinone is the main active compound found in Nigella sativa L. seeds, one of the most promising medicinal plants with many therapeutic effects. Among these effects, a potential treatment was confirmed by its ability to maintain kidney function, prevent the formation of urinary stones, reduce retention crystals in the kidney tissue and stimulate their excretion in urine. It also appears that the cure effect is less effective than preventive. The mechanism underlying this effect is probably controlled by an antioxidant.

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