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ARTICLE INFO

Article history:
Received 12 Aug 2015
Received in revised form 28 Aug 2015
Accepted 18 Oct 2015
Available online 17 May 2016

Keywords:
Nephrolithiasis
Cuminum cyminum
Mice

ABSTRACT

Objective: To investigate the protective effect of Cuminum cyminum (C. cyminum) essential oil on ethylene glycol induced nephrolithiasis in mice.

Methods: The study comprised of the following four different groups of six mice: ethylene glycol group, C. cyminum group, treatment group and normal group. The levels of blood urea nitrogen and creatinine were analyzed and the kidney samples from all the animals of each group were stained with haematoxylin and eosin.

Results: Treatment group revealed mild tubular degeneration without formation of calcium oxalate crystals and protein deposition. There were no significant differences between serum levels of blood urea nitrogen and creatinine in treatment and normal groups.

Conclusions: It seems that C. cyminum essential oil significantly decreased formation of calcium oxalate crystals and the growth of renal calculi in different parts of the tubules.

1. Introduction

Urinary calculi are the third prevalent disorder in the urinary system and may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary system. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones[1,2]. Kidney stone formation consists of several stages including supersaturation, nucleation, growth, aggregation and retention within renal tubules[3]. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures which are highly cost-effective may cause severe complications[4]. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms[5].

Cumin is a strong aromatic dried ripe fruit seed of Cuminum cyminum L. (C. cyminum). It belongs to the Apiaceae family (Parsley family). Cumin seeds are ancient spices with a strong aromatic smell and warm bitter taste. Cumin not only is a spice but also has great medicinal value. Cumin is widely used in traditional medicine to treat flatulence, digestive disorders, diarrhea and wounds. It is valuable in dyspepsia, diarrhea and hoarseness, and used as a remedy against indigestion and colic[6]. In recent years, herbal medical researches have focused on therapeutic effects of C. cyminum. The researches showed that fruit of the plant have anticonvulsant and analgesic activity[7-9]. There are some reports regarding the antibacterial, anti-diabetic and estrogenic activities of this plant[10-12].

The present study was conducted to investigate the protective effect of C. cyminum essential oil on ethylene glycol induced nephrolithiasis in mice.

2. Material and methods

2.1. Animals

The experimental protocols were approved by the Research Ethic Committee of the Shahid Bahonar University of Kerman, Iran. A total of 24 male NMRI mice were purchased from the Animal Laboratory of Kerman University of Medical Sciences, Kerman, Iran and kept in the Center for Laboratory Animal Care at the Veterinary Medicine Faculty of Shahid Bahonar University of Kerman for 1 week before treatment. The mice weighed 25–30 g and were at the same age of 1.5–2 months old. The experimental animals were randomly divided into four groups of 12 animals and were housed in standard polypropylene cages with wire mesh top, at 21 °C in a 12 h/12 h dark-light cycle. During the study, the animals
received water and pellet food (Javaneh Khorasan Co, Iran) ad libitum.

2.2. Experimental design

The study comprised of following four different groups of six mice: ethylene glycol group was received 0.1 mL normal saline by gavage and 1% ethylene glycol (Merck, Darmstadt, Germany) in drinking water for 35 days, *C. cyminum* group was received 0.1 mL *C. cyminum* essential oil (Barij Essence, Kashan, Iran) at a dose of 1 mg/kg by gavage and normal saline as drinking water for 5 weeks, treatment group was received 1% ethylene glycol (Merck, Darmstadt, Germany) in drinking water and treated by *C. cyminum* essential oil (1 mg/kg) during experimental period and normal group was received the same volume of normal saline during mentioned period.

2.3. Biochemical assays

The blood samples were collected directly from the animals by heart puncturing. The samples were centrifuged at 10000 r/min for 15 min and the clear serum were collected and stored in a -20 °C freezer. The levels of blood urea nitrogen (BUN) and creatinine were analyzed. All biochemical assays were determined according to Pars Azmon kits (Pars Azmon Co, Iran) by Autolab auto-analyzer (Rome, Italy).

2.4. Histopathological assays

After necropsy, the kidney samples from all the animals of each group were preserved in 10% neutral buffered formalin solution for histological examination at the end of the experiment. Formalin-fixed samples were processed by the standard paraffin wax technique, and sections of 5 cm thickness were cut and stained with haematoxylin and eosin (H&E).

2.5. Statistical analysis

All data were expressed as mean ± SE. Statistical analysis was performed using One-way ANOVA, followed by post-hoc Tukey HSD test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Biochemical assay

Results of serum biochemical parameters were presented in Table 1. The data obtained revealed that serum levels of BUN and creatinine in ethylene glycol group were significantly increased ($P < 0.05$) in comparison with normal group and there were no significant differences between treatment and normal groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal group</th>
<th>Group Cc</th>
<th>Group E</th>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>56.70 ± 3.10</td>
<td>61.80 ± 1.70</td>
<td>211.50 ± 5.70*</td>
<td>68.60 ± 4.20</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.76 ± 0.12</td>
<td>0.74 ± 0.20</td>
<td>2.34 ± 0.41*</td>
<td>0.71 ± 0.09</td>
</tr>
</tbody>
</table>

*: A significant difference in comparison with normal group ($P < 0.05$);
Group Cc: *C. cyminum* group; Group E: Ethylene glycol group.

3.2. Histopathological examination

Histopathological examination of kidney revealed the normal glomeruli and tubules with the absence of CaOx crystals in the normal group (Figure 1). In ethylene glycol group, some renal corpuscles had atrophied glomerular tufts and dilated urinary space. The epithelium of renal tubules showed degenerative changes including cell swelling and vacuolar degeneration (Figure 2). Excreted proteins were mostly deposited in the lumen of tubules in the cortex. These depositions were eosinophilic or basophilic due to mixed with calcium compounds (Figure 3). Depositions of CaOx crystals were observed in some tubules (Figure 4). Treatment group revealed mild tubular degeneration without formation of CaOx crystals and protein deposition (Figure 5).
According to the Khan and Thamilselvan, CaOx crystals can produce damages in the epithelial cells of nephrons, and consequently, the cells may produce some products which cause the aggregation of crystals[13]. Some of the constituents of the essential oil of C. cymimum such as α-pinene and β-pinene have been reported to possess anti-inflammatory activity. Another constituent, myrcene, has peripheral analgesic effect acting by the stimulation of nitric oxide pathway[9]. Also the seeds of C. cymimum have a high antioxidant activity[6].

El-Dakhakhny et al., stated that anti-inflammatory and antioxidant effects of the different compounds of the essential oil of C. cymimum play an important role in the treatment of CaOx calculi and related nephritis[14]. It seems that CaOx crystals can damage epithelial cells and induce inflammation[14].

The exposure of CaOx crystals induces the production of some inflammatory molecules with no apparent role in crystal formation. These indicate a relationship between nephrolithiasis and inflammation[15].

Kramer et al. reported that CaOx calculi may have a bacterial origin[16]. Antibacterial activity of the essential oil of C. cymimum may be effective in treatment of calculi formation[10].

Obstruction of the outflow of urine due to calculi formation decreases the glomerular filtration rate and the accumulation of waste products in blood[17]. In the present research, the nephrotoxicity induced by ethylene glycol was characterized by elevation of BUN and serum creatinine. But above mentioned parameters were decreased in the treatment group compared to the normal group. It seems that mentioned repair is due to protective effects of the essential oil of C. cymimum.

It seems that C. cymimum essential oil significantly decreased the formation of CaOx crystals, and the growth of renal calculi in different parts of the tubules. The results confirm folk information regarding antiurolithiatic activity of the medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was financially supported by Research Council of Shahid Bahonar University of Kerman, Iran, (Grant No. 9406).

References