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Prevention of Selenite-induced Cataractogenesis by *Origanum vulgare* Extract

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**Abstract:** The present study sought to assess antioxidant effect of *Origanum vulgare* extract in preventing selenite-induced cataractogenesis. This study was performed on Young white rats received sodium selenite (30 nmol g⁻¹ birth weight) subcutaneously on day 13 post partum during two months in 2009. Cataract formation and intensity was detected and measured by slit-lamp. *Origanum vulgare* (Ov) extract (2 g kg⁻¹) was given (1-2 times) intraperitoneal at different times with respect to the selenite administration lens opacification was analyzed in selenite, selenite-Ov, Ov and control groups on day 7 after selenite administration. Ov extract have revealed a significant protective effect against selenite induced cataract when injected 1 and 2 day (2 times) before selenite injection. There is a protective effect of Ov against selenite induced cataract formation. It is supposed that the anticataract effect of Ov extract could be based on direct or indirect antioxidant mechanisms.

**Key words:** Selenite, cataractogenesis, *Origanum vulgare*, white rats, extract

**INTRODUCTION**

Cataract is a progressive opacification of the lens of the human eye that impairs vision and may cause blindness (Aeschbach et al., 1994). Cataractogenesis is a multifactorial pathological process in which many risk factors and different causes are involved (Bakkali et al., 2008; Devamanoharan et al., 1991). Age related cataract remains a major cause of blindness, affecting over 20 million of the nearly 45 million blind people worldwide with the highest incidence occurring in developing countries (Doganay et al., 2002; Geraldine et al., 2006; Gupta et al., 2003). Oxidative stress is a common initiator of many age-related conditions. The aging ocular lens is susceptible to oxidative insult and physiological damage through photocatalytic generation of various oxygen radicals (Gupta et al., 2005) and this is probably the most important mechanism in cataractogenesis. There are effective surgical procedures to combat this problem, but the requirement for highly trained personnel and the cost of surgery pose a significant economic problem. Thus, there is need for chemical and pharmacological solutions for cataract prevention as well.

There are several endogenous defense mechanisms, which protect the lens against oxidative damage and these include the enzymes or components of the redox system (Gupta et al., 2005; Hockwin, 1997). The model of scavenging of the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. The DPPH is a stable nitrogen-center free radical, the color of which changes from violet to yellow upon reduction, by either the process of hydrogen or electron donation substances able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Lee et al., 2003) (Table 1).

Selenite-induced cataract is a cataract model mainly dependent on oxidative stress, in which oxidation of the critical sulfhydryl group is essential for the initiation of cataractogenesis (Kokkin, 1997; Kulusic et al., 2004).

**Table 1: Antioxidant activity of Diospyros lotus L. fruits extract and butylated hydroxyl toluene (BHT) against 1,1-diphenyl-2-picrylhydrazyl stable radical (DPPH)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg mL⁻¹)</th>
<th>Inhibition % (Mean±SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diospyros lotus L</td>
<td>0.5</td>
<td>32.47±3.08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>45.71±2.11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>59.12±1.18</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>71.61±2.34</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>86.43±1.27</td>
</tr>
<tr>
<td>BHT</td>
<td>0.01</td>
<td>34.11±2.48</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>57.91±0.86</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>75.16±1.91</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>91.31±0.8</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>95.57±0.51</td>
</tr>
</tbody>
</table>

*Each value in the table was obtained by calculating the average of 3 experiments±SD*

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Selenium-induced oxidative stress mediated cataractogenesis has been shown to be prevented by antioxidative agents such as caffeic acid phenethyl ester (Lagouti et al., 1993) 2-ketoglutarate (McCay, 1985), lycopene (Muranov et al., 2004) and Ocimum sanctum (Nirmalan et al., 2003). However, the biochemical mechanisms for these activities have not been completely elucidated.

Recently, emphasis has been laid on exploring the possibility of using natural resources to delay the onset and progression of cataract. One of such natural products is Origanum vulgare extract. The genus Origanum belongs to the family of Labiatae (Orhan et al., 1999).

Recently, this plant has drawn more attention due to the antimicrobial, anti-fungal, insecticidal and antioxidative effects of this herb on human health (Shearer et al., 1987; Shearer et al., 1992; Skoula and Harborne, 2002).

Results of various studies indicated that the antioxidant effects of oregano might be related to the dominant components, carvacrol and thymol, of the essential oil (Thylefors, 1995, 1999; Varma et al., 1984).

In this study, an attempt has been made to determine whether Origanum vulgare extract can retard or prevent selenite-induced cataractogenesis in an experimental in vivo setting.

**MATERIALS AND METHODS**

**Preparation of Origanum vulgare extract:** After collection of Origanum vulgare from Northern Iran, upper crust of beans was separated and then the green beans grinded to the smaller segment, put into the glass container, then put into the oven and powdered in temperature of 45°C. After drying, the powder of beans was obtained from the dried beans by grinder. Aqueous ethanol (70%) was added to the powdered beans (250 g) and stirred for one hour. The mixture was kept at room temperature for 48 h. After filtration, methanol was evaporated under reduced pressure at 40°C. Finally, 26.5 g of extract powdered extract was obtained.

**Animal care and cataract injection:** White rat mothers and their litter were kept in separate cages. They were fed a laboratory chow rodent diet and water. Temperature was maintained at 20°C and light was turned on and off at 12 h intervals. To initiate cataract, the rat pups were injected subcutaneously on day 13 post partum with a solution of sodium selenite, Na$_2$SeO$_3$ dissolved in 0.9% NaCl to give dose of 30 nmol g$^{-1}$ b.wt. of selenium. Following selenite injection, opacification progressed rapidly to maturity by day 4 or 5 post injection. Observations of lens opacification were made on day 7 after selenite administration under photo slit-lamp microscope and photographed. The pupils were dilated with a drop of 1% atropine. All of injections were done in laboratory of sari medicinal college and observation of lenses were done in Boosali hospital, Ophthalmology ward.

**Classification of cataract:** Cataract was graded from 0 to 4 according to following explanation (Fig. 1-4).

- Grade 0: clear lens
- Grade 1: swollen fibers and subcapsular opacities observed
- Grade 2: nuclear cataract in lens and swollen fibers in lens cortex
- Grade 3: strong nuclear cataract with perinuclear area opacity in lens
- Grade 4: total opacity of lens

**Origanum vulgare extract administration:** Rat pups received a single daily intraperitoneal injection (2 g kg$^{-1}$)

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**Fig. 1:** Grade 0: Clear lens

**Fig. 2:** Grade 1: Swollen fibers and subcapsular opacities observed
Table 2: Schemes of *Oreganum vulgare* extract and sodium selenite application

<table>
<thead>
<tr>
<th>Group</th>
<th>Post partum (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: Control group (G1)</td>
<td>11, 12</td>
</tr>
<tr>
<td>G2: Only 0.1% injection</td>
<td>13</td>
</tr>
<tr>
<td>G3: One injection</td>
<td>14</td>
</tr>
<tr>
<td>G4: One injection</td>
<td>15</td>
</tr>
<tr>
<td>G5: Two injection</td>
<td>16</td>
</tr>
<tr>
<td>G6: Three injection</td>
<td>17</td>
</tr>
<tr>
<td>G7: Four injection</td>
<td>18</td>
</tr>
</tbody>
</table>

G1: No material injected, G2: Only NaS injection, G3: One injection of OV (2 g/kg/day) in 11, 12 and 13 post partum day. G4: One injection of selenite (30 mmol g⁻¹ b.wt.) in 11, 12 and 13 post partum day. G5: Two injection of selenite + 2 injection of OV in 11, 12 and 13 post partum day. G6: One injection of OV in 11, 12 and 13 post partum day + one selenite injection at 11, 12 and 13 post partum day.

Table 3: Influence of Ov extract on cataract formation

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Grade (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Control (NS)</td>
<td>30</td>
<td>0.03±0.18</td>
</tr>
<tr>
<td>OV only</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Se only</td>
<td>5</td>
<td>2.2±0.84</td>
</tr>
<tr>
<td>2 OV+Se</td>
<td>5</td>
<td>0.4±0.55</td>
</tr>
<tr>
<td>3 OV+Se</td>
<td>5</td>
<td>0.6±0.55</td>
</tr>
</tbody>
</table>

8 mg mL⁻¹ were added, at an equal volume, to a methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. Butylated Hydroxy Toluene (BHT) was used as an antioxidant standard. Statistical analysis of data was carried out using descriptive statistics, the Mann-Whitney U-test and Kruskal Wallis nonparametric test.

RESULTS

**DPPH radical-scavenging activity:** It was found that the radical-scavenging activities of both *Oreganum vulgare* extract and BHT increased with increasing concentration. The maximum scavenging effects were obtained in 66.43% and 95.57% at 0.4-8 mg mL⁻¹ for *Oreganum vulgare* extract and BHT, respectively (Table 1).

Table 3 shows the cataract grades in rats that received Na₂SeO₃ and *Oreganum vulgare* extract according to the schemes shown in Table 1. Single and double Ov extract injection decreased lens opacity significantly (p<0.01). Kruskal Wallis shows there is statistically significant difference among the Mean Ranks and p<0.05. The lenses of control group 1 and 2 rats did not show any opacity except a very slight haziness in one lens in control group 2 (n = 35). The lenses of Ov-treated rats did not show any opacity (n = 5). Selenite injection (30 mmol g⁻¹ body weight) caused formation of severe nuclear cataract as a rule, but cortical cataracts without nuclear opacity were found too (n = 5). As shown in Table 3 Ov extract significantly protects rat lenses from selenite-induced cataract when injected on 1 and 2 days before selenite administration.

**Measurement of free radical scavenging activity:** Different concentrations of *Oreganum vulgare* (0.5 to
DISCUSSION

Nowadays, cataract is one of the main ophthalmic problems in the world and yet there is no effective preventive drug for it. The data of the present study demonstrated that *Origanum vulgare* extract can protect against selenite-induced cataract formation. The pathogenesis of selenite-induced cataract is strongly related to oxidative damage. Selenite causes oxidation of protein and non-protein sulfhydryl groups, which leads to ion pump damage and electrolyte imbalance. The intracellular calcium level increases, which activates the calcium-dependent protease calpain. Calpain partially hydrolyzes intracellular proteins, especially β-crystallin. Protein aggregates scatter light and lens opacity increases (Kokkini, 1997; Kulisic *et al.*, 2004). Low oxidative stress induced by a low selenite dose (<15 nmol kg⁻¹) causes biochemical processes before calpain activation, such as disturbance of the lens membranes’ ion permeability, water influx and swelling of the fiber cells. Cortical opacity is a marker of these events. Moderate oxidative stress (20-30 nmol kg⁻¹ of selenite) causes nuclear opacity through that calpain proteolysis of lens proteins. High oxidative stress (>30 nmol kg⁻¹) causes intensified injury, i.e., damage of both nucleus and perinuclear area and eventually, of the whole lens (Bakkali *et al.*, 2008). In this study, Ov extract protected the lens against the selenite-induced nuclear opacity (Table 3). We assume that the molecular mechanism of the Ov extract effect is connected with protection against mild or high oxidative stress induced by selenite.

We propose that the protective effect of Ov extract may be related to protection against oxidative stress induced by selenite.

Antioxidant activity of Ov extract demonstrated with DPPH test, but the molecular mechanism is unknown. Two mechanisms were suggested (Aeschbach *et al.*, 1994) direct influence on free radical oxidation in the lens (Bakkali *et al.*, 2008) an indirect effect through activation of a system that increases the antioxidant potential in the lens.

Therefore, augmentation of antioxidants is necessary to maintain a constant protective effect. For instance, several daily injections of water-soluble ascorbic acid after selenite exposure were effective against selenite-induced cataract (Varma and Hedge, 2004), as well as other antioxidants effectively prevent the selenite-induced cataract (Yanishlieva *et al.*, 1999).

In conclusion, a protective influence of Ov extract against selenite cataract development can be assumed, based probably on direct or indirect antioxidant mechanisms.

REFERENCES


