Effects of *Nigella sativa* L. on Lipid Peroxidation and Reduced Glutathione Levels in Erythrocytes of Broiler Chickens

Yasin TÜLÜCE¹, Halil ÖZKOL¹, Bünyamin SÖĞÜT², İsmail ÇELİK²

¹ Department of Medical Biology, Medical Faculty, Yuzuncu Yıl University, Van, Turkey
² Department of Animal Science, Faculty of Agriculture, Bingöl University, Bingöl, Turkey

**INTRODUCTION**

Black cumin (*Nigella sativa* L.) is a spice plant belonging to the family Ranunculaceae (Davis, 1965). It is a herbaceous plant grows in Asian and Mediterranean countries. The seed of black cumin has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma (El-Tahir et al., 1993). Black cumin seed is also used in folk medicine as an antispasmodic, antihelminthic, anti-septic, antirheumatic, nerve tonic, appetizer, emmenagogue and for the treatment of ascites, asthma and pustular dermatitis (Al-Yahya, 1986; Ageel et al., 1987).

The effects of *Nigella sativa* on different livings have been investigated, but reports concerning healthful vertebrates are very limited. *Nigella sativa* L. contains >30 of a fixed oil and 0.40-0.45 w/w of a volatile oil, alkaloids, sterols, saponins and quinines (Al-Yahya, 1986; Daba and Abdel Rahman, 1998; El-Kamali et al., 1998). The volatile oil has been shown to contain 18.4-24 % thymoquinone and 46 % many monoterpenes such as p-cymene and α-pinene (El-Tahir et al., 1993). Black cumin seed has antioxidant (Burits and Bucar, 2000; Meral et al., 2001), digestive, appetite stimulant (Gilani et al., 2004; Guler et al., 2006), analgesic (Khan et al., 1999), hepatoprotective (Mahmoud et al., 2002), renal protective (Khán and Sultaná, 2005), insecticide, bronchodilator, immunomodulative (El-Kadi and Kandil, 1987), anti-inflammatory (Houghton et al., 1995), antibacterial (Hanafy and Hatem, 1991), hypotensive (Zaoui et al., 2000; Mahfouz et al., 1962), choleretic, antitumoral (Salomi et al., 1992; El-Daly, 1998), antiadiabetic (Al-Hader et al., 1993; El-Shabrawy and Nada, 1996; Kanter et al., 2003a), antifungal, antihelminthic, anti-inflammatory (El-Tahir et al., 1993) and antiulcerogenic (El-Dakhakhny et al., 2002; El-Abhar et al., 2003; Kanter et al., 2005) effects.

On the other hand, it has been reported that the oil of black cumin seed inhibited the lipid peroxidation of biological membranes. It decreases the lipid peroxidation, activities of liver enzymes and contributes the antioxidant defense system in the CCl₄-treated rats (Kanter et al., 2003b). It has also reported that thymoquinone which is the major active component of the volatile oil of black cumin seeds protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism (Nagi et al., 1999) and is a potent superoxide anion scavenger (Badary et al., 2003). In our previous study, while liver MDA content

**ABSTRACT**

We aimed to determine the effect of *Nigella sativa* L. on reduced glutathione (GSH) and lipid peroxidation (as malondialdehyde, MDA) in erythrocytes of broiler chickens.

We used 100 Ross 308 chickens and they were equally divided into four groups namely control, 0.5 %, 1 % and 1.5 % containing *Nigella sativa* L. The control group received control broiler fattening feed whereas, the treatments groups were fed by the feed containing 0.5 %, 1 % and 1.5 % grinded *Nigella sativa* L. seeds for 6 weeks, respectively.

Erythrocyte MDA levels were significantly lower in 0.5 % (p<0.002) and 1 % (p<0.005) of *Nigella sativa* L. groups than in control although GSH levels were significantly (p<0.005) higher in 0.5 % and 1 % of *Nigella sativa* L. groups. The 1.5 % of *Nigella sativa* L. did not induce on MDA and GSH levels in the animals.

In conclusion, *Nigella sativa* L. caused protective effects on the oxidative stress-induced erythrocyte injury by inhibiting free radical production and regulation of GSH.

**Key words:** *Nigella sativa* L., lipid peroxidation, antioxidants, broiler chicks.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>BC</td>
<td>Black cumin</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>CCl₄⁻</td>
<td>Carbon teta chloride</td>
</tr>
<tr>
<td>DTNB</td>
<td>5, 5’-dithio-bis-(2-nitrobenzoic acid)</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine teta acetic acid</td>
</tr>
<tr>
<td>GSH</td>
<td>Reduced glutathione</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
</tbody>
</table>

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Ô₂⁻</td>
<td>Super oxide radical</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Polysaturated fatty acids</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
</tbody>
</table>

Corresponding Author: Yasin TÜLÜCE, Department of Medical Biology, Medical Faculty, Yuzuncu Yıl University, Van, Turkey.
yasintuluce@yahoo.com

Received : November, 7, 2009
Accepted : November, 24, 2009
decreased, GSH level and antioxidant enzyme activities including catalase, glutathione -s- transferase increased in applications with 3, 5 and 7 % black cumin seed supplementation (Sogut et al., 2008). Guler et al. (2007) demonstrated that when the diet was supplemented with 2 and 3 % black cumin seed, MDA concentration in serum, breast muscle, liver and heart muscle was significantly more reduced compared to birds fed 1 and 0.5 % black cumin seeds and control diet. In another study, it has also been reported that the oil of black cumin seed inhibited the lipid per-oxidation of biological membranes (Houghton et al., 1995).

Erythrocytes like the brain may be vulnerable to oxidative stress induced by epilepsy and are exposed to reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide and hydroxyl radical continuously generated via the auto-oxidation of hemoglobin (Cimen, 2008). Erythrocytes are extremely susceptible to oxidative damage induced by the ROS because erythrocytes contain polyunsaturated fatty acids (PUFAs) which can readily be peroxidized (Kovacic and Somanathan, 2008). Lipid peroxidation causes injury to cell and intracellular membranes and may lead to cell destruction and subsequently cell death (Kovacic and Somanathan, 2008). Erythrocytes are protected by antioxidants from peroxidative damage (Cimen, 2008). Glutathione (GSH) in erythrocytes is a hydroxyl radical and singlet oxygen scavenger and it participates wide range of cellular functions (Wu et al., 2004).

In the current study, we aimed to evaluate whether there would be protective effect of Black cumin seeds on lipid peroxidation and GSH status in erythrocytes of chicken.

MATERIALS AND METHODS

Preparation of Plant Feeds
Black cumin seeds were obtained from a local herb store, Van, Turkey, authenticated by Dr. Fevzi Ozgokce, Department of Biology, University of Yuzuncu Yil, washed, air-dried and grounded. 0.5, 1 and 1.5 % proportional of black cumin seeds in the feeds were prepared fresh daily. A voucher specimen (F-5427b) has been kept in the VANF herbarium for future reference. The diet composition of broiler fattening feed was given in Table 1.

Treatment of Animals
Totally 100 Ross 308 birds, 3-d-old, were used. Chicks were equally divided into four groups. The control group received normal broiler fattening feed whereas, the treatment groups were fed with the feed containing 0.5, 1 and 1.5 % grounded black cumin seeds and all groups fed and watered ad libitum for 6 weeks, and received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institutes of Health. The animals were housed at 20±2 °C and 23 h light and 1 h dark cycle from beginning to end of the treatment.

Blood collection and preparation of blood samples
At the end of the treatments, chicken’ blood was taken into anticoagulated tubes containing sodium EDTA, protected against light after 12 hours fast. The anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1500 g for 10 min at +4 °C. Erythrocytes samples were washed three times in cold isotonic saline (0.9 %, v/w), then haemolyzed with a nine-fold volume of phosphate buffer (pH 7.4). The haemolyzed erythrocytes and plasma were stored at -30 ºC for < 3 months pending on measurement of MDA and GSH levels.

GSH analysis
The erythrocyte GSH concentration was measured using the method described by Beutler et al. (1963). Briefly, 0.2 ml of erythrocyte pellet was added to 1.8 ml of distilled water. 3 ml of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 ml distilled water) was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered. 2 ml of the filtrate was taken and added into another tube, and then 8 ml of the phosphate solution and 1 ml of 5,5′-di-thiobis-(2-nitrobenzoic acid) (DTNB) were added. A blank was prepared with 8 ml of phosphate solution, 2 ml of diluted precipitating solution (three parts to two parts distilled water), and 1 ml of DTNB reagent. A standard solution of the glutathione was prepared (40 mg/100 ml). The optical density was measured at 412 nm in the spectrophotometer.

MDA analysis
The erythrocyte MDA concentration was determined using the method described by Jain et al. (1989), based on thiobarbituric acid (TBA) reactivity. Briefly, 0.2 ml of erythrocyte packets, 0.8 ml of phosphate buffer (pH 7.4), 0.025 ml of butylated hydroxytoluene (BHT) and 0.5 ml of 30 % trichloroacetic acid (TCA) were added to the tubes and mixed. After 2 h incubation at -20 °C, the mixture was centrifuged (4000g) for 15 min. Afterwards, 1 ml supernatant was taken and added to each tube, and then 0.075 ml of 0.1 M EDTA and 0.25 ml of 1 % TBA were added. These tubes with caps were incubated at 90 °C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 and 600 nm in a
spectrophotometer for erythrocyte MDA.

**Analysis of Data**

The data were expressed as mean ± standard deviation (SD). Statistical analyses were made using the Minitab 13 for windows packet program. Means and standard deviation were calculated according to the standard methods for all parameters. One-way ANOVA analyses of variance statistical test was used to determine differences between means of the treatments and the control group accepting the significance level at p< 0.05.

Vitamin premix: Vitamin concentration per kg of diet: vit. A 12,500 i.u., vit. D3 2500 i.u., vit. E (91% a-tocopherol acetate) 20•9 mg, vit. B1 1•25 mg, vit. B2 5•5 mg, vit. B6 3•5 mg, vit. B12 18 m g,

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow maize</td>
<td>429.1</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>259.3</td>
</tr>
<tr>
<td>Wheat</td>
<td>200</td>
</tr>
<tr>
<td>Animal fat</td>
<td>30</td>
</tr>
<tr>
<td>Fish meal</td>
<td>49.6</td>
</tr>
<tr>
<td>Limestone</td>
<td>13.3</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>8.5</td>
</tr>
<tr>
<td>Salt (sodium chloride)</td>
<td>1.9</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.8</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>3</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1: Diet composition.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>0.5 % black cumin</th>
<th>1 % black cumin</th>
<th>1.5 % black cumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/dl)</td>
<td>35.30 ±3.50</td>
<td>40.94 ±1.71⁹</td>
<td>43.33 ±1.66⁹</td>
<td>37.52 ±3.44</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>3.21 ±0.33</td>
<td>1.82 ±0.211.71⁹</td>
<td>2.47 ±0.42⁹</td>
<td>2.87 ±0.40</td>
</tr>
</tbody>
</table>

⁹ p <0.005 versus control. The p <0.002 versus control.

Table 2: Effects of black cumin seed on erythrocyte GSH and MDA levels in hemolyzed erythrocytes of broiler chickens (mean ± SD and n:25)

pantothenic acid 9 mg, folic acid 0.09 mg, biotin 60 mg, vit. PP 31 mg, vit. K 25 mg. Mineral premix: Trace mineral concentration per kg of diet: Fe 35 mg, Cu 7 mg, Zn 50 mg, Mn 80 mg, I 150 mg, Co 400 mg

**RESULTS AND DISCUSSION**

GSH and MDA levels were shown in Table 2. The results of experiment showed that treatment with black cumin seed decreased the erythrocyte MDA concentration, increased the levels of GSH significantly in feed containing 0.5 and 1 % black cumin seeds compared to those of control chicks (Table 2, Figure 1 and 2). Erythrocyte MDA levels were significantly lower in 0.5 % (p<0.002) and 1 % (p<0.005) of black cumin seeds groups than in control although GSH levels were significantly (p<0.005) higher in 0.5 % and 1 % of black cumin seeds groups. The 1.5 % of black cumin seeds did not induce on MDA and GSH levels in the animals.

We investigated whether black cumin seed could pre-
vent the lipid peroxidation and increase GSH in erythrocytes of broiler chickens. We observed protective effects of 0.5% and 1.0% black cumin seed on GSH and lipid peroxidation although 1.5 cumin seed has no effect on the values.

In the current study, we determined that the treatment of black cumin seeds increased the levels of GSH, whereas they decreased the production of lipid peroxides significantly in 0.5 and 1% dosages compared with control. However, increase of GSH and decrease of MDA in dose of 1.5% black cumin seed did not change significantly. Similar to this study, elevated level of GSH and reduced level of MDA in liver tissues in applications consisting of 3, 5 and 7% black cumin seeds were determined (Sogut et al., 2008). Guler et al. (2007) demonstrated that supplementing the diet with 2 and 3% black cumin seed reduced MDA levels in the liver, breast muscles and heart muscles of broilers. They claimed that this positive effect of black cumin seeds could be due to the main active constituent thymoquinone and other components: carvacrol, anethole, and 4-terpinol of black cumin essential oil. This data supported current study. Our results are consistent with the result of some studies in which effect of black cumin seeds’ oil were investigated (Nagi et al., 1999; Burits and Bucar, 2000; Meral et al., 2001; Kanter et al., 2003). To our knowledge, there is no study examining the effect of black cumin on concentrations of GSH and MDA in chicken erythrocyte.

The possible protective effect of NS was investigated in a few animal models like rat, mouse and rabbit. In the hepatotoxicity (Meral et al., 2001; Badary and Gamal El-Din, 2001; Mansour et al., 2001; Al-Johar et al., 2008), nephrotoxic (Badary, 1999; Ali, 2004; Sayed-Ahmed and Nagi, 2007; Khattab and Nagi, 2007), cardiotoxic (Ebru et al., 2008), carcinogenic (Mabrouk et al., 2002), parasitic (El Shenawy et al., 2008), diabetic (Kanter et al., 2004; Al-Enazi, 2007), ischaemia/reperfusion (Hosseinizadeh et al., 2007; Bayrak et al., 2008) and gastric mucosal injury (Kanter et al., 2005) studies, it has been shown that NS treatment decreased lipid peroxidation and increased GSH levels in various tissues such as liver, kidney heart, stomach, brain and serum.

The present study indicated that black cumin seed has a preventive effect on lipid peroxidation and GSH level. The reasons for the effect of black cumin seed haven’t been exactly understood till today, but it may be thought that black cumin seed exerted decisive impact like other antioxidants. It also might decreased hydrogen peroxide (H2O2), hydroxyl (OH) and super oxide (O2−) radicals that occur as result of aerobic condition in the organisms, leading to an increase in lipid peroxidation. Comprehensive experiments are needed to show its antioxidant affect mechanism on the erythrocytes in vivo.

References


Effects of Nigella sativa on Antioxidant Parameters


