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The Effects of Diet Supplemented with the Black Cumin (*Nigella sativa* L.) upon Immune Potential and Antioxidant Marker Enzymes and Lipid Peroxidation in Broiler Chicks

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Abstract: This study was carried out to investigate whether *Nigella sativa* (NS) could decrease the lipid peroxidation [Malondialdehyde = MDA], increase the anti-oxidant defence system [Reduced Glutathione (GSH), Catalase (CAT) and Glutathione-S-Tranferase (GST)] and also posses the immunopttential [Adenosine Deaminase (ADA) and Myeloperoxidase (MPO)]. Totally, 100 Ross 308 birds, 1 day old, were used. Chicks were divided into 4 experimental groups, (control, 3, 5 and 7% containing *Nigella sativa*) each containing 25 broiler chicks. While, the control group received normal broiler fattening feed, the treatments groups were fed with the feed containing 3, 5 and 7% grinded *Nigella sativa* seed and all groups fed and watered *ad libitum* for 6 weeks during experiment. At the end of the 6 weeks experiment, liver samples were taken to measure MDA, GSH, CAT, GST, ADA and MPO. While, treatment with *N. sativa* decreased the liver MDA content, increased the activities of CAT, GST, ADA and MPO significantly, but increased the level of GSH lightly in comparison to control. It was concluded that *N. sativa* might be used to potent of antioxidant and immune system and also prevent liver from oxidative stress resulting lipidperoxidation.

Key words: *Nigella sativa* L., antioxidants, immune potential, malondialdehyde, broiler chicks

INTRODUCTION

Today's world is increasingly seeking ways to replace the synthetic drugs with the therapeutic power of natural products. The traditional folk medicine had already found the secret of healing in the nature. Medicinal plants have been used for therapeutic purposes since the beginning of civilization. Following a recent period in Western medicine when plant medicines were shunned, there has been a resurgence of interest in plant compounds with beneficial pharmacological properties (Schulman and Heather, 2003). The most commonly pursued treatments are antibacterial, antiviral, antitumor, anti-inflammatory, antihypertensive, antioxidant properties.

Nigella sativa is a spice plant belonging to the family *Ranunculaceae* (Davis, 1965). It is a medical plant has got black seeds and has been used as a natural remedy for a variety of illnesses (Meral *et al*., 2001).

The effects of *Nigella sativa* on different livings have been investigated, but reports concerning healthful vertebrates are very limited. In the literature, it is reported that the seeds oil of *Nigella sativa* anti-bacterial (Toppozade *et al*., 1965), bronchodilator (El-Dakhakhny, 1965), decreased the elevated glucose and MDA concentrations, strengthened non-enzymatic antioxidant defence (Meral *et al*., 2001), hypotensive (Mahfouz *et al*., 1962), immunopo-tentiating (El-Kadi and Kandil, 1987), Gastroprotective (El-Abhar *et al*., 2003) and *Nigella sativa* essential oil Antioxidant (Burits and Bucar, 2000) activities. On the other hand, it has reported that the oil of *Nigella sativa* inhibited the lipid per oxidation of biological membranes. *Nigella sativa* oil decreases the lipid peroxidation and liver enzymes and increase the antioxidant defence system activity in the Ccl_4 treated rats (Kanter *et al*., 2003). It has also reported that thymoquinone which is the major active component of the volatile oil of *Nigella sativa* 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). It has also been reported (Houghton *et al*., 1995) that the oil of *Nigella sativa* inhibited the lipid peroxidation of biological membranes.

Today, because the effects of plant on animal illnesses are well known, they are used widely in medicine.

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There are few study examined the improve effects of these plants on animals; therefore, this subject has attracted the interest of many researchers recently.

In this study, our attention is focused on chemo preventive and anti-oxidant activity of the natural products, since these properties are closely related to prevention and therapy of cancer. In order to achieve a more rational design of plants, it is necessary to clarify the mechanism of plants' improve effects comprehensively. For this aim, the treatment of *Nigella sativa* was done orally because the effect of plants represents a well characterized *in vivo* model system. The liver was chosen due to its important role in metabolism of eaten nutrition's.

MATERIALS AND METHODS

Preparation of plant feeds: Briefly, *Nigella sativa* seeds were purchased from a local herb store, Van, Turkey, authenticated by Dr. Fevzi Ozgokce, Department of Biology, University of Yuzuncu Yil, washed, air-dried and grinded. Three, five and seven percent proportional of *Nigella sativa* seeds in the feeds were prepared fresh daily. A voucher specimen (F-5427b) has been kept in the VANF herbarium for future reference.

Treatment of animals: Totally, 100 Ross 308 birds, 1 day old, were used. Chicks were divided into four experimental groups, (control, 3, 5 and 7% containing *Nigella sativa*) each containing 25 broiler chicks. While, the control group was received normal broiler fattening feed, the treatments groups were fed with the feed containing 3, 5 and 7% grinded *Nigella sativa* seed 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 after 3 weeks of age and 23 h dark and 1 h light cycle from beginning to end of the treatment.

At the end of the treatments, the chicks were sacrificed and after liver samples were obtained. The livers were dissected and put in Petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples taken and kept at -87°C until analysis. The tissues were homogenized for 5 min in 0.115 M potassium chloride (KCl) solution (1:5 w/v) using a glass-porcelain homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 × g for 15 min. All processes were carried out at 4°C. Supernatants were used to determine MDA, GSH, CAT, GST, ADA and MPO.

Biochemical analysis: The liver MDA concentration was determined using the method described by Jain *et al.* (1989), based on thiobarbituric acid (TBA) reactivity. Briefly, 0.2 mL supernatant obtained from tissues, 0.8 mL phosphate buffer (pH 7.4), 0.025 mL butylated hydroxytoluene (BHT) and 0.5 mL 30% trichloroacetic acid (TCA) were added to the tubes and mixed. After 2 h incubation at -20°C, the mixture was centrifuged (400 g) for 15 min. After this, 1 mL supernatant was taken and added to each tube and then 0.075 mL of 0.1 mol ethlenediaminetetraacetic acid (EDTA) and 0.25 mL of 1% TBA were added. These tubes with Teflon-lined screw 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 for tissues MDA concentration (Novaspec II Pharmacia-Biotech, Biochrom Ltd., UK).

Liver GSH concentration was measured using the method described by Beutler *et al.* (1963). Briefly, 200 μL supernatant was added to 1.8 mL distilled water. There millilitres of the precipitating solution was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered. Two millilitres of filtrate was taken and added into another tube and then 8 mL of the phosphate solution and 1 mL 5,5'dithiobis-(2-nitrobenzoic acid) (DTNB) were added. A blank was prepared with 8 mL of phosphate solution, 2 mL diluted precipitating solution (three parts to two parts distilled water) and 1 mL 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.

The liver CAT activity was determined using the method described by Beutler (1984), Briefly, 1 M Tris-HCl, 5 mM EDTA (pH = 8), 10 mM H_2O_2 and H_2O were mixed and the rate of H_2O_2 consumption at 240 nm and the 37°C was used for quantitative determination of CAT activity. GST was assayed at 25°C spectrophotometrically by following the conjugation of glutathione with 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm as described by Mannervic and Guthenberg (1981). ADA was assayed by the method described by Giusti (1974); ADA assay is based on indirectly measuring the formation of NH_3 produced when ADA acts in excess of adenosine. The release of ammonia was determined colorimetrically at 630 nm after the development of an intense blue colour with hypochlorite and phenol in an alkaline solution. MPO was assayed by the method described by Bradley *et al.* (1982).

Analysis of data: The data were expressed as mean± Standard Deviation (SD). For statistical analysis the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was

used. For all parameters, means and SD were calculated according to the standard methods and analysed with Analysis of Variance (ANOVA). Tukey's test was used to test differences among means for which ANOVA indicated a significant ($p \leq 0.05$) F-ratio.

RESULTS AND DISCUSSION

The results of experiment showed that treatment with *Nigella sativa* decreased the liver MDA concentration, increased the activities of CAT, GST, ADA and MPO significantly, but increased the level of GSH lightly in liver in comparison to those of control chicks (Table 1). To find out the significance of decreases and increases in different groups MDA, CAT, GSH GST, ADA and MPO on treatment with *Nigella sativa* for 6 weeks, the data obtained have been subjected to Turkey's test.

The first aim of this study was to investigate whether *Nigella sativa* could prevent the lipid peroxidation and increase the anti-oxidant defence system in broiler chicks. The data collected in this study were all from one time-point of the experiment.

In this study, *Nigella sativa* caused a significant alteration in the immune potential and antioxidant marker enzyme activities, GSH level and MDA content in the liver tissue almost all percent supplemented with the *Nigella sativa* (Table 1). The result of present study indicates that *Nigella sativa* possesses the antioxidant and immune potential agent properties. This is evidenced from our observation that, upon *Nigella sativa* treatment of chick *in vivo*, the decrease level of MDA, which may correlate to the decrease of reactive oxygen radicals and the increase activities of immune potential marker enzymes. The reasons for such affect of *Nigella sativa* are not understood at the present decisively, but it is conceivable that *Nigella sativa*, being antioxidant like other antioxidant. It also might be decrease hydrogen peroxide (H_2O_2), hydroxyl (OH) and super oxide (O_2^-) radicals as result of aerobic condition in the organisms, leading to an increase in lipid per oxidation. *Nigella sativa* may also lead to the activation of this enzyme synthesis into plasma as result of mRNA synthesis or cellular transcription mechanisms. Inhibition or activation of the enzyme ADA by *Nigella sativa*, as well as the inhibition of activation adenosine transport and metabolism, might be decreases or increased levels of adenosine. The increase may indicate stimulated cell-mediated immunity. As know, ADA is essential for the proper functioning of the vertebrates' body immune system. Because ADA is the major enzyme responsible for the degradation of Ado, the inhibition of its activity should represent one of the best ways to increase accumulation of Ado in tissues under chemicals stress conditions.

On the other hand, the presence of abnormal levels of enzymes in tissues is used in clinical practice to indicate whether or not tissue damage which organ has been affected. Also, activation of MPO by *Nigella sativa* in the liver might be cause the immune potential. In phagolysosomes, this enzyme works together with other oxidases and proteases to cause the destruction of ingested organisms (Deby-Dupont *et al*., 1999). Low MPO levels are the most common neutrophilic lysosomal deficiency, but usually occur without a noticeable increase susceptibility to infection or reduced immune response (Deby-Dupont *et al*., 1999; Aratani *et al*., 1999). MPO detection also has been used as a marker of neutrophil infiltration into tissues (Haqqani *et al*., 1999; McConnico *et al*., 1999).

So far, no study examining the affect of *Nigella sativa in vivo* has been made on chicks' liver MDA content, GSH level, antioxidant enzymes and immune potential marker enzymes as diet supplemented with the *Nigella sativa* study model. Because of this, we couldn't have the chance to compare with the previous results. In addition, due to inconsistent factors like treatment time and manner, purity and species tissue differences etc., it is difficult to compare data provided from different laboratories such as the test plant for biological effect. However, there are a few studies although the treatment, materials of studies and the setting of studies are different. These results are almost in accordance with our result and each other. Our results are accordance with the result of some previous studies which are investigating effect of *Nigella sativa* seeds oil too (Meral *et al*., 2001; Burits and Bucar, 2000; Kanter *et al*., 2003; Nagi *et al*., 1999).

Table 1: Effects of *Nigella sativa* on liver MDA, GSH, antioxidant and immunopotential enzymes of chicks

Parameters	Control X±S.D.	3 (%) X±S.D.	5 (%) X±S.D.	7 (%) X±S.D.
MDA nmol g^{-1}	109.3±8.73	31.32±6.14*	26.92±3.2*	25.72±6.73*
GSH mg g^{-1}	28.11±2.44	29.31±2.65	30.71±3.58	30.81±3.56
CAT U g^{-1}	36.62±2.48	50.04±3.5*	51.12±3.72*	41.33±3.18*
GST U g^{-1}	124.75±11.54	149.71±5.74*	172.12±13.69*	160.13±13.39*
ADA U g^{-1}	18.81±1.35	20.22±2.91	31.2±2.7*	37.08±4.66*
MPO U g^{-1}	74±30.3	105.9±54.6*	134.9±25.4*	155.2±75.1*

Each value represents the Mean±S.D.* are significantly different from control at the $p \leq 0.05$ level

CONCLUSION

Nigella sativa treatment in chicks and other animals could prevent lipid peroxidation, increase the activity of immune potential and antioxidant defence system and protect from liver damage. In addition, the diet supplemented with the black cumin could be used the antioxidant and immune potential defense system for poultry and further studies are required to demonstrate its anti-oxidant and immune potential affect mechanism on the liver the *in vivo*.

REFERENCES

Aratani, Y., H. Koyama, S. Nyui, K. Suzuki, F. Kura and N. Maeda, 1999. Severe impairment in early host defense against Candida albicans in mice deficient in myeloperoxidase. Inf. Immun., 67: 1828-1836.

Badary, O.A., R.A. Taha, A.M. Gamal el-Din and M.H. Abdel-Wahab, 2003. Thymoquinone is a potent superoxide anyon scavenger. Drug Chem. Toxicol., 26: 87-98.

Beutler, E., O.B. Dubon and M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Beutler, E., 1984. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd Edn. Grune and Startton, New York, pp: 105-106.

Bradley, P.P., D.A. Priebat, R.D. Christensen and G. Rothstein, 1982. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J. Invest. Dermatol., 78: 206-209.

Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res., 14: 323-328.

Davis, P.H., 1965. Flora of Turkey and the East Aegean Islands, Edinburgh Univ. Press., Edinburgh, Vol. 1.

Deby-Dupont, G., C. Deby and M. Lamy, 1999. Neutrophil myeloperoxidase revisited: It's role in health and disease. Intensivmedizin-und-Notfallmedizin, 36: 500-513.

El-Dakhakhny, M., 1965. Egyptian *Nigella sativa*. Arzneimittel-Forsch., 15: 1227-1229.

El-Kadi, A. and O. Kandil, 1987. The black seed (*Nigella sativa*) and immunity: Its effect on human T cell subset. Fed. Proc., 46: 1222.

El-Abhar, H.S., D.M. Abdallah and S. Saleh, 2003. Gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. J. Ethnopharmacol., 84: 251-258.

Giusti, G., 1974. Methods of Enzymatic Analysis. In: Bergmeyer, H.U. (Ed.). New York: Academic Press, 2: 1092-1099.

Haqqani, S., J.K. Sandhu and H.C. Birnboim, 1999. A myeloperoxidase-specific assay based upon romidedependent chemiluminescence of luminol. Analyt. Biochem., 273: 126-132.

Houghton, P.J., R. Zarka, B. De las Heras and J.R. Hoult, 1995. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med., 61: 33-36.

Jain, S.K., R. McVie, J. Duett and J.J. Herbst, 1989. Erythrocyte membrane lipid peroxidation and glycolylated hemoglobin in diabetes. Diabetes, 38: 1539-1543.

Kanter, M., I. Meral, S. Dede, H. Gunduz, M. Cemek, H. Ozbek and I. Uygan, 2003. Effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl_4-treated rats. J. Vet. Med. A., 50: 264-268.

Mahfouz, M., M. Dakhakhny, A. Gemei and H. Moussa, 1962. Choleretic action of *Nigella sativa* seed oil. Egyp. Pharmacol. Bull., 44: 225-229.

Mannervic, B. and C. Guthenberg, 1981. Glutathione-S-transferase (Human placenta). Method enzymol., 77: 231-235.

McConnico, R.S., D. Weinstock, M.E. Poston and C. Malcolm, 1999. Myeloperoxidase activity of the large intestine in an equine model of acute colitis. Am. J. Vet. Res., 60: 807-813.

Meral, I., Z. Yener, T. Kahraman and N. Mert, 2001. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. J. Vet. Med. A., 48: 593-599.

Nagi, M.N., K. Alam, O.A. Badary, O.A. Al-Shabanah, H.A. Al-Sawaf and A.M. Al-Bekairi, 1999. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int., 47: 153-159.

Schulman, R.N. and S.O. Heather, 2003. Plants provide new drug leads: The natural medicinal properties of plants could save millions in drug development costs. Prepared Foods, 172: 13-18.

Toppozade, H., H. Mazloum and M. El-Dakhakhny, 1965. The antibacterial properties of *Nigella sativa* seeds: Active principle with some clinical applications. J. Egypt Med. Assoc., 48: 187-202.