0163-4984/05/10703–0277 $30.00

Effect of Black Cumin *(Nigella sativa)* on Cadmium-Induced Oxidative Stress in the Blood of Rats

MEHMET KANTER,*[,1] OMER COSKUN,[1] AND AHMET GUREL[2]

[1]*Department of Histology and Embryology, Faculty of Medicine, Trakya University, Edirne, Turkey;* [2]*Department of Biochemistry, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*

Received January 3, 2005; Revised January 28, 2005;
Accepted February 7, 2005

ABSTRACT

The protective effect of black cumin (*Nigella sativa* = NS) on cadmium-induced oxidative stress was studied in rats. The rats were randomly divided into three experimental groups: A (conrol), B (Cd treated), and C (Cd + NS treated), each containing 10 animals. The Cd-treated and Cd + NS-treated groups were injected subcutaneously daily with $CdCl_2$ dissolved in isotonic NaCl in the amount of 2 mL/kg for 30 d, resulting in a dosage of 0.49 mg Cd/kg/d. The control group was injected with only isotonic NaCl (2 mL/kg/d) throughout the experiment (for 30 d). Three days prior to induction of $CdCl_2$, the Cd + NS-treated group received a daily intraperitoneal injection of 0.2 mL/kg NS until the end of the study. Cd treatment increased significantly the malondialdehyde levels in plasma and erythrocyte ($p<0.01$ and $p<0.05$, respectively) and also increased significantly the antioxidant levels (superoxide dismutase, glutathione peroxidase, and catalase) ($p<0.05$) compared to the control group. Cd + NS treatment decreased significantly the elevated malondialdehyde levels in plasma and erythrocyte ($p<0.01$ and $p<0.05$, respectively) and also reduced significantly the enhanced antioxidant levels ($p<0.05$). Cd treatment increased significantly the activity of iron levels ($p<0.05$) in the plasma compared to the control group. Cd + NS treatment decreased the activity of iron levels ($p<0.05$) in the plasma compared to the Cd-treated group. In the control group with no treatment, histology of erythrocytes was normal. In the Cd-treated group, there were remarkable membrane destruction and hemolytic changes in erythrocytes. In the Cd + NS-treated group, these changes were less than in the Cd-treated group. Our results show that *N. sativa* exerts a protective effect against cadmium toxicity.

Index Entries: *Nigella sativa;* cadmium; erythrocyte; oxidative stress; hemolysis; rat.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Cadmium, a potent toxic metal, is very harmful to the environment and to humans because of in vivo accumulation in the liver, kidney, and other tissues. The toxicity of cadmium as an industrial pollutant, a food contaminant, and one of the major components in cigaret smoke has been well established *(1).* Cadmium accumulates and proves to be very toxic in many organs, such as the kidney, liver, lung, testis, brain, bone, blood system, and so forth *(2,3).* The molecular mechanisms of its toxicity are not yet well defined. Recent studies on mammals have shown that Cd stimulates the formation of reactive oxygen species (ROS), including the oxygen free anion radical *(4),* hydrogen peroxide *(5),* and, probably, hydroxyl radical *(6).* As a consequence, enhanced lipid peroxidation, DNA damage, as well as marked disturbances of the antioxidant defense system occur *(7,8).* After the intake and resorption, Cd enters the blood, where it binds to the erythrocyte membranes and plasma albumin *(9).* In the blood and tissues, Cd stimulates the formation of metallothioneins *(10)* and ROS, thus causing oxidative damage in erythrocytes and in various tissues, which result in a loss of membrane functions *(11).* Cd also induces the onset of anemia, decreases the red blood cell (RBC) count, hemoglobin concentration, and hematocrit value, as well as reduced blood iron levels *(12).* Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported *(13,14).* Fariss has shown that free-radical scavengers and antioxidants are useful in protecting against Cd toxicity *(15).*

The black seed of *Nigella sativa* (NS), an annual Ranunculaceae herbaceous plant, has been used traditionally for centuries in the Middle East, northern Africa, the Far East, and Asia for the treatment of asthma. NS contains >30% of a fixed oil and 0.40–0.45% (w/w) of a volatile oil. The volatile oil has been shown to contain 18.4–24% thymoquinone (TQ) and 46% monoterpenes such as *p*-cymene and α-piene *(16).* Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects, such as bronchodilatation, immunomodilative *(17),* antibacterial *(18),* hypotensive *(19),* hepatoprotective *(20),* and antidiabetic *(21,22).*

The aim of this study was to investigate a possible protective effects of NS treatment on the selected biochemical parameters and histological changes of RBCs in rat exposed to Cd.

MATERIAL AND METHODS

Plant Material and Extraction Procedure

The NS seeds were purchased from a local herb store in Van, Turkey. Voucher specimens have been kept at the Department of Histology and Embryology, Yuzuncuyil University, Van, Turkey for the future reference.

The seeds of NS were powdered in a mixer and placed in a distillation flask, and the volatile oil with 0.2% yield was collected by steam distillation.

Treatment of Rats

Thirty healthy male Wistar albino rats weighing 200–250 g (age: 4 mo) and averaging 16 wk old were supplied from The Center of Medical Investigations of Yuzuncu Yil University. The animals were given standard rat pellets (Murat Animal Food Product Co., Ankara, Turkey) and tap water *ad libitum*. The rats were housed singly in temperature-controlled (20–25°C) cages. They were housed in macrolon cages under standard laboratory conditions (light period 7.00 AM to 7.00 PM, 21±2°C, relative humidity = 55%). The rats were randomly divided in 3 experimental groups: A (conrol), B (Cd treated), and C (Cd + NS treated), each containing 10 animals. The Cd-treated and Cd + NS-treated groups were injected subcutaneously daily with $CdCl_2$ dissolved in isotonic NaCl in the amount of 2 mL/kg for 30 d, resulting in a dosage of 0.49 mg Cd/kg/d *(23).* The conrol group was injected with only isotonic NaCl (2 mL/kg/d) throughout the experiment. (for 30 d). Three days prior to induction of $CdCl_2$, the Cd + NS treated group received a daily intraperitoneal (ip) injection of 0.2 mL/kg NS until the end of the study. The dose of NS volatile oil was chosen on the basic of a previous study *(22).*

At the end of the experiment, blood samples were obtained for biochemical and histopathological investigations. The rats in all groups were starved overnight for 12 h and sacrificed under ketamin hydrocloride (Ketalar®, Eczacibasi Farma; 10 mg/kg ip) anesthesia. After entering the abdominal and thoracic cavities blood from the heart was collected into potassium–EDTA tubes using 10-mL syringes (10.5 mg/7 mL). Blood samples were centrifuged at 3000 rpm for 5 min in a refrigerated centrifuge. Plasma and buffy coat were discarded. Packed erythrocytes were washed twice with normal saline after 1/10 dilutions to remove leftover leukocytes and plasma components. All reagents were of analytical grade. Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), and hexane were purchased from Merck Chemical Co. (Darmstadt, Germany). *n*-Heptane and α-α'dipyridyl were purchased from Sigma (St. Louis, MO, USA). All animals received care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.

Biochemical Analysis

Malondialdehyde (MDA) was determined by the double-heating method of Draper and Hadley *(24).* This method is based on the fact that lipid peroxides and TBA react to form a pink pigment with absorption maximum at 532 nm. For this purpose, 2.5 mL of 100 g/L TCA solution was added to 0.5 mL hemolysate/plasma in each centrifuge tube and placed in

a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000*g* for 10 min, and 2 mL of the supernatant was added to 1 mL of 0.67% (w/v) TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 (Japan) spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex, 1.56×10^5 cm^{-1} M^{-1}, and are expressed in micromoles per gram of hemoglobin (Hb) and in plasma as micromoles per liter. All samples were assayed in duplicate.

Blood for the determination of antioxidant status was centrifuged to separate plasma and RBCs. Isolated RBCs were washed three times with 3 vol of ice-cold 155 mmol/L NaCl, and hemolysates containing about 50 g Hb/L prepared according to McCord and Fridovich *(25)* were used for the determination of catalase (CAT) and glutathione peroxidase (GSH-Px) activities. CAT activity was determined according to Beutler *(26)*, whereas the activity of GSH-Px was assayed by the subsequent oxidation of NADPH at 340 nm with *t*-butyl-hydroperoxide as a substrate *(27)*. For determination of superoxide dismutase (SOD) by the method of Misra and Fridovich *(28)*, lysates were diluted with distilled water (1 : 7 [v/v]) and treated by chloroform–ethanol (0.6 : 1 [v/v]) in order to remove hemoglobin. The clinical chemical analysis in plasma was performed on a Cobas Integra 800 automatic analyzer from Roche Diagnostic (USA). Analysis in blood was carried out at 37°C with potassium–EDTA. Analysis of iron was done according to the instructions of the manufacturer of the automatic analyzer. Hb measurement was made by mixing blood with Drabkin solution, which contains potassium ferricyanide and potassium cyanide.

Morphological Evaluation

Blood samples were taken using sterile potassium–EDTA tubes. Immediately after mixing with blood by inverting the tubes, blood smears were made by spreading one drop on a slide and then staining with the Giemsa solution. RBC morphology was determined using a Nikon Optiphot-2 microscope (Tokyo, Japan) with an oil immersion 100/1.25 objective.

Image Analysis

The system used consisted of a PC with hardware and software (Image-Pro Plus 5.0, Media Cybernetics, USA) for image acquisition and analysis, a Spot Insight QE (Diagnostic Instruments, USA) camera, and an optical microscope. The method requires preliminary software procedures of spatial calibration (micrometer scale) and setting of color segmentation for quantitative color analysis. The image analysis program was used for morphologic evaluation of the samples. Twenty areas from each group were chosen randomly. The number of intact and hemolyzed erythrocytes were measured. The investigator who performed these measurements was unaware of the experiment.

Table 1
Comparison of Plasma MDA and Erythrocyte MDA, SOD, GSH-Px, and CAT of Groups A, B, and C

Parameters	A	B	C	P
Erythrocyte MDA (μmol/g Hb)	8.82±1.06 [a]	13.72±1.93 [b]	11.31±1.12 [c]	P<0.05
Erythrocyte **SOD (U/g Hb)x10^3**	5.08±0.61 [a]	12. 24±1.32 [b]	8.06±1.15 [c]	P<0.05
Erythrocyte **GSH-Px (nmol NADPH/min/**g Hb) **x10^3**	60.58±1.93 [a]	80.24±2.33 [b]	71.30±2.04 [c]	P<0.05
Erythrocyte **CAT** (μmol H_2O_2/ min/g Hb) **x10^3**	72.34±1.23 [a]	94.02±3.12 [b]	83.27±2.72 [c]	P<0.05
Plasma MDA (nmol/ml)	2.96±0.16 [d]	6.49±0.66 [e]	4.79±0.56 [f]	P<0.01

Note: Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and $n = 10$ for all groups.
[a,b,c] Means in the same row with different superscripts significantly differ ($p<0.05$).
[d,e,f] Means in the same row with different superscripts significantly differ ($p<0.01$).

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) and analyzed using repeated measures of variance. The Tukey test was used to test for differences among means when the analysis of variance (ANOVA) indicated a significant ($p<0.05$) *F*-ratio.

RESULTS

Plasma MDA and erythrocyte MDA, SOD, GSH-Px, and CAT are presented in Table 1. As shown in Table 1, Cd treatment increased significantly the MDA levels in plasma and erythrocytes ($p<0.01$ and $p<0.05$, respectively) and also increased significantly the antioxidant levels (SOD, GSH-Px, and CAT) ($p<0.05$) compared to the control group. Cd + NS treatment decreased significantly the elevated MDA levels in plasma and erythrocytes ($p<0.01$ and $p<0.05$, respectively) and reduced significantly the enhanced antioxidant levels ($p<0.05$).

As shown in Table 2, Cd treatment increased significantly the activity of iron levels ($p<0.05$) in the plasma compared to the control group. Cd + NS treatment decreased the activity of iron levels ($p<0.05$) in the plasma compared to the Cd- treated group. It was found that the RBC counts decreased ($p<0.05$) in Cd-treated rats. NS treatment increased ($p<0.05$) the lowered RBC counts (Table 2). It was also found that the hemolyzed RBC

Table 2
Values of RBCs and Iron Concentration of Plasma in the Blood Samples of Groups A, B, and C

	A	B	C	P
RBC (x $10^6/\mu l$)	5.7 ± 0.6^a	3.1 ± 0.4^b	4.9 ± 0.5^a	$P<0.05$
Iron ($\mu g/dl$)	62.7 ± 5.7^a	88.3 ± 7.5^b	70.1 ± 6.2^a	$P<0.05$

Note: Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and $n = 10$ for all groups.
^{a,b} Means in the same row with different superscripts significantly differ ($p<0.05$).

Table 3
Image Analysis of Groups A, B, and C

Groups	Intact RBC (%)	Hemolysed RBC (%)	P
A	97 ± 1.13^a	3 ± 0.16^d	$P<0.001$
B	57 ± 2.29^b	33 ± 1.07^e	$P<0.001$
C	83 ± 3.17^c	17 ± 0.99^f	$P<0.001$

Note: Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and $n = 10$ for all groups.
$^{a–f}$ Means in the same column with different superscripts significantly differ ($p<0.001$).

counts increased ($p<0.05$) in Cd-treated rats. NS treatment reduced ($p<0.05$) hemolyzed RBC counts number (Table 3).

In the control group with no treatment, histology of erythrocytes was normal (*see* Fig. 1). In the Cd-treated group, there were remarkable membrane destruction and hemolytic changes in erythrocytes (*see* Fig. 2). In the Cd + NS-treated group, these changes were less than in the Cd-treated group (*see* Fig. 3).

DISCUSSION

Previous investigations showed that chronic treatment with cadmium induced oxidative damage in erythrocytes of rats and goldfish, causing destruction of cell membranes and increased lipid peroxidation, as well as alteration of the antioxidant defense system, energy metabolism, and the appearance of anemia *(12,29–33).* The data of other authors showed that Cd caused damage of the erythrocyte membrane resulting in hemolysis.

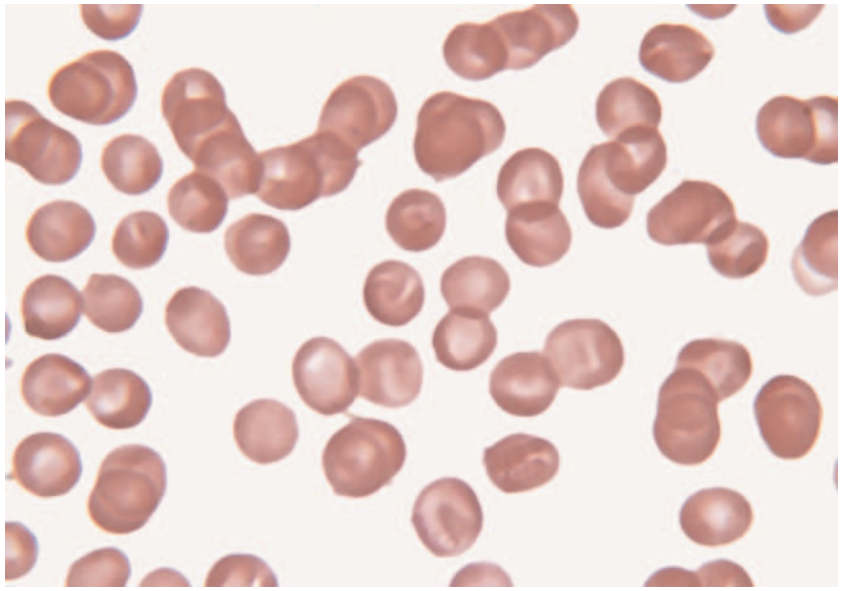

Fig. 1. The morphology of normal RBCs in control rats (Giemsa; ×1150).

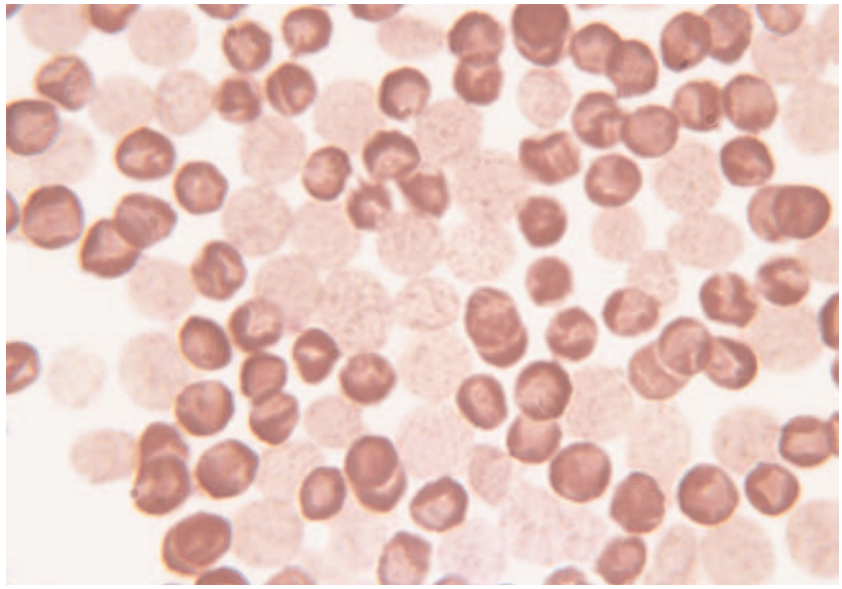

Fig. 2. In the Cd-treated group, remarkable membrane destruction and hemolytic changes are seen in the erythrocytes (Giemsa; ×1150).

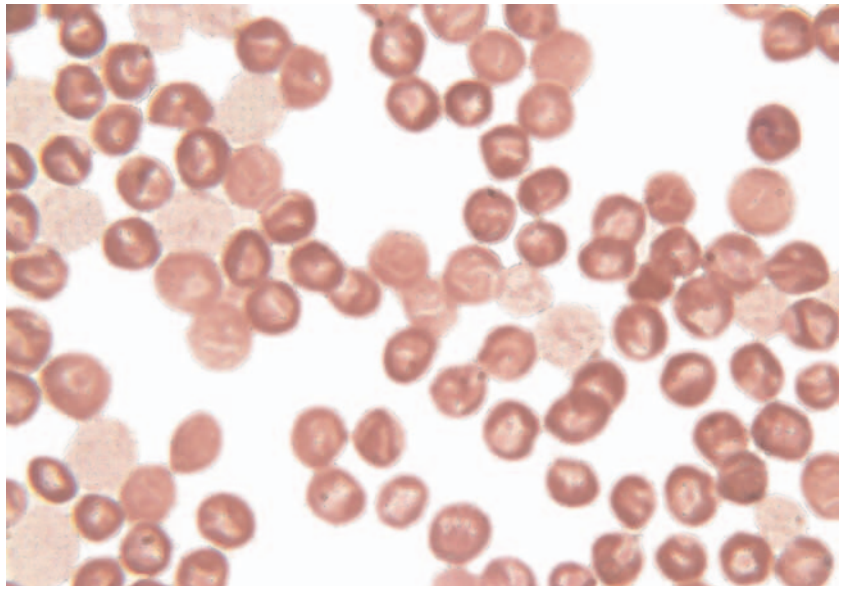

Fig. 3. Reduced hemolysis of RBCs in Cd + NS-treated rats are seen (Giemsa; ×1150).

Some antioxidants can exert a protective role against Cd-induced destruction of RBCs *(34).*

Treatment with cadmium increased the lipid peroxide concentration in the blood of rats, which was accompanied by increased formation of ROS *(35,36).* As a consequence, enhanced lipid sulfhydryl homeostasis as well as marked disturbances of the antioxidant defense system occurred *(37–39).* Pretreatment with vitamin E exhibited a protective role on the toxic effects of cadmium on the hematological values, lipid peroxide concentration, as well as on enzymatic and nonenzymatic components of the antioxidant defense system *(33).* In our study, Cd treatment increased significantly the MDA levels in plasma and erythrocytes compared to the control group. Cd + NS treatment, decreased significantly the elevated MDA levels in plasma and erythrocyte.

Activities of SOD, CAT, and GSH-Px were significantly increased in the RBCs of Cd-treated rats. It is known that Cd induces the formation of superoxide anion radicals in erythrocytes and it is reasonable to expect an increased activity of SOD *(12,32,33,38).* Cadmium induced an increase in CAT and GSH-Px activities, which might be explained by their influence on hydrogen peroxide as a substrate formed in the process of dismutation of superoxide anion radicals *(34).* The pretreatment with vitamin E prior to Cd administration decreased erythrocyte SOD, CAT, and GSH-Px activities, indicating that vitamin E eliminates the toxic effects of Cd on the activity of these enzymes *(33).* In the present study, Cd treatment increased significantly the antioxidant levels (SOD, GSH-Px, and CAT) in plasma

and erythrocytes compared to the control group. Cd + NS treatment decreased significantly the elevated antioxidant levels in plasma and erythrocytes. In contrast, Sarkar et al. *(35)* indicated that the erythrocyte SOD and CAT activities decreased significantly with Cd, and the pretreatment with vitamin E and/or Se prior to Cd administration partially reversed these changes. In addition, Beytut et al. *(40)* observed that the Cd treatment decreased the GSH-Px activity of RBCs.

The iron concentration of plasma is elevated in hemolysis because the iron in hemoglobin. Similar to these results, our present data show that Cd causes a significant increase of iron levels. However, cotreatment of rats with Cd and NS combined caused a significant decrease in the levels of iron. However, Kostić et al. *(12)* indicated that the chronic exposure of Cd decreases the level of iron in the blood and causes the decrease of the RBC count, hemoglobin concentration, and hematocrit value. The decrease of hematocrit value in hemolyzed plasma of rats exposed to Cd indicates the increased destruction of erythrocytes *(41,42).* It was found in this work that the RBC counts decreased in Cd-treated rats. NS treatment increased the lowered RBC counts. It was also found that the hemolyzed RBC counts increased in Cd-treated rats. NS treatment reduced hemolyzed RBC counts number.

It can be concluded from the presented results that cadmium-induced oxidative damage in erythrocytes leads to anemia, loss of membrane function by enhancing of lipid peroxide concentration, as well as alteration of the activity of antioxidant defense system enzymes. Our results show that NS expressed a protective role against toxic influence of cadmium on all of the examined parameters in rat blood.

REFERENCES

1. A. F. Morselt, Environmental pollutant and diseases, *Toxicology* **70,** 1–132 (1991).
2. WHO, *Environmental Health Criteria 134, Cadmium,* World Health Organization, Geneva (1992).
3. US Departmant of Health Services, Toxicological profile for cadmium, Draft for public comment, Agency for Toxic Substances and Disease Registry, Atlanta, GA (1997).
4. M. A. Amoruso, G. Witz, and B. D. Goldstein, Enhancement of rat and human phagocyte superoxide anion radical production by cadmium in vitro, *Toxicol. Lett.* **10,** 133–138 (1982).
5. Z. Zhong, W. Troll, K. L. Koenig, and K. Frenkel. Carcinogenic sulfide salts of nickel and cadmium induce H_2O_2 formation by human polymorphonuclear leukocytes, *Cancer Res.* **20,** 7564–7570 (1990).
6. T. Ochi, F. Otkusa, K. Takahashi, and M. Oshawa, Glutathione and metallothioneins as cellular defense against cadmium toxicity in culture chinese hamster cells, *Chem. Biol. Interact.* **65,** 1–14 (1988).
7. S. J. Stohs and D. Bagchi, Oxidative mechanism in the toxicity of metal ions, *Free Radical Biol. Med.* **18,** 321–336 (1995).
8. M. Sugiyama. Role of cellular antioxidants in metal-induced damage, *Cell. Biol. Toxicol.* **10,** 1–22 (1994).
9. J. W. Bauman, J. Liu, and C. D. Klaassen, Production of meallothionein and heat-shock proteins in response to metals, *Fundam. Appl. Toxicol.* **21,** 15–22 (1993).

10. C. Simpkins, T. Lloyd, S. Li, and S. Balderman, Metallothionein-induced increase in mitochondrial inner membrane permeability, *J. Surg. Res.* **75,** 30–34 (1998).
11. S. Sarkar, P. Yadav, R. Trivedi, A. K. Bansal, and D. Bhatnagar, Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues, *J. Trace Elements Med. Biol.* **9,** 144–149 (1995).
12. M. M. Kostić, B. Ognjanović, R. V. Zikić, et al., Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats: in vivo effects, *Eur. J. Haematol.* **51,** 86–92 (1993).
13. R. V. Zikić, A. Stajn, B. Ognjanović, et al., The effect of cadmium and selenium on the antioxidant enzyme activities in rat heart, *J. Environ. Pathol. Toxicol. Oncol.* **17,** 259–264 (1998).
14. M. M. Kostić, B. Ognjanović, R. V. Zikić, et al., Effects of Cadmium on antioxidant enzymes, glutathione and lipid peroxidation in brown adipose tissue, *Iugoslov Physiol. Pharmacol. Acta* **29,** 137–145 (1993).
15. M. W. Fariss, Cadmium toxicity: unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes, *Toxicology* **69,** 63–77 (1991).
16. K. E. H. El-Tahir, M. M. S. Ashour, and M. M. Al-Harbi, The respiratory effects of the volatile oil of the black seed *(Nigella sativa)* in guinea pigs: elucidation of the mechanism(s) of action. *Gen. Pharmacol.* **24,** 1115–1122 (1993).
17. A. El-Kadi and O. Kandil, The black seed *(Nigella sativa)* and immunity: its effect on human T cell subset, *Fed. Proc.* **46,** 1222 (1987).
18. M. S. M. Hanafy and M. E. Hatem, Studies of antimicrobial activity of *Nigella sativa* seed (black cumin). *J. Entopharmacol.* **34,** 275–278 (1991).
19. A. Zaoui, Y. Cherrah, M. A. Lacaille-Dubois, et al., Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat, *Therapeutics* **55,** 379–382 (2000).
20. M. K. Turkdogan, Z. Agaoglu, Z. Yener, et al., The role of antioxidant vitamins (C and E), selenium and *Nigella sativa* in the prevention of liver fibrosis and cirrhosis in rabbits: new hopes, *Dtsch. Tierarztl. Wochenschr.* **108,** 71–73 (2001).
21. M. Kanter, I. Meral, Z. Yener, et al., Partial regeneration/proliferation of the beta-cells in the islets of langerhans by *Nigella sativa* L. in streptozotocin-induced diabetic rats, *Tohoku J. Exp. Med.* **201,** 213–219 (2003).
22. M. Kanter, O. Coskun, A. Korkmaz, and S. Oter, Effects of *Nigella sativa* on oxidative stress and β-cell damage in streptozotocin-induced diabetic rats, *Anat. Rec.* **279,** 685–691 (2004).
23. M. Kanter, M. Yoruk, A. Koç, et al., Effect of cadmium exposure on morphological aspects of pancreas, weights of fetus and placenta in streptozotocin-induced diabetic pregrant rats, *Biol. Trace Element Res.* **93,** 189–200 (2003).
24. H. H. Draper and M. Hadley, Malondialdehyde determination as index of lipid peroxidation, *Methods Enzymol.* **186,** 421–431 (1990).
25. J. M. McCord and I. Fridovich, Superoxide dismutase. An enzymatic function for erythrocupreine (hemocupreine), *J. Biol. Chem.* **244,** 6049–6056 (1969).
26. E. Beutler, Catalase, in *Red Cell Metabolism: A Manual of Bichemical Methods,* E. Beutler, ed., Grune and Stratton, New York, pp. 105–106 (1982).
27. J. Maral, K. Puget, and A. M. Michelson, Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals, *Biochem. Biophys. Res. Commun.* **77,** 1525–1535 (1977).
28. H. P. Misra and I. Fridovich, The role of superoxide anion in autoxidation of epinephrine and simple assay for superoxide dismutase, *J. Biol. Chem.* **247,** 3170–3175 (1972).
29. B. I. Ognjanović, S. Z. Pavlovic, R. V. Zikić, et al., The effect of olive oil on the plasma transaminase activities and blood hematological values of rats exposed to cadmium, *Kragujevac J. Sci.* **22,** 93–99 (2000).

30. R. V. Zikić, A. S. Stajn, B. I. Ognjanović, et al., Activities of superoxide dismutase and catalase in erythrocytes and transaminases in the plasma of carps (*Cyprinus carpio* L.) exposed to cadmium, *Physiol Res.* **46,** 391–396 (1997).
31. S. Z. Pavlovic, B. I. Ognjanović, A. S. Stajn, et al., The effect of coenzyme Q10 on blood ascorbic acid, vitamin e, an lipid peroxide in chronic cadmium intoxication, *J. Environ. Pathol. Toxicol. Oncol.* **20,** 133–140 (2001).
32. R. V. Zikić, A. S. Stajn, S. Z. Pavlovic, et al., Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. *Physiol. Res.* **50,** 105–111 (2001).
33. B. I. Ognjanović, S. Z. Pavlovic, S. D. Maletic, et al., Protective influence of vitamin e on antioxidant defense system in the blood rats terated with cadmium, *Physiol. Res.* **52,** 563–570 (2003).
34. Z. A. Shaikh, T. T. Vu, and K. Zaman, Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants, *Toxicol. Appl. Pharmacol.* **154,** 256–263 (1999).
35. S. Sarkar, P. Yadav, and D. Bhatnagar, Lipid peroxidative damage on cadmium exposure and alteration in antioxidant system in rat erythrocytes: a study with relation to time, *J. Trace Elements Med. Biol.* **11,** 8–13 (1997).
36. H. Shi, N. Noguchi, and E. Niki, Comparative study on dynamics of antioxidative action of α-tocopheryl hydroquinone, ubiquinol, and α-tocopherol against lipid peroxidation, *Free Radical Biol. Med.* **27,** 334–346. (1999).
37. A. Skoczynska and R. Smolik, The effect of combined exposure to lead and cadmium on serum lipids and lipid peroxides levels in rat, *Int. J. Occup. Med. Environ. Health* **7,** 263–271 (1994).
38. S. Sarkar, P. Yadav, and D. Bhatnager, Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time, *Biometals* **11,** 153–157 (1998).
39. S. J. Stohs, D. Bagchi, E. Hassoun, et al., Oxidative mechanisms in the toxicity of chromium and cadmium ions, *J. Environ. Pathol. Toxicol. Oncol.* **19,** 201–213 (2000).
40. E. Beytut, A. Yuce, N. N. Kamiloglu, et al., Role of ditary vitamin E in cadmium-induced oxidative damage in rabbit's blood, liver and kidneys, *Int. J. Vitam. Nutr. Res.* **73,** 351–355 (2003).
41. A. Shukla, G. S. Shukla, and R. C. Srimal, Cadmium-induced alterations in blood–brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat, *Hum. Exp. Toxicol.* **15,** 400–405 (1996).
42. T. Hamada A. Tanimato, N. Arima, et al., Pathological study of splenomegaly associated with cadmium-induced anemia in rats, *Sangyo Ika Daigaku Zasshi* **30,** 2343–2349 (2000).