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Protective Effect of *Nigella Sativa* on Lipid Peroxidation and Antioxidant Enzymes System in Cadmium-Treated Rats

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The effect of cadmium chloride on Nigella sativa and their combination were investigated on lipid peroxidation and antioxidant enzymes system in male Sprague-Dowely rats. Oral administration of CdCl2 resulted in a significant increase of lipid peroxidation concentrations measured by thiobarbituric acid reacting substance assay, significant decreased of reduced glutathion levels in liver and kidney. Also, the activities of glutathione reductase, glutathione transferases, glutathione peroxidase and superoxide dismutase in liver were significantly decreased by 92%, 45 %, 48% and 69.6%, respectively while that of kidney were decreased by 80 % , 31.7%, 39.5% and 48%, respectively in cadmium group compared to control group. Treatment of animals with Nigella sativa prior to oral administration of cadmium resulted in a significant increased in the activities of all enzymes in both liver and kidney to be nearly close to that of control animals. Glutathione reductase was partially purified from liver carried using 75% saturation ammonium Sulphate, CM-cellulose and Sephadex-G100 chromatography. On testing the inhibitory effect of $CdCl_2$ on pure glutathione reductase, it was found that the $CdCl_2$ inhibited the enzyme non-competitively where the apparent V_{max} was decreased greatly to 183.5 and 20.0 units on using different concentrations glutathione disulfide and NADPH respectively.

Keywords: *Cadmium chloride, Nigella sativa, lipid peroxidation, antioxidant enzymes system.*

1. INTRODUCTION

Cadmium (Cd) is a naturally occurring metallic element widely present in environmental sources and industrial wastes. It is absorbed in human body from the grown plants, especially grain, leafy vegetables [1, 2] because it is readily taken up by the cells of different plant species [3, 4]. Cd is also present in cigarette fumes, fumes from vehicles [5] and contamination of drinking or well water due to leaching of industrial wastes. It has no essential biological function and is extremely toxic to humans [6]. When Cd is absorbed from the alimentary tract, it forms durable combinations with the protein thionein, forming metallothioneins which play an important role in further metabolism of this metal. Kidneys (mainly renal cortex) and liver are considered to be the most susceptible organs in the case of exposure to cadmium [7, 8].

Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of membrane polyunsaturated fatty acids, a phenomenon termed lipid peroxidation (LPO), in numerous tissues both in vitro and in vivo [9, 10] and initiating various pathological conditions in humans and animals [11]. The Cd-induced increase in LPO is associated with reductions in glutathione peroxidase [10] and catalase activities [12] and with reduced glutathione levels [13]. Fariss [14] has shown that free radical scavengers and antioxidants are useful in protecting against Cd toxicity.

A larger number of plants such as *Nigella sativa* (NS) and their purified constituents have been shown to exhibit potentially beneficial therapeutic effects due to their antioxidant and free radical scavenging characters. NS, a member of the family of ranunculaceae, contains more than 30% of fixed oil and 0.4-0.45 % wt/wt of volatile oil. The volatile oil contains 18.4-24% thymoquinone (TQ) and 46% many monoterpenes such as *p*-cymene and á-piene [15]. It has also been shown that compounds isolated from NS (including thymoquinone, carvacol, tanethole and 4-terpineol) have appreciable free radical scavenging properties [16].

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In addition, the seeds of NS have long been used in the Middle East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleretic, antispasmodic and uricosuric [17-19].

The aim of our study was to investigate a possible protective influence of NS pretreatment on antioxidant defense system and lipid peroxide (LP) concentration on rats treated with cadmium.

2. MATERIALS AND METHODS

40 Male Sprague-Dowely rats 200 ± 25 g were used. Rats were housed in cages with 10 animals in each cage at random and kept on a 12:12h day-night cycle with free access to standard food and water.

Experimental design: Animals were grouped into the following 4 groups:

- **Control group:** Rats were fed on basal rat diet and normal water.
- **$CdCl_2$ group:** Rats were fed on basal rat diet and water contains $CdCl_2$ at concentration of 2 mg of cadmium chloride/kg body weight/ day.
- **Nigella sativa group:** Rats were fed on basal rat diet containing 15 g of fresh finally crushed NS seeds per kg basal diet and normal water.
- **Nigella sativa + $CdCl_2$ group:** Rats were fed on basal rat diet containing 15 g of fresh finely crushed NS seeds per kg diet and water contains CdCl2 at concentration of 2 mg of cadmium chloride/kg body weight/ day.

At the end of the experimental period (4 weeks), the animals were sacrificed and their livers and kidneys were immediately excised the tissues were minced and homogenized separately in 0.1 M Tris-HCl buffer (pH 7.4) using Teflon homogenizer. The homogenate was centrifuged at 10.000 rpm for 30 min at 4 °C and the supernatant was used for antioxidant enzyme assays.

Standardized procedures were followed for various estimations. Lipid peroxidation in tissues was measured by the thiobarbituric acid reacting substance (TBARS) method of Placer *et al* [20] and was expressed in terms of the malondialdehyde (MDA) content, which served as the standard of 1,1,3,3-tetraethoxypropane. Samples assayed for MDA contained 1.0 mM butylated hydroxytoluene (BHT) in order to prevent artefactual LPO during the boiling step. Values were expressed as MDA equivalents in nmol/g tissue. Reduced glutathione level (GSH) was estimated spectrophotometrically by Sedlak and Lindsay's method [21], using Ellman's reagent [5,5-dithio-bis(2-nitrobenzoic acid); DTNB]. Method is based upon the reduction of Ellman's reagent by sulfhydryl groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. Aliquots of homogenates were mixed with 4 ml distilled water and 1 ml of 50% TCA. Solutions were centrifuged for 15 min at 3000 rpm. Then 0.4 M tris buffer (pH 8.9) and DTNB were added to the supernatant and then shaken. The absorbance was measured at 412 nm. GSH levels are expressed as µmol/g wet weight.

Glutathione transferase (GST) was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with glutathione (GSH) at 340nm at 37oC [22]. The assay mixture contained 0.5mM CDNB, 1mM GSH and 100mM phosphate buffer, pH 7. The CDNB was dissolved in ethanol and added to the phosphate buffer before use. The ethanol concentration in the assay mixture was 2%. The phosphate buffer-CDNB mixture was preincubated for 10mins at 37 °C and the reaction was started by adding GSH followed immediately by an aliquot of the supernantant. The rate of increase in absorbance at 340nm was measured for 10mins at 37 °C against a blank containing the reaction mixture without supernatant. Glutathione peroxidase (GPX) activity was measured according to Chiu *et al* [23], with a modified reaction mixture. The reaction mixture contained 0.1 M phosphate buffer (pH 7.0), 1.5 mM EDTA, 1.5 mM sodium azide, 1 mM GSH, 0.25 mM NADPH, 0.68 units of glutathione reductase (GR), 1 mM cumene hydroperoxide, and an appropriate amount of enzyme. The enzyme activity was measured by absorbance at 340 nm and was defined as nmol NADPH oxidized per min per mg protein.

Activity of superoxide dismutase (SOD) was determined by monitoring the decrease in absorbance due to NADH oxidation at 340 nm using the method of Paoletti *et al*. [24]. Enzyme activity is expressed as nmole NADH oxidized/ min/mg protein.

The activity of GR was determined spectrophotometrically [25]. The decrease of absorbance due to the oxidation of NADPH was recorded at 340 nm. The standard reaction mixture contained 100 mM phosphate buffer, 1 mM EDTA, 1 mM GSSG, and 0.2 mM NADPH (pH 7.0). The activity that catalyzes the consumption of 1 mM of substrate per minute was defined as 1 U. For determining the Km values of GR for NADPH and GSSG, the second cosubstrate was used in a concentration of 1 mM glutathione disulfide (GSSG) and 0.2 mM (NADPH), respectively. GR was purified according to the method of Gutterer *et al* (25) with some modifications. The purification procedure carried out at 4 °C. The liver was homogenized in a Waring Blendor with 20 mM phosphate buffer and 2 mM EDTA (pH 7.4) (buffer A) using 3 ml of buffer per gram of wet tissue, followed by treatment in a homogenizer. After centrifugation (10000 rpm, 30 min), the pellet was submitted to a further cycle of homogenization and centrifugation. Under vigorous stirring, powdered ammonium sulfate was added to the combined supernatants until 35% saturation was reached. The resulting suspension was stirred for an additional 60 min and centrifuged as described above. The supernatant was brought to 75% saturation with ammonium sulfate. Again, the suspension was stirred for additional 45 min and centrifuged as above. The pellet was dissolved in 50 mM phosphate buffer and 2 mM EDTA (pH 7.4) (buffer B) and dialyzed twice against 4 L of buffer B overnight. The dialyzate was applied to CM-cellulose column equilibrated with buffer B. The enzyme was eluted by application of a linear gradient of buffer B containing 0.5 M NaCl. The effluent containing GR was pooled, concentrated and applied to Sephadex-G100 gel filtration column equilibrated in buffer B supplemented with GSH (2 mM) (buffer C). The affinity column was then eluted with a linear gradient (0–0.5 mM) of NADPH in buffer C. The fractions containing GR activity were pooled, concentrated and saved for kinetic study. The protein was determined by the method of Lowry *et al* [26] using bovine serum albumin as standard. During purification the protein was estimated by measuring its absorbance at 280 nm.

Statistical Analysis: The results were expressed as mean valves ± SD. The significances between mean values were determined according to student t test.

3. RESULTS AND DISCUSSION

3.1. Oxygen radicals and other activated oxygen species are common products of cellular metabolism. Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics [27].

The results obtained in this study indicated that oral administration of cadmium resulted in a significant increases of lipid peroxidation concentrations measured by TBARS assay (MDA) in liver and kidney (32.1±2.3 and 24.5±1.29 nmol/g dry wt, respectively) in $CdCl_2$ group compared to control group (9.97±1.2 and 8.90±0.896 nmol/g dry wt, respectively) as shown in Table 1. On the other hand, NS was very effective in the prevention of oxidative damage which resulted in significantly lower MDA level in liver and kidney (4.1±0.2 and 3.77 ±0.17 nmol/g dry wt, respectively). Beytut & Aksakal [28] reported that increased MDA level by 40% and 17% in the liver and kidneys respectively than in control animal when rabbits treated with Cd for 28 weeks. Also Ognjanoviæ *et al* [29], Tandon *et al,* [30] and El-Demerdash *et al*[31] showed that chronic intoxication with cadmium causes lipid peroxidation in numerous tissues both in vivo and in vitro.

Table 1
Levels of MDA and GSH in Control and Treated Groups Values are Expressed as mean ± SD. Groups were Compared as Follows: Group 2 Vs Group 1; Group 3 Vs Group 1; Group 4 Vs Group 2; * P<0.001, **P<0.005, *#P<0.01, ## P<0.05

Group	*MDA (nmol/g dry wt)*		*GSH µmol/g*	
	Liver	*Kidney*	*Liver*	*Kidney*
Control	9.97±1.24	8.90±0.896	46±3.4	36.4±2.22
CdCl2	32.1±2.34*	24.5±1.29*	30.2±4.99**	24.2±3.77**
Nigella Sativa	4.1±0.2*	3.77±0.17*	53±3.65##	43.5±3.11*#
Nigella Sativa + CdCl2	16.9±1.79*	12.5±1.27*	38.8±2.99##	29.5±1.29##

The results presented in Table 1 showed that the concentrations of GSH in liver (30.2±4.99 µg/g protein) and kidney (24.2±3.77 µg/g protein) homogenates were significantly decreased in cadmium treated rats in comparison with the

control animals (46±3.4 and 36.4±2.22 µg/g protein respectively). Pretreatment of rats with NS prior to Cd administration increased the concentrations of GSH in both liver (38.8±2.99 µg/g protein) and kidney (29.5±1.29 µg/g protein) compared to GSH concentrations in livers and kidneys of Cd treated group (30.2±4.99 and 24.2±3.77 µg/g protein, respectively). Stohs *et al.* [32] indicated that Cd depletes GSH and protein-bound sulfhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide. Similarly Ognjanovic *et al.,* [29] and Tandon *et al.*, [30] have shown that chronic exposure to Cd decreases the level of GSH in the liver and kidneys of rats.

The antioxidant enzymes and other antioxidants provide the cells with a protection against oxidative damage and protect cells from Cd-induced toxicity [30,33]. The role of antioxidants in reversing the oxidative stress has been of long-standing interest to basic scientists and clinicians [34].

The data presented in Table 2 showed a significant change in the activity of antioxidant enzymes when rats were treated with Cd, NS and their combination. In the rats treated with Cd alone, the activities of GR, GST, GPX and SOD in liver were significantly decreased by 92%, 45 %, 48% and 69.6%, respectively while that of kidney were decreased by 80% , 31.7%, 39.5% and 48% respectively compared to control group. Feeding animals with NS alone resulted in a significant increase in the activities of all antioxidant enzymes in the liver and kidney compared to control animals. In addition, feeding animals with NS prior oral administration of Cd resulted in a significant increase in the activities of GR, GST, GPX and SOD in both liver and kidney to be nearly close to that of control animals.

These results agree with that of Congiu *et al.* [35], who reported a decrease in the activity of GPX in the livers and kidneys of Cd treated rats. The decreased activity of GPX can be explained by competition of cadmium metallothioneins and GPX for sulfur containing aminoacids [36]. Studies of other authors have shown that Cd inhibits the activity of majority of antioxidant enzymes [37, 38] inducing an increased production of free radicals, lipid peroxidation and destruction of cell membranes [29].

Houghton *et al.* [39] and Burits & Bucar [40] have been shown that both the fixed oil of NS as well as compounds isolated from NS (including thymoquinone, carvacol, tanethole and 4-terpineol), have appreciable free radical scavenging properties and inhibit nonenzymatic lipid peroxidation in liposomes. Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of NS may explain its claimed usefulness in folk medicine.

3.2. Glutathione Reductase Purification: From the previous results we noticed that GR is the most enzyme inhibited in Cd treated rats. So, the study was continued for partial purification of GR from liver of control animal and studying its kinetic and the type of Cd inhibition (Table 3).

The Partial purification of liver GR was carried out using 75% saturation ammonium sulfate (NH_4 $(SO_4)_2$)and most of the enzyme activity was preserved in the precipitate. The specific activity was increased to 64. 53 U/mg protein while the total protein was decreased from 383.75 to 46.8 mg (Table 3). The dialyzed ammonium sulfate fraction was applied to CM-cellulose column (Fig. 1). It was found that ammonium sulfate fraction contained different protein molecules but it showed only one peak for enzyme activity. Table 3 revealed that GR was enriched after the CM-cellulose and the specific activity decreased to be 145.37 U/mg protein with a purification factor of 14.03 and 64% yield. The affinity of the enzyme to CM-cellulose column was low since it eluted with lower concentration of NaCl gradient.

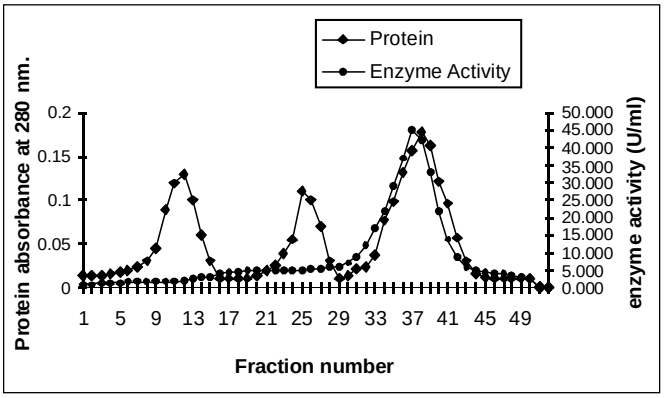

Figure 1: CM- Cellulose cation exchange chromatography of rat liver glutathione reductase. The separation occurred in buffer B (pH 7.4). Elution was started from fraction Number 1 till number 50 using linear gradient of buffer B containing 0.5 M NaCL

Table 2
Antioxidant Enzymes Activity in Liver and Kidney in Control and Treated Groups Values are Expressed as Mean ± SD. Groups were Compared as Follows: Group 2 Vs Group 1; Group 3 Vs Group 1; Group 4 Vs Group 2; * P<0.001, **P<0.005, ***P<0.01, *# P<0.05

Group	*GR (U/mg)*		*GST(U/mg)*		*GPX (U/mg)*		*SOD (U/mg)*	
	Liver	*Kidney*	*Liver*	*Kidney*	*Liver*	*Kidney*	*Liver*	*Kidney*
Control	10.9±0.82	8.95±0.77	65.8±3.77	43.2±5.12	15±1.41	9.5±1.29	9.4±1.05	6.1±0.52
CdCl2	1.35±0.3*	1.85±0.25*	36±3.37*	29.5±1.29**	7.75±0.96*	5.75±0.96##	2.85±0.68*	3.17±0.92**
Nigella Sativa	15.8±1.63**	12.1±0.87**	80.2±3.4**	58.2±4.99*#	19±1.41*#	12±1.83##	12.8±0.89**	7.38±0.41*#
Nigella Sativa + CdCl2	7.85±0.99*	3.55±0.44*	49.2±5.5*#	36.8±4.79##	10.8±0.96**	7.5±1*#	5.28±0.65**	4.62±0.46##

Table 3
Purification Scheme of Rat Liver Glutathione Reductase

Purification step	*Volume*	*Activity (U/ml)*	*Protein (mg/ml)*	*Total activity*	*Total protein (mg)*	*Specific activity (U/mg)*	*fold*	*Yield %*
Crude	125	31.8	3.07	3975	383.75	10.36	1.00	100
Ammonium Sufate (75%)	40	75.5	1.17	3020	46.8	64.53	6.23	75.97
CM Cellulose	25	101.76	0.70	2544	17.5	145.37	14.03	64
SephadexG-100	19	119.25	0.23	2265.75	4.37	518.48	50.05	57

GR purification was completed by applying the active fraction on Sephadex G-100 gel filtration column chromatography. The elution profile (Fig. 2) showed that this purification step gave only one protein peak overlapped with peak of GR activity. The specific activity was increased to 518.48 U/mg proteins with a purification factor 50.05 (Table 3).

The enzyme kinetic studies indicated that GR activity depends on their substrate concentration. Lineweaver Burk plot of GR was done for both GSSG and NADPH substrates as shown in Figs. 3 & 4. The results revealed that the Km values of GR for GSSG and NADPH were 1.43 mM and 0.48 mM respectively. However V_{max} values were calculated as 277 U/mg protein and 40 U/mg protein respectively.

The K_m values of GR for its substrates NADPH (8 mM) and GSSG (36 mM) are lower than those reported for enzyme purified from sheep brain (NADPH: 60.9 mM; GSSG: 116.2 mM; [41], but in the range (NADPH: 8–60.9 mM; GSSG: 26–116.2 mM) of the Km values reported for GR from several species and tissues [41, 42]. A heterogeneity among these K_m values has been attributed to differences in the solutions used during measurement, as the K_m values of GR for its substrates depend on the ion strength of the buffer used [43].

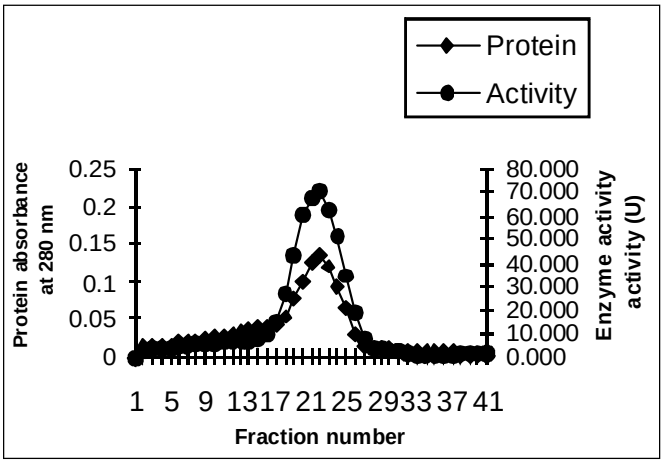

Figure 2: Sephadex G-100 gel filtration column chromatography of rat liver glutathione reductase. Sephadex-G100 gel filtration column equilibrated and washed with buffer C (pH 7.4) and eluted with a linear gradient (0-0.5 mM) of NADPH in buffer C

On testing the inhibitory effect of $CdCl_2$ on pure GR, it was found that the $CdCl_2$ inhibited the enzyme non-competitively where the apparent V_{max} decreased greatly to become 183.48 U/mg protein on using different concentrations of GSSG at constant concentration of NADPH (Fig. 3). Also, the apparent V_{max} was decreased to become 20 U/mg protein on using different concentrations of NADPH at constant concentration of GSSG (Fig. 4). Similar results are obtained by Ulusu *et al* [44] who investigated the in vitro inhibitory effects of Cd on

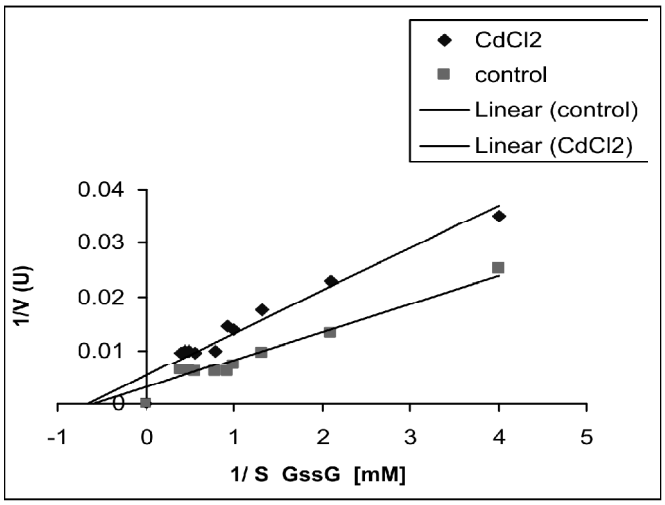

Figure 3: Lineweaver-Burk plot of glutathione reductase in presence and absence of CdCl2

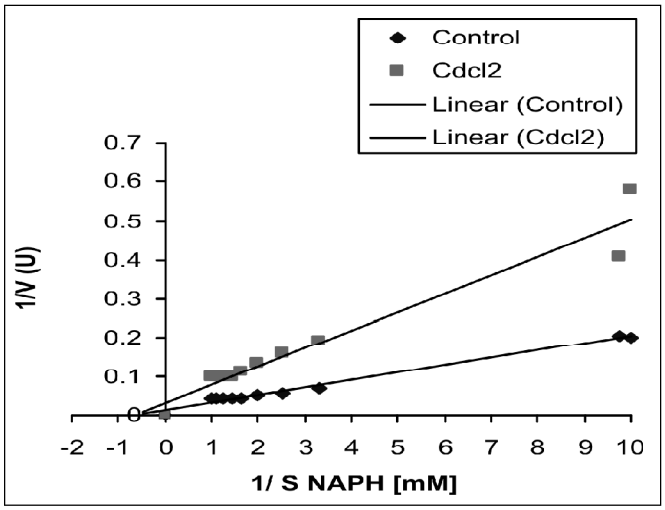

Figure 4: Lineweaver-Burk plot of glutathione reductase in presence and absence of Cd Cl2

GR activity of liver and brain tissues. Lyubenova *et al.* [45] reported that GR that partially purified from *Calystegia sepium* is greatly inhibited by Cd. Also, Mukhopadhyay *et al.* [46] observed platelet GR activity was decreased by almost 50% after incubation with Cd.

CONCLUSION

Our results show that treatment with NS exhibited a protective role on the toxic effects of Cd on the lipid peroxidation as well as on the antioxidant defense system in livers and kidneys of the rats. Also, on testing the inhibitory effect of $CdCl_2$ on pure GR, it was found that the $CdCl_2$ inhibited the enzyme non-competitively.

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References

[1] Hu H. Heavy Metal Poisoning. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL, eds. *Harrison's: Principles of Internal Medicine* 1998; Vol 1. 3rd ed. New York, PA: McGraw-Hill: 2564-73.

[2] Williams F, Robertson R, and Roworth M. Detailed Profile of 25 Major Organic and Inorganic Substances, (SCEIH, Glasgow) 1999; pp. 12-19.

[3] Gomes-Júnior RA, Moldes CA, Delite FS, *et al.* Antioxidant Metabolism of Coffee Cell Suspension Cultures in Response to Cadmium. Chemosphere 2006; 65: 1330-1337.

[4] Liu Y, Wang X, Zeng G, *et al.* Cadmium-Induced Oxidative Stress and Response of the Ascorbate–Glutathione Cycle in Bechmeria nivea (L.) Gaud. Chemosphere 2007; 69: 99-107.

[5] Baldwin DR, and Marshall WJ. Heavy Metal Poisoning and Its Laboratory Investigation. *Ann. Clin. Biochem* 1999; 36 (3): 267-300.

[6] Rodriguez-Gabriel MA, and Russell P. Distinct Signaling Pathways Respond to Arsenite and Reactive Oxygen Species in Schizosaccharomyces pombe. Eukaryot. Cell 2005; 4(8): 1396-402.

[7] Ryan PB, Huet N, and Maclntosh DL. Longitudinal Investigation of Exposure to Arsenic, Cadmium and Lead in Drinking Water. Environ Health Perspect 2000; 108: 731-5.

[8] Yamano T, Kosanke SD, and Rikans LE. Attenuation of Cadmium-Induced Liver Injury in Senescent Male Fischer 344 rats: Role of Metallothionein and Glutathione. *Toxicol Appl Pharmacol* 1999; 161: 225–30.

[9] Muller L. Consequences of Cadmium Toxicity in Rat Hepatocytes: Mitochondrial Dysfunction and Lipid Peroxidation. *Toxicology* 1986; 40: 285.

[10] Jamal IS, and Smith JC. Effects of Cadmium on Glutathione Peroxidase, Superoxide Dismutase and Lipid Peroxidation in the Rat Heart: A Possible Mechanism of Cadmium Cardiotoxicity. Toxicol. *Appl. Pharmacol* 1985; 80: 33-42.

[11] Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and Cellular Mechanisms of Cadmium Carcinogenesis: a review. *Toxicology* 2003; 192: 95-117.

[12] Jolly SR, Kane WJ, Bailie MB, Abrams GD, and Lucchesi BR. Canine Myocardial Reperfusion Injury: Its Reduction by the Combined Administration of Superoxide Dismutase and Catalase. *Circ Res* 1984; 54: 277-284.

[13] Siegers CP, Sharma SC, and Younes M. Hepatotoxicity of Metals in Glutathione-Depleted Mice. *Toxicol Lett.* 1986; 34: 185-191.

[14] Fariss M W. Cadmium Toxicity: Unique Cytoprotective Properties of Alpha Tocopheryl Succinate in Hepatocytes. Toxicology 1991; 69: 63-77.

[15] Aboutabl EA, El-Azzouny AA, and Hammerschmidt FJ. Aroma Volatiles of Nigella sativa L. seeds. In: Brunke, E.J. (Ed.), Progress in Essential Oil Research. Proc. Int. Symposium on Essential Oils. Berlin, DeGuyter 1986; pp 44–55.

[16] Burits M, and Bucar F. Antioxidant Activity of Nigella sativa essential oil. Phytother Res 2000; 14: 323–328.

[17] Merfort I, Wray V, Barakat HH, *et al*. Flavonoid Triglycerides from Seeds of Nigella Sativa. Phytochemistry 1997; 46: 359-363.

[18] Aboutabl EA, El-Azzouny AA, and Hammerschmidt FJ. Aroma Volatiles of Nigella sativa L. seeds. Progress in Essential Oil Research 1986; pp. 49-55. Walter de Gruyter & Co, Berlin, New York.

[19] Hashem FM, and El-Kiey MA. Nigella Sativa Seeds of Egypt. *Journal of Pharmaceutical Sciences of the United Arab Republic* 1982; 3: 121-133.

[20] Placer ZA, Cushman LL and Johnson BC. Estimation of Product of Lipid Peroxidation (Malonyl Dialdehyde) in Biochemical Systems. *Anal. Biochem* 1966; 16: 359-364.

[21] Sedlak J and Lindsay RH. Estimation of Total, Protein-Bound, Nonprotein Sulfhydryl Groups in Tissue with Ellman's Reagent. *Anal. Biochem* 1968; 25: 192-205.

[22] Habig W H, Pabst MJ and Jakoby WB. Glutathione-S-transferase. *J. Biol Chem* 1974; 249: 7130-7139.

[23] Chiu D, Fredrick H and Tappel AL. Purification and Properties of Rat Lung Soluble Glutathione Peroxidase. *Biochemica Biophysica Acta* 1976; 445: 558-566.

[24] Paoletti F, Aldinucci D, Mocali A *et al*. A Sensitive Spectrophotometric Method for the Determination of Superoxide Dismutase Activity in Tissue Extracts. *Anal Biochem* 1986; 154: 536-541.

[25] Gutterer JM, Dringen R, Hirrlinger J and Hamprecht B. Purification of Glutathione Reductase from Bovine Brain, Generation of an Antiserum, and Immunocytochemical Localization of the Enzyme in Neural Cells. *J. Neurochem* 1999; 73(4): 1422-1430.

[26] Lowry OH, Rosebrough NJ, Farr AL, *et al*. Protein Measurement with the Folin Reagent. *J. Biol. Chem*. 1951; 193: 265-275.

[27] Anane R, and Creppy EE. Lipid Peroxidation as Pathway of Aluminium Cytotoxicity in Human Skin Fibroblast Cultures: Prevention by Superoxide Dismutase and Catalase and Vitamins E and C. Hum Exp Toxicol 2001; 20: 477-481.

[28] Beytut E, and Aksakal M. The Effect of Long-Term Supplemental Dietary Cadmium on Lipid Peroxidation and the Antioxidant System in the Liver and Kidney of Rabbits. *Turkish Journal of Veterinary and Animal Science* 2002; 26:1055-1060.

[29] Ognjanovic B, Pavlovic SZ, Maletic SD, *et al*. Protective Influence of Vitamin E on Antioxidant Defense System in the Blood of Rats Treated with Cadmium. *Physiol Res* 2003; 52: 563-570.

[30] Tandon S K, Singh S, Prasad S, *et al*. Reversal of Cadmium Induced Oxidative Stress by Chelating Agent, Antioxidant or their Combination in Rat. Toxicol Lett 2003; 145: 211-217.

[31] El-Demerdash FM, Yousef MI, Kedwany FS and Baghdadi HH. Cadmium-Induced Changes in Lipid Peroxidation, Blood Hematology, Biochemical Parameters and Semen Quality of Male Rats: Protective Role of Vitamin E and Beta-Carotene. *Food Chem Toxicol* 2004; 42: 1563-1571.

[32] Stohs SJ, Bagchi D, Hassoun E and Bagchi MM. Oxidative Mechanisms in the Toxicity of Chromium and Cadmium Ions. *J. Environ Pathol Toxicol Oncol* 2000 19: 201-213.

[33] Ognjanovic B, Markovic SD, Pavlovic SZ, *et al*. Combined Effects of Coenzyme Q10 and Vitamin E in Cadmium Induced Alterations of Antioxidant Defense System in the Rat Heart. *Environ Toxicol Pharmacol* 2006 22: 219-224.

[34] Mates M. Effects of Antioxidant Enzymes in the Molecular Control of Reactive Oxygen Species Toxicology. *Toxicology* 2000; 153: 83-104.

[35] Congiu L, Chicca M, Pilastro A, Turchetto M and Tallandini L. Effects of Chronic Dietary Cadmium on Hepatic Glutathione Levels and Glutathione Peroxidase Activity in Starlings (Sturnus vulgaris). *Arch Environ Contam Toxicol* 2000; 38: 357-361.

[36] Olsson U. Selenium Deficiency and Detoxication Functions in the Rat: Short-Term Effects of Cadmium. *Drug Nutr. Instruct.* 1986; 4: 309-319.

[37] Casalino E, Calzaretti G, Sblano C and Landriscina C. Molecular Inhibitory Mechanisms of Antioxidant Enzymes in Rat Liver and Kidney by Cadmium. *Toxicology* 2002; 179: 37-50.

[38] Patra RC, Swarup D, and Senapati SK. Effects of Cadmium on Lipid Peroxides and Superoxide Dismutase in Hepatic, Renal and Testicular Tissue of Rats. Vet Hum Toxicol 1999; 41: 65-67.

[39] Houghton PJ, Zarka R, Heras B, and Hoult RS. Fixed Oil of Nigella Sativa and Derived Thymoquinone Inhibit Eicosanoid Generation in Leukocytes and Membrane Lipid Peroxidation. *Planta. Med.* 1995; 61, 33–36.

[40] Burits M, and Bucar F. Antioxidant Activity of Nigella Sativa Essential Oil. *Phytother Res.* 2000; 14: 323–328.

[41] Acan NL, and Tezcan EF. Kinetic Properties of Sheep Brain Glutathione Reductase. *Enzyme* 1991; 45: 121–124.

[42] Carlberg I, and Mannervik B. Purification and Characterization of Glutathione Reductase from Calf Liver. An Improved Procedure for Affinity Chromatography on 29, 59-ADP–Sepharose 4B. *Anal Biochem* 1981; 116: 531–536.

[43] Williams C H J. Flavin-Containing Dehydrogenases. *Enzymes* 1976; 13: 89–173.

[44] Ulusu NN, Acan NL, Turan B and Tezcan EF. Inhibition of Glutathione Reductase by Cadmium Ion in Some Rabbit Tissues and the Protective Role of Dietary Selenium: Biological Trace Element Research 2003; 91 (2): 151-156.

[45] Lyubenova L, Götz C, Goldhirsh AG, and Schrder P. Direct Effect of CD on Glutathione S-Transferase and Glutathione Reductase from Calystegia Sepium. I J Phytoremediation 2007; *http://www.informaworld.com/smpp/title~content=t713610150~db=all~tab=issueslist~branches=9 - v99* (6): 465 – 473.

[46] Mukhopadhyay S, Mukhopadhyay S, Addya S, *et al.* Effects of Cadmium Treatment in Vitro on the Antioxidant Protection Mechanism and Activation of Human Blood Platelets. *Thromb-Res.* 1988; 50(3):419-27.