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Effects of Sodium Selenite in Cadmium Chloride Induced Hepatoxicity in Male Sprague-Dawley Rats

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Abstract.- The present study aimed to assess the protective role of selenium against cadmium induced hepatotoxicity. The rats were given subcutaneous doses of 1mg/Kg body weight of either normal saline or cadmium chloride or sodium selenite or cadmium chloride plus sodium selenite on alternate days for four weeks. The results showed that cadmium treatment increased hepatic levels of lipid peroxidation, DNA damage and metals. Conversely, the levels of antioxidant system and hemoglobin were decreased as compared to the control and selenium groups of rats. Ameliorating effects of selenium were observed in rats treated with the co-administration of cadmium and selenium. The hepatoprotective effects of selenium against cadmium induced toxicity, oxidative stress and tissue damage in this study could be attributed to its antioxidant and possible chelating effects on cadmium. This study provides a possible elucidation of hepatotoxicity in rats that may have resulted from their exposure to cadmium in the environment.

Keywords: Lipid peroxidation, antioxidant system, cadmium, selenium, hepatotoxicity.

INTRODUCTION

Cadmium (Cd) being a ubiquitous contaminant of dietary products (WHO, 1992; Vromman *et al*., 2008; Satarug *et al*., 2003; Satarug and Moore, 2004) and the natural as well as occupational environment (WHO, 1992; Palus *et al*., 2003; Yoshioka *et al*., 2008), can pose a risk of health damage. Habitual cigarette smoking is also an important source of exposure to this metal (Gałaz˙yn-Sidorczuk *et al*., 2008). Although Cd is toxic to several tissues such as liver, kidneys and testis, the basis for its toxicity is not yet fully understood. Cd initially accumulates in the liver and therefore acute exposure to toxic doses of Cd produces apoptosis and necrosis in the liver (Rikans and Yamano, 2000; Tzirogiannis *et al*., 2003; Takamure *et al*., 2006). Overproduction of reactive oxygen species (ROS) has been considered as the primary mechanism for Cd toxicity (Rikans and Yamano, 2000; Tzirogiannis *et al*., 2003; Takamure *et al*., 2006; Casalino *et al*., 2002). It has been reported that administration of Cd via different routes caused increased lipid peroxidation in the membranes of erythrocytes and tissues where malondialdehyde (MDA) is used as an indicator of oxidative damage (Gutteridge, 1995). Intake of Cd results in the consumption of glutathione and protein binding sulfhydryl groups and subsequently the levels of free radicals such as hydrogen peroxide, hydroxide and superoxide are increased. Increased lipid peroxidation results in changes in the intracellular stability, DNA damage and apoptosis (Stohs *et al*., 2001). Bagchi *et al*. (1996) reported that the levels of glutathione peroxidase (GSHPx) were increased whereas a reduction was observed in the activity of glutathione reductase in the experimental Cd-induced toxicity. More beneficial effects were documented for the combined treatment with chelating agent and antioxidant against Cd-induced oxidative stress in rat livers than if they were applied separately for the same purpose (Tandon *et al*., 2003).

Selenium (Se) is generally recognized as an important antioxidant with numerous biological functions. It is well known that Se is an antagonist that moderates the toxic effects of many heavy metals such as arsenic, cadmium, mercury, and lead in various organisms. In spite of the intense research during recent years, the role of this microelement needs further elucidation. The hepatotoxic effects of Cd have been extensively studied in various animals (Sheweita, 1998; Grawe *et al*., 2004; Shimada *et al*., 2004). In addition, Cd toxicity may be linked to the

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alterations in the antioxidant defense system (Ognlanovic *et al*., 1995) and since little information is available on the protective effects of Se against Cd-induced hepatotoxicity, the present study was designed to investigate the ameliorating effect of sodium selenite on the cadmium chloride induced hepatoxicity in male Sprague-Dawley rats. Hepatic toxicity was assessed by measuring the hepatic levels of malondialdehyde (MDA) an index of lipid peroxidation, lipid hydroperoxides (LHP), reduced glutathione (GSH), catalase (CAT) activities, DNA damage and hepatic concentrations of Zn, Cd, Fe and Se.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents were purchased from Sigma-Aldrich Ltd, UK.

Animals and treatments

Following the approval by the Quaid-i-Azam University Islamabad, Pakistan's Ethics Committee, twenty post weaning male Sprague-Dawley rats (28 days old) were housed at the animal unit of this University. The rats were acclimatized to their housing and feeding for two weeks before the commencement of this completely randomised study. The rats were housed in steel cages (38x23x10cm) which were maintained at 25±2°C in a room with dark to light cycle of 14 to 10 h. The same commercial diet and fresh water was available *ad libitum* to these rats throughout this study. The rats were weighed and distributed into four groups of 5 rats with similar initial mean body weight (BW) per group. Each rat received a subcutaneous injection of relevant treatment over four weeks period as follows: (I) Control group: saline solution; (II) Cd-treated group: cadmium chloride ($CdCl_2$) in saline solution at a dose of 1 mg/kg body weight on alternate days; (III) Se- treated group: sodium selenite ($Na_2Se_2O_3$) in saline solution at a dose of 1mg /kg body weight on alternate days and (IV) Cd + Se-treated group: $CdCl_2$ + $Na_2Se_2O_3$ in saline solution at a dose of 1mg /kg body weight on alternate days. All rats were weighed before their sacrifice on the 29th day post-injection. At the end of treatment, rats were fasted for 12 hours before their sacrifice. Each rat was dissected immediately to collect blood sample directly from the heart. Liver tissues were isolated, cleaned, weighed immediately, washed with saline solution, and stored at −70 °C for the biochemical studies as described below.

Estimation of hemoglobin

Hemoglobin (Hb) was determined by the method described by Drabkins and Austin (1932).

Preparation of liver homogenate

The liver was quickly removed, washed in ice-cold, isotonic saline solution and blotted individually on ash-free filter paper. The tissues were then homogenized in 0.1 M Tris-HCl buffer, pH 7.4 using a Potter-Elvejham homogenizer at 4°C with a diluting factor of 4, the crude tissue homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C, the supernatant was kept at -20°C for the estimation of MDA, LHP, GSH and CAT activity.

Estimation of lipid peroxidation

The concentration of lipid peroxidation end product (MDA) in the liver homogenate was determined by the method of Okhawa *et al.* (1979). In brief, the reaction mixture contained 0.2 mL of 10% (w/v) tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20 % acetic acid and 1.5 mL of 0.8% aqueous solution of thiobarbutaric acid. The pH of 20% acetic acid was pre-adjusted with 1 M NaOH to 3.5. The mixture was made up to 4 mL with distilled water and heated at 95°C for 1 hour with anti-bumping glass beads in a water bath. After cooling in tap water, 1 mL of distilled water and 5 mL of mixture of n-butanol and pyridine (15:1) were added and mixture was shaken vigorously on a vortex mixer (Bio Vortex; peQ Lab, UK). After centrifugation at 4000 rpm for 10 minutes the absorbance of the upper organic layer was read at 532 nm. Tetramethoxy-propane was used as an external standard, and the level of lipid peroxidation was expressed as nmol of MDA. The values of lipid peroxidation were expressed in nM/g of tissues.

Estimation of lipid hydroperoxides (LHP)

The estimation of LHP was done by the

method of Jiang *et al.* (1992), in which 0.1 mL of 10% (w/v) tissue homogenate was treated with 0.9 mL of fox reagent (88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange and 9.8 mg of ammonium iron sulfate which were added to 90 mL methanol and 10 mL 250 mM sulphuric acid) and incubated at 37°C for 30 minutes. The colour developed was then read at 560 nm and the LHP were expressed as mM/g of tissues.

Estimation of reduced glutathione (GSH)

The GSH content of the liver homogenate was measured at 412 nm by using the method of Sedlak and Lindsay (1968). The homogenate was precipitated with 50% trichloroacetic acid (TCA) and then centrifuged at 1000 rpm for 5 min. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of Tris-EDTA buffer (0.2 M; pH 8.9) and 0.1 mL of 0.01 M 5'5'- dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 minutes and then read at 412 nm on the spectrophotometer (Biochrom, Libra S12 UV/Vis Spectrophotometer). The values were expressed as µM /g of tissues.

Estimation of catalase (CAT) activity

CAT activity was assayed according to the method of Aebi (1974). 50 µL of 10 % (w/v) tissue homogenate (supernatant) were measured into a 3.0 mL cuvette that contained 1.95 mL of 50 mM phosphate buffer (pH 7.0). About 1 mL of 30 mM hydrogen peroxide was added and the changes in absorbance were followed for 30 sec at 240 nm at 15 sec intervals. CAT activity was expressed as unit per mL of tissue homogenate.

Quantification of DNA fragmentation with diphenylamine

DNA fragmentation was examined by using the method of Pernadones *et al.* (1993). 50 mg of freeze dried ground liver tissues were mixed in 1mL of hypotonic lysis buffer (0.2% triton X-100, 10mM Tris, 1mM EDTA, pH 7.4) which was then shaken vigorously to obtain cell lysates. The cell lysates were centrifuged at 13,000g for 20 minutes at 4ºC. The supernatant containing small DNA fragments were separated immediately into a new tube labelled as S. The pellet containing large pieces of DNA and cell debris was resuspended in 1ml of hypotonic lysis buffer. Large fragments were extracted at 13000 g for 20 minutes at 4 ºC and supernatant was transferred into a new tube labelled as P. Then 1ml of 10% TCA was added to both supernatant (S) and pellet extracted DNA (P), vortexed vigorously to precipitate the DNA and then incubated at 4ºC over night. After incubation the samples were centrifuged at 20,000 g for 10 minutes and precipitates were resuspended in 330µL of 5% TCA. The tubes were incubated at 80ºC for 30 minutes and blank was prepared with 330 µL of 5% TCA. Two volumes of DPA solution [0.088 M diphenylamine, 98% v/v glacial acetic acid, 1.5 v/v sulphuric acid and 0.5 v/v of 1.6% acetaldehyde solution] were added to one volume of extracted DNA. The samples were stored at 37°C for 4 h. The blue colour was quantified spectrophotometrically at 600nm by using spectrophotometer (Biochrom, Libra S12 UV/Vis Spectrophotometer). The percent of DNA fragmentation in each sample was expressed by the formula:

$$\text{\% DNA fragmentation} = \frac{\text{OD supernatant}}{\text{OD supernatant} + \text{OD pellet}} \times 100$$

Metal analysis in liver

The freeze dried liver tissues were digested in concentrated HNO_3 by using 2g of a ground sample in 10 ml of concentrated HNO_3 (VWR, UK) in digestion blocks at 80°C. Each sample was evaporated to about 1 ml, cooled, diluted to 10 ml with distilled water and filtered through Whatman filter paper 1. These samples were then analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) by using Unicam 701machine. The machine was calibrated for the relevant concentrations using individually certified standards obtained from Sigma-Aldrich, UK. The metal concentrations in liver tissues were expressed as mg /kg dry matter (DM) which provides a more stable basis than the wet weights (WW) for presenting information on chemical compositions.

Statistical analysis

The data were statistically analysed by using ANOVA in Minitab software to determine the treatment effects on different parameters. The

Table I.- Initial body weight, final body weight, weight of liver as g/100 g body weight and as % dry matter and hemoglobin (g/dl) among different treatment groups of male sprague-dawley rats

Parameters	Control group	Cd group	Se group	Cd+Se group	SEM
Initial body weight (g)	86.2 ±4.4*	86.6±6.5	86.2±6.4	83.6±4.7	2.8
Final body weight (g)	217.6 ±4.7	229.4 ±8.8	225.0±6.9	217.8±12.2	4.3
Liver weight (g)	3.9± 0.1	3.4 ±0.1	4.1±0.2	4.3± 0.6	0.1
Liver dry matter (%)	20.4± 0.6^{c}	27.9± 0.4 ^{a}	23.4± 0.8^{b}	26.6±0.6 ^{a}	0.3***
Haemoglobin (g/dl)	12.3± 0.2 ^{b}	10.4± 0.3 ^{c}	14.1± 0.3 ^{a}	12.3± 0.4 ^{b}	0.2***

*Mean±SD; SEM=standard error of means; Means which do not bear any letter in the same row did not differ significantly, P>0.05; ***= significance at P<0.001.

Table II.- Effect of cadmium and selenium on levels of lipid peroxides (MDA), hydroperoxides (LHP), reduced glutathione (GSH), catalase (CAT) and DNA fragmentation in liver of different treatment groups of male sprague-dawley rats.

Parameters	Control group	Cd group	Se group	Cd+Se group	SEM
MDA(nM/g of tissue)	462 ±21.9^{b}	1726.2±129.4^{a}	522.9±29.8^{b}	701.6±41.8^{b}	35.3***
LHP (mM/g of tissue)	7.9±0.3^{b}	9.1 ±0.2^{a}	7.7±0.4^{b}	8.2 ±0.3b^{a}	0.1*
GSH (µM/g of tissue)	2245± 115^{a}	1262±43.7^{b}	2448±247.9 ^{a}	2729± 66.6^{a}	71.3***
CAT (U/ml of tissue homogenate)	2.5± 0.2^{a}	1.9± 0.03^{b}	2.1±0.1b^{b}	2 ±0.1b^{b}	0.1*
DNA fragmentation (%)	37.4± 0.1^{d}	61.5±0.2^{a}	40.5 ±0.2^{c}	42.7± 0.3^{b}	0.1***

Means with same letter in same row did not differ significantly, P>0.05; * and **** = significance at P<0.05 and P<0.0001 respectively

analysis compared the effect of the above mentioned treatments on body weight, liver weight, metals in liver tissues, lipid peroxidation and oxidative stress parameters and hemoglobin concentrations, at P<0.05. Tukey's test was used to compare treatments means at P<0.05.

RESULTS

The effects of Cd, Se and Cd+Se on body weight, weight of liver, liver dry mass and Hb levels in male Sprague-Dawley rats are shown in Table I. There was no significant difference in the body weight as well as relative weight of liver in all treatment groups of animals (P>0.05). Dry matter of liver showed significant difference among different treatment and control groups (P<0.001). The rats receiving Cd showed highly significant decreases in Hb, whereas the Se treated rats showed significantly increased Hb than the control rats (P<0.001). Co-administration of Cd and Se significantly increased the lower levels of Hb as compared to the rats receiving Cd alone (P<0.001). Table II presents the effects of Cd, Se and Cd+Se on lipid peroxidation end product (MDA, LHP, GSH, CAT activity and DNA fragmentation in rat livers. MDA levels were significantly higher in rats that were exposed to Cd than the control rats (P<0.001), whereas Cd+Se treated rats showed decreased MDA levels than the Cd treated rats. There was no significant difference between the Se and control groups of rats for the MDA levels (P>0.05). The LHP levels were significantly higher in both the Cd and Cd+Se treated rats than the control group of rats (P<0.01). There was no significant difference between the Control and Se treated rats for the liver LHP (P>0.05). While the GSH levels were significantly lowered by the administration of Cd than the control group, these were increased by the administration of Cd+Se than the Cd treated group (P<0.001). CAT activities were significantly lowered in Cd, Se and Cd+Se treated groups than the control group of rats (P<0.05). The treatment groups differed significantly for the extent of DNA fragmentation (P<0.001).The livers of Cd treated rats showed 1.63 fold increase in the liver DNA fragmentation than the control group (Table II).

Table III showed the concentrations of Zn,

Table III.- Effect of cadmium and selenium on concentration of selected minerals (mg /kg DM) in livers of male sprague-dawley rats.

Minerals	Control group	Cadmium (Cd) group	Selenium (Se) group	Cd+Se	SEM
Zn	97.3±2.7[b]	265.7±31.4[a]	106.3±0.8[b]	232.7±1.6[a]	7.9***
Cd	1.9±0.3[c]	289.3±36.6[a]	0.9±0.2[c]	157.2±1.8[b]	9.2***
Fe	360.4±29.3[b]	616.4±95.3[a]	354.2±7.6[b]	411.9±1.6[b]	24.9**
Se	2.5±0.1[d]	4.2±0.2[c]	6.4±0.3[b]	16.9±0.9[a]	0.2***

SD, standard deviation; DM, Dry Matter; Means with same letter in same row did not differ significantly, P>0.05; **=P<0.01; ***=P<0.001

Cd, Fe and Se in the livers of rats treated with Cd, Se and Cd+Se or untreated as control. The Zn levels were significantly higher in rats treated with either Cd or Cd+Se than the control and the Se treated groups of rats (P<0.001). The mean Cd concentration in the livers of Cd treated rats was significantly higher than the control and Se treated groups. In contrast the Cd concentration in the liver of rats was reduced by the co-administration of Se and Cd than with the Cd treated group. The liver Fe concentration was significantly increased in Cd treated rats than those of the control and other groups of rats (P<0.001). The Se concentration was significantly increased in the Cd+ Se treated rats than the control and other treated groups of rats (P<0.001).

DISCUSSION

This study was conducted in order to investigate the ameliorating effects of Se on Cd induced toxicity to lipid peroxidation, antioxidant enzyme activities, DNA damage and levels of Zn, Cd, Fe and Se in liver of male Sprague Dawley rats. The increase in the dry mass of the liver might be due to the intensity of the damage or excess of collagen /lipid accumulation in rat that was exposed to Cd. In this study the blood Hb concentration might have been diminished due to the haemorrhage or haemolysis or because of impaired blood formation in bone marrow when rats were treated with Cd (Table I). Moreover, Cd may have had a direct effect on blood Hb by decreasing its formation due to two basic red cell defects, shortened life span and impaired heme synthesis (Moore *et al*., 1985). Cd may compete with iron to cause anaemia due to iron deficiency to reach the bone marrow where Hb is synthesized. Cd inhibits the bone marrow to make Hb by interfering with several enzymatic steps in the heme pathway (Abd-El-Baset and Abd El-Reheem, 2009; McDowell, 1992) which is also reflected in the present investigations where reduced levels of Hb were observed in Cd treated rats. The Cd treated rats showed not only a significant increase in the MDA and LHP levels, but also decreased in the activity of CAT as well as GSH which may have lead to the production of oxygen reactive forms. These results correlate well with other reports where Cd has shown to up-regulate oxidative stress marker such as MDA and decrease the activity of antioxidants such as GSH and CAT (Stohs *et al*., 2001; Nigma *et al*., 1999; Al-Hashem *et al*., 2009). Subcutaneous co-administration of Se and Cd significantly reduced the lipid peroxidation biomarker (MDA and LHP) and returned them to their normal levels; it also normalized the levels of liver antioxidants (GSH and CAT). The elevated levels of lipid peroxidation end product (MDA) and LHP in the livers from the Cd than the control group of rats of this study agreed well with the findings of Stohs and Bagachi (1995). These researchers indicated that Cd might induce phagocytic cells for the production of reactive oxygen species which might be involved in the initiation of lipid peroxidation and oxidative stress in different tissues. Increased MDA and LHP levels and depressed antioxidant status in the livers of rats receiving Cd suggest that the cytotoxic effect was imposed by this oxidative insult. Peroxidation of cellular membranes leads to molecular disorganization of lipids resulting in increased membrane permeability and leakage of cellular enzymes into circulation (Mason *et al*., 1997). The decreased levels of MDA in Cd+Se treated groups

than the Cd treated group were also a strong evidence of the antioxidant role of Se. In the present study GSH levels were decreased in the liver extracts of Cd treated group than the control group which may be due to its consumption by the scavenging free radicals generated by Cd (Koyuturk *et al*., 2006; Al-Hashem *et al*., 2009). Moreover, the sulfhydryl group of cysteine moiety of glutathione has a high affinity for metals, forming thermo dynamically stable mercaptide complexes with several metals, *e.g.*, Cd. These complexes are inert and excreted via bile, so decreased GSH level may be due to its consumption during the Cd detoxification (Mohanpuria *et al*., 2007). The CAT activities were decreased in the liver extracts of Cd treated group than the control rats and this agreed with the earlier studies (Al-Hashem *et al*., 2009). Inhibition of catalase after Cd treatment may be due to the depletion of Se in Cd detoxification. This suggestion is supported by Lazarus *et al*., (2006), that Se forms a complex with Cd giving inert cadmium-selenide (Cd-Se). In the present study, inhibitions of CAT activities were recovered by Se treatment of rats during Cd administration. On the other hand, Se supplementation increased the activities of selenoproteins, *e.g.*, GPx and TrxR. Increased GPx and TrxR activities caused by Se treatment could be due to an increased incorporation of selenocysteine in such selenoproteins (Berggren *et al*., 1999; Saito and Takahashi, 2002). This may decrease free radical-mediated lipid peroxidation and regenerate the GSH (Gan *et al*., 2002). The action of Se to prevent lipid peroxidation and enhance the GSH and CAT levels in rat livers prompted us to study whether there were any antigenotoxic effects.

The principal mechanisms of Cd genotoxicity, mutagenicity and carcinogenicity are: (i) generation of reactive oxygen species (Hartwig, 1995; Lloyd *et al*., 1998; O'Brien and Salacinski, 1998); (ii) inhibition of DNA repair (Hartwig, 1995; Calsou *et al*., 1996; Hartwig, 1998; Hartmann and Hartwig, 1998); (iii) depletion of glutathione (Hartwig, 1995, Stohs and Bagchi, 1995); and (iv) possibly also suppression of apoptosis (Shimada *et al*., 1998). The results of this study confirmed such changes in DNA where significant increase in the % DNA damage was observed in the livers of rats treated with Cd than those of the other groups. Malondialdehyde, 4-hydroxy-2-nonenol and several reactive mutagenic and genotoxic lipid peroxidation products bind to DNA causing strand breakage and distortion (Eder *et al*., 2006), which is in line with the present findings. This might be the major reason for increased severity of DNA damage in Cd treated group. The present data also suggest that Se might act as a chain breaking antioxidant to inhibit the binding of the mutagenic products to the DNA.

Several studies have suggested that the interactions between Cd and Zn in an organism result in a high degree of affinity of both metals to Metalothionine (Mt) and their ability to induce its synthesis. They can induce Mt synthesis in various tissues, especially in intestine, liver and kidney (Prasad and Nath, 1995; Brzo´ska *et al*., 2000). Cd is about eight times more potent than Zn in increasing the hepatic Mt concentration (Eaton *et al*., 1980). Mt is a low-molecular weight, thiol-rich, metal-binding protein, which was first identified as a Cd binding protein and was later shown to be a Zn and Cu binding protein. Because of its higher affinity to Mt, Cd displaces Zn from the cysteine binding sites on this protein. The increasing concentration of free Zn^{2+} ions may further induce synthesis of new molecules of Mt (Chang and Huang, 1996). By displacing Zn, Cd interferes with the Zn absorption, distribution into tissues and transport into cells or several intracellular structures and may inhibit its activities at various stages. These studies are in line with the present investigations where high Zn levels were recorded in Cd treated groups. Many toxic effects of Cd occur through a disruption of Zn-mediated or Zn-dependent metabolic processes, including cellular production of DNA, RNA and protein (Sunderman and Barber, 1988). Disturbances in Zn function and metabolism may have serious consequences for the animal and human health. This element plays an important role in the growth, development and functioning of all living cells (Bray and Bettger, 1990; Nishi, 1996; Okano, 1996). It is involved as a co-factor in many metalloenzymes (over 200) and regulatory proteins, including enzymes of both DNA and RNA biosynthesis and repair. The Cd induced retention of Zn in the liver of Cd treated groups is due to the Cd accumulation and Mt induction in this organ which

is in line with the previous findings (Tandon *et al*.,1994; Brzo´ska *et al*., 2000). Se concentration in rat livers was significantly increased in Se and Cd+Se treated groups ($P<0.0001$). Se was found to have a protective effect by decreasing Cd content in liver. However, it has also been observed that co-administration of Cd and Se (200 ppm + 0.1 ppm, respectively) in drinking water for five weeks did not decrease Cd concentration in the liver nor in the kidney, and only diminished the toxic effects of Cd in these organs (Jihen *et al*., 2008). In contrast, in our study, Se administration decreased the Cd concentration in the liver in the Cd+Se exposed groups of rats (Table III). The mechanism of protective effects of Se on Cd toxicity and increased Se content in liver after the Cd intake can be explained by their antagonism to Cd induced DNA damage and by the fact that Se is an essential constituent of a number of enzymes, some of which have antioxidant functions. Deficiency of the element in animals makes them susceptible to injuries by certain types of oxidative stresses (Burk, 2002). This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (Combs and Gray, 1998; Ognjanovic *et al*., 2008). The lipid peroxidation, one of the main manifestations of the oxidative damage plays an important role in the toxicity of many xenobiotics. Intoxication with Cd causes a significant increase of lipid peroxidation in liver and kidneys of rats (Ognjanovic *et al*., 2008) which are also the main organs accumulating the Cd which is supported by the present findings. Therefore, increase in Se content in liver can be connected with the protective role of Se in oxidative stress induced by Cd.

CONCLUSIONS

The hepatoprotective effect of Se against Cd induced toxicity, oxidative stress and tissue damage in this study could be attributed to its antioxidant and possible chelating effects on Cd. It appeared that the subcutaneous administration of Se in the form of 1mg sodium selenite/kg body weight was able to curtail the toxic effects of Cd on male Sprague-Dawley rats that were exposed to 1mg cadmium chloride/kg body weight. This study clearly indicated the hepatoprotective effect of Se on Cd induced hepatoxicity in male Sprague-Dawley rats.

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