

Full Length Research Paper

Methidathion-induced hematological, biochemical and hepatohistological alterations in rat: Ameliorative effects of selenium and zinc

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The aim of this study was to determine the effect of a sub-acute 4-week exposure to methidathion (MD) on the hematological system and hepatic integrity of rats. We also assessed whether co-administration of micronutrients such as selenium (Se) and zinc (Zn) prevented MD-induced hepatic damage. Rats were randomly divided into four groups of six each: the first group served as a control which received standard diet, the second group received both Se (0.5mg/kg of diet) and Zn (0.227 g/l drinking water), the third group received only MD (5 mg/kg b.w. by gavage using corn oil as a vehicle), and the fourth group received MD and combined selenium and zinc. After four weeks, exposure to MD induced a significant increase in plasmatic activities of AST, ALT, ALP, LDH, and liver malondialdehyde level. In contrast, reduced glutathione level (GSH), and the activities of catalase (CAT), superoxide dismutase (SOD), and the glutathione peroxidase (GPx) content of hepatic tissue decreased significantly. Moreover, treatment with Se and Zn in MD-treated rats maintained all the biochemical parameters cited above. In conclusion, the obtained results indicate the ability of Se and Zn to attenuate the MD-induced liver and erythrocytes oxidative damage.

Key words: Biochemical studies, liver, methidathion, oxidative stress, rat, selenium, zinc.

INTRODUCTION

Organophosphate insecticides (OPIs) are widely used in agriculture, in order to enhance food production against

insects and micro-organisms throughout the world. The predominant action of OPIs is to cause inhibition of acetyl

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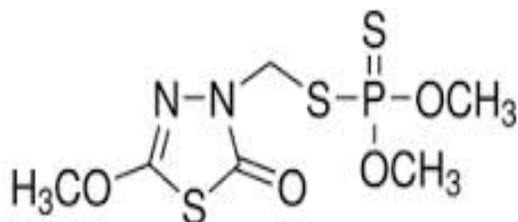


Figure 1. Chemical structure of methidathion.

cholinesterase (AChE) activity in the target tissues leading to the accumulation of acetylcholine substance. The latter represent the chemical mediator of nervous system, and it is responsible for the transmission of nervous function by binding to post synaptic acetylcholine receptors, resulting in muscle contraction or glands secretions (Lauwerys and Lisson, 2007; Abou-Donia, 2010). Chronic and subchronic exposure of OPIs may induce oxidative stress leading to the generation of free radicals and alteration in antioxidants or reactive oxygen species (ROS) scavenging enzymes (Attia et al., 2012). Methidathion (ultracide, GS 13005) S-[(2, 3-dihydro-5-methoxy-2-oxo-1, 3, 4-thiadiazol 3(2H)-yl) methyl] O,O dimethyl phosphorodithioate) (Figure 1) is one of the most widely used OPIs for agriculture and public health programmes (Yavuz et al., 2004). It has been reported that the major route of methidathion metabolism in rat liver was through glutathione-S-transferase and that the predominant metabolite was desmethyl methidathion (Beauvais, 2005). Some previous studies showed the *in vivo* and *in vitro* effect of MD on lipid peroxidation (LPO), and antioxidant enzyme in which a single dose of MD caused elevation in erythrocytes LPO (Altuntas et al., 2002a). In addition, the sub chronic administration of MD may induce biochemical and histopathological changes in different tissues such as liver, kidney and heart. However, the supplementation of antioxidants can be useful to constrain the oxidative damage. The interest in selenium pharmacology and biochemistry has increased since it possesses biological and antioxidant properties as those of vitamin C and D and some micronutrients (Altuntas et al., 2002b; Gokalp et al., 2003; Sulak et al., 2005; Guney et al., 2007a). Also, antioxidant enzymes, that is, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), operate in concert together with several other non-enzymatic molecules to contrast the ROS actions and to avoid oxidative damage.

SOD catalyzes the reduction of superoxide anion into hydrogen peroxide, which is subsequently detoxified by CAT and GPx at both intra and extra-cellular levels (Ghalekandi et al., 2013). In particular, an isoform of SOD has been identified and shown to include Se and Zn at its catalytic site. Consequently, Zn and Se are considered essential for the correct functioning of an organism. Additionally to being a co-factor of SOD, Zn is required, as a functional component, in more than 200

enzymes and transcription factors (Sfar et al., 2009). Zinc is a structural constituent of many proteins, hormones and hormone receptors, which plays a fundamental role in a wide range of biochemical processes, and it is an important modulator in the mammalian central nervous system (CNS). In addition, zinc exhibits both neurotoxic and neuroprotective effect (Nowak, 2000). Zn also functions as an antioxidant; it is a constituent of the antioxidant enzyme superoxide dismutase (Shankar and Prasad, 1998) and maintains the physiological roles of metallothioneins. Furthermore, Zn prevents the reactions between thiols and iron, which give rise to free radicals, and is also an essential constituent of the nucleic acid-repairing enzymes and a membrane stabilizing factor (Savarino et al., 2001). Selenium is also an essential trace element, which plays an antioxidant role, as a selenocysteine residue. The best known biochemical role of Se is to be a part of the glutathione peroxidase active site (Kralj and Gorup, 2004). Selenium was demonstrated to be a modulator of oxidative stress response, where its supplementation could prevent chronic degenerative diseases (Rayman, 2002).

Furthermore, some investigators confirms that the interactions between zinc and selenium are not really apparent, and the mode of interaction could be by the competition for a common ligand or transport sites, so, one element may be involved in the metabolism of the other (House and Welch, 2014). However, it has been established that the co administration of Se and Zn together exercise a synergistic effect against the oxidative stress induced by cadmium (El Heni et al., 2009). Most previous reports in the field of pesticides and oxidative stress were performed by using single intoxication on animal models. For this reason, the present study was undertaken to evaluate the effect of a sub-chronic exposure of methidathion not only administered alone, but also in combination of two micronutrients Se and Zn to male rats. Therefore, the aim of this study was to (i) evaluate the effect of methidathion on biochemistry, lipid peroxidation and enzyme activities and to (ii) investigate the combined administration of selenium and zinc in alleviating the methidathion induced toxicity.

MATERIALS AND METHODS

Chemicals and reagents

A commercial formulation of methidathion, named 'Supracide[®], Syngenta Crop Protection Pty Limited)' was used in the experiments. All other chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

Animals and treatment

Twenty eight (28) male Wistar rats (weighing 270 to 290g) were obtained from the Pasteur Institute (Algiers, Algeria). Animals were acclimated for two weeks under the same laboratory conditions of

photoperiod, relative humidity (40%) and temperature ($24\pm 2^\circ\text{C}$). A standard diet, supplied by the "ONAB, El-Harrouch", Algeria and water *ad libitum* were provided. Rats were randomly divided into four groups of seven individuals each as follows. The first group was served as a control. The second group (Se+Zn) was given daily selenium as sodium selenite (Na_2SeO_3) at a dose of 1.5 mg/kg diet, and Zinc ($\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$) at a dose of 0.227 g/l water. The third group (MD) was administered at a dose of 5 mg/kg b.w. by gavage using corn oil as a vehicle. Rats in the fourth group (MD+Se+Zn) were treated with MD, Se and Zn. Only corn oil was given in the same way to the control group. The dose of MD used in this study represents 1/15 of LD_{50} (5 mg/kg bw) which has been used previously by other investigators since it is toxic but not lethal to rats (Sutcu et al., 2006; Güney et al., 2007). The doses of Na_2SeO_3 and ZnSO_4 were selected based on the clinical application and on results from previous experiments in human and experimental animals against heavy pesticide poisoning (Goel et al., 2005; Mansour and Mossa, 2009; Ben Amara et al., 2011). Rats were administered, their respective doses every day for four weeks. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of our Institution. The amount of ingested diet was calculated as the difference between the weight of feed that remained in the food bin (D_1) and the amount placed 1 day before (D_2). These data were then used to calculate the daily average feed intake, according to the formula:

Average feed intake: $D_2 - D_1$

At the end of the experiment, body weights of rats were recorded. Animals were then sacrificed by cervical decapitation without anesthesia to avoid animal stress and liver was immediately removed and weighed; the organ weight ratio was then calculated. The relative weight of organs (%) was calculated as g/100 g body weight. Blood samples were collected in EDTA tubes and centrifuged at 2200 g for 15 min at 4°C alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Hematological variables

Red blood cells (RBCs) count, hemoglobin (Hb) concentration, hematocrit, mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration were measured by electronic automate coulter MAXM (Beckman Coulter, Inc., Fullerton, California, USA).

Tissue preparation

About 1 g of liver was homogenized in 2 ml of buffer solution of phosphate buffered saline 1:2 (w/v; 1 g tissue with 2 ml PBS, pH 7.4). Homogenates were centrifuged at 10,000 g for 15 min at 4°C , the supernatants were divided into aliquots, then stored at 20°C , and then used for the determination of malondialdehyde (MDA), reduced glutathione (GSH) levels and the activities of the antioxidant enzymes SOD, CAT, and GPx.

Estimation of plasma biochemical parameters

Different biochemical parameters were assayed spectrophotometrically using commercially available kits from Spinreact (Spain, Refs: Bilirubin-1001044, total protein-1001291GOT-1001165, GPT-1001175, ALP-1001131, and LDH-SP41214).

Estimation of lipid peroxidation

The lipid peroxidation level of liver homogenate was measured as malondialdehyde (MDA), which is the end product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex, which has peak absorbance at 532 nm according to Buege and Aust (1984) 125 μl of supernatant was homogenized by sonication with 50 μl of PBS, 125 μl of TCA-BHT (trichloroacetic acid- butylhydroxytoluene) in order to precipitate proteins, and then centrifuged (1000 g, 10 min, and 4°C). After that, 200 μl of supernatant were mixed with 40 μl of HCl (0.6 M) and 160 μl of TBA dissolved in Tris, and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The amount of MDA was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M/cm}$ (Draper and Hadley, 1990).

Estimation of reduced glutathione

Liver GSH content was estimated using a colorimetric technique, as mentioned by Ellman (1959), and modified by Jollow et al. (1974) based on the development of a yellow colour when DTNB [(5,5 dithiobis-(2-nitrobenzoic acid)] is added to compounds containing sulfhydryl groups. Briefly, 0.8 ml of liver supernatant was added to 0.3 ml of 0.25% sulphosalicylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg protein.

Measurement of glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) (EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig et al. (1974). P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 340 nm at 30 s intervals for 3 min.

Measurement of antioxidant enzyme activities

Measurement of glutathione peroxidase activity

Glutathione peroxidase (GPx) (E.C.1.11.1.9) activity was measured by the procedure of Flohe and Gunzler (1984). Supernatant obtained after centrifuging 5% liver homogenate at 1500 g for 10 min followed by 10000 g for 30 min at 4°C was used for GPx assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of H_2O_2 (1 mM) and 0.3 ml of liver supernatant. After incubation at 37°C for 15 min, reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500 g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Measurement of catalase activity

The catalase (E.C.1.11.1.6) activity was measured according to Aebi (1974). This assay is based on the ability of the enzyme to induce the disappearance of hydrogen peroxide. The reaction mixture consist of 780 μl phosphate buffer (pH 7.5), 200 μl of

Table 1. Changes in body, absolute and relative liver weights, and the food intake of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD+Se+Zn) after 4-week treatment.

Parameter	Treatment groups			
	Control	Se+Zn	MD	MD+Se+Zn
Initial body weight (g)	315.62±3.18	303.25±2.71	316 ± 3.9	304.62±4.14
Final body weight (g)	347.5±5.86	342.75±3.89	296.6±3.9*	311±4.34
Absolute liver weight (g)	10.93 ± 0.34	11.88±1.36	12.22±1.07*	11.61±1.97
Relative liver weight (g/100 g b.w.)	3.674±0.12	3.96±0.45	4.07±0.3**	3.87± 0.66
Food intake (g/day/rat)	20.68±1.17	19.43±3.0	16.38±2.17*#	19.47± 2.72

Values are given as mean ± SEM for group of 7 animals each. Significant differences: *Se+Zn, MD, MD+Se+Zn groups compared with control group (*p≤0.05; **p≤0.01). # MD+Se+Zn group compared with MD group (#p≤0.05).

hydrogen peroxide (500 mM) and 20 µl supernatant in a final volume of 1 ml. Absorbance was recorded at 240 nm every 15 s for 1 min. The enzyme activity was calculated by using an extinction coefficient of 0.043 mM cm⁻¹.

Measurement of superoxide dismutase activity

The method of the determination of SOD (E.C.1.15.11) activity by the nitroblue tetrazolium (NBT) test is a method of photoreduction of riboflavin/methionine complex that generates superoxide anion. The oxidation of the NBT by the anion superoxide O₂⁻ is used as the basis for the presence of SOD detection. In aerobic environments the riboflavin, methionine, and NBT mixture gives a blue color. The presence of SOD inhibited the oxidation of the NBT. SOD activity was estimated by the procedure of Beauchamp and Fridovich (1971). Briefly, 5 µl of the supernatant was combined with 1 ml of EDTA/methionine (0.3 mM), 1890 µl phosphate buffer (pH 7.8), 85 µl of 2.6 mM NBT and 22 µl of riboflavin (0.26 mM) was added as the last and the light was switched. The reaction changes in absorbance at 560 nm were recorded after 20 min.

Measurement of protein content

The protein contents of various samples were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Histopathological analysis

For histopathological analysis, the liver tissues were dissected and the tissue samples were immediately fixed in formalin solution, embedded in paraffin. The paraffin sections were cut into 5 µm thick slices and stained with hematoxylin and eosin (H&E) for light microscopic examination. The sections were viewed and photographed by a compound binocular light microscope (Hould, 1984).

Statistical analysis

Data are expressed as means ± SEM. Data comparisons were carried out by using one way analysis of variance followed by Student's t-test to compare means between the different treatment groups. Differences were considered statistically significant at p≤ 0.05.

RESULTS

Effects of treatment on general rat health

In our study, there were no deaths in any of the groups during the treatment period (4 weeks). During the experiment, rats in the control group and in the Se+Zn-treated group did not show any sign of toxicity. However, MD-treated group of rats showed varying degrees of clinical signs including huddling, depression (Diarrhea, changes in appetite weight, balance disorder), mild tremor and dyspnea. The observed signs were related to the cholinergic crisis, a consistent sign in acute organophosphate poisoning. None of these clinical manifestations were observed in the MD+se+Zn treated rats. As shown in Table 1, the decrease in body weight of MD group was associated with the reduction in food intake by 21%. Beside, co-administration of Se plus Zn with MD showed an improvement of the consumption in food intake by 20% as compared to the MD-treated group.

Effects of treatments on body and liver weights

The variations in the body and relative liver weights of male rats in control and treatment groups are given in Table 1. During the course of present work, it was observed that the body weights of control and Se+Zn treated group, increased progressively throughout the study. However, the exposure of MD resulted in the loss of body weight by 15%. Treatment with Se plus Zn led to an improvement in the body weight of the MD-treated rats by 10% as compared to the control group. Beside, a significant increase of MD-treated rats in absolute and relative liver weights was recorded (11.75 and 11%, respectively) compared to the controls, and by 5 and 4.8%, respectively, compared to the MD-treated group.

Effects of treatments on hematological parameters

Hematological parameters in control and treated groups

Table 2. Changes of hematological parameters of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD+Se+Zn) after 4-week treatment.

Parameter	Treatment groups			
	Control	Se+Zn	MD	MD+Se+Zn
RBC count(10^6 /ml)	5.94±0.6	4.77±1.32	4.087 ± 0.57**	5.23±0.14
WBC (10^3 /ml)	4.61±0.1	6.02±0.17	4.83±0.18	7.48±0.51*
Hb (g/dl)	9.48±0.12	8.4±0.23	7.68 ±0.21*	8.98±0.27
PLT (10^3 /ml)	365.1±7.32	376±12.47	349.7±8.53	397.75±20.5 [#]
HT (%)	25.21±0.5	23.74±0.71	28.61±0.74	26.15±0.76
VMC (mm^3 /RBC)	50.04±1.05	49.4±2.32	40.46± 1.78	49.76±2.42
TCMH (pg/RBC)	16.8±0.35	17.68±0.12	17.17± 0.19	17.06±0.83
CCMH (g/dl)	34.53±0.45	35.85±0.27	34.03±0.28	34.36±0.43

Values are given as mean ± SEM for group of 7 animals each. Significant differences: * Se+Zn, MD, MD+Se+Zn groups compared with control group (* $p \leq 0.05$; ** $p \leq 0.01$). [#] MD+Se+Zn group compared with MD group ([#] $p \leq 0.05$).

Table 3. Changes in biochemical parameters of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD+Se+Zn) after 4-week treatment.

Parameter	Treatment groups			
	Control	Se+Zn	MD	MD+Se+Zn
Bilirubin (mg/dl)	4.6±0.25	3.75±0.7	2.7±0.12*	3.0±0.92
Total protein (g/dl)	6.88±0.32	6.78±0.63	4.7 ± 0.38**	6.37±0.51
Cholesterol (mg/dl)	102.09±2.35	102.07±5.16	116±3.32**	106.11±6.28##
Triglycerides (mg/dl)	92.5±4.24	96.6±6.24	82.21±4.14*	95.7 ± 3.3
AST (U/L)	191.12±6.02	170.75±5.91	267.57±6.35***	153.25±1.98***, ##
ALT (U/L)	111.87±2.73	117.62±1.66	202±1.51***	189.5±1.48 [#]
ALP (U/L)	260.66±12.1	239.2±11.03	368.75±10.09***	300.71±13.34*
LDH (U/L)	1827.26 ± 63.82	1792.6±50.23	2585±45.4**	1353.86±32.23***, #

Values are given as mean ± SEM for group of 7 animals each. Significant differences: *Se+Zn, MD, MD+Se+Zn groups compared with control group (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$), [#]MD+Se+Zn group compared with MD group ([#] $p \leq 0.05$; ## $p \leq 0.01$).

are shown in Table 2. Red blood cells (RBCs), haemoglobin (Hb) content in MD-treated group were significantly decreased compared to those in the controls. While, co-administration of Se plus Zn with MD showed an improvement of RBCs and Hb levels by 29 and 8%, respectively, as compared to control and by 22 and 7%, respectively, as compared to the MD-treated group.

Effects of treatments on biochemical parameters

Total protein, bilirubin, cholesterol, triglycerides, AST, ALT, ALP, and LDH are indicators of hepatic function. As shown in Table 3, compared to the control group, the MD-treated group had significantly lower total protein and bilirubin levels, and significantly elevated ($P < 0.001$) AST, ALT, ALP, and LDH activities ($P < 0.01$). In the current experimental study, MD treatment caused significant increase in the levels of cholesterol and a significant increase in triglyceride level as compared to the control group. Supplementation of Se and Zn to the MD-treated group produced recovery in the above mentioned biochemical variables.

Effects of treatments on hepatic oxidative stress parameters

Table 4 summarizes the changes of liver oxidative injury. Exposure to methidathion in rats produced significant adverse effects on the redox status of liver, which is evidenced by a significant reduction in antioxidant enzymes activities as seen with GPx (21%), CAT (16%) and SOD (26%) and a significant decrease (19%) in GST activity in liver compared with control group. These changes were accompanied by a significant increase (23%) in MDA level and depletion (33%) in GSH content. On the other hand, co-administration of selenium plus zinc with MD produced recovery in the above mentioned hepatic oxidative stress parameters.

Histopathological changes of liver

Liver of the control group had regular histological structure with a characteristic pattern of hexagonal lobules (Figure 4A). Accordingly, no histological alterations were observed in the liver of Se+Zn-treated

Table 4. Activities of antioxidant enzymes of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD+Se+Zn) after 4-week treatment.

Parameter	Treatment group			
	Control	Se+Zn	MD	MD+Se+Zn
Glutathione peroxidase ^a	16.44±0.33	16.54±0.51	12.33±0.164 [*]	18.17±0.326 [#]
Catalase ^b	36.48±0.73	38.97±0.27	30.74±0.42 [*]	40.6±1.08 [#]
Superoxide dismutase ^c	25.24±0.62	20.01±1.28	18.58±0.13 [*]	23.17±0.42 [#]

Values are given as mean ± SEM for group of 7 animals each. Significant differences: ^{*}Se+Zn, MD, MD+Se+Zn groups compared with control group (^{*}p≤0.05). [#]MD+Se+Zn group compared with MD group ([#]p≤0.05). ^aGlutathione peroxidase: nmoles of GSH/min/mg protein. ^bCatalase: μmoles H₂O₂ degraded/min/mg protein. ^cSuperoxide dismutase: units/mg protein.

Table 5. Semiquantitative scoring of histopathological changes in the liver sections of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD+Se+Zn) after 4-week treatment.

Parameter	Treatment groups			
	Control	Se+Zn	MD	MD+Se+Zn
Mononuclear cell infiltration	-	-	++	-
Hepatic hemorrhage	-	-	++	+
Necrosis	-	-	++++	-
Dilation of sinusoids	-	-	++++	-
Vascular congestion	-	-	+++	-

(-) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++ indicates extremely severe.

group (Figure 4B). In contrast, in MD-treated animals, sub chronic of MD administration for four weeks (Figure 5), led to mononuclear cell infiltration, necrosis, dilation of sinusoids and vascular congestion (Figure 4C to D). Whereas, in MD+Se+Zn-treated group, dilation of sinusoids and vascular congestion were less in comparison to the MD treated group (Figure 4E). The histopathological changes are graded and summarized in Table 5. Histological grading was made according to five severity grades: (-) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++ indicates extremely severe.

DISCUSSION

Pesticide hazards have been accentuated by the sharp rise in their agricultural, industrial and domestic use. Acute exposure to pesticides can cause oxidative damage. The current study investigated the potential ability of Se and Zn, to reduce oxidative damage induced by MD, an organophosphate pesticide. OPs cause a reduction of body weight in experimental animals (Güney et al., 2007; Kalender et al., 2007; Ogutcu et al., 2008). The findings from the present work indicate that excessive MD exposure has changed body weight, absolute and relative liver weights, leading however to significant decrease in animal growth and production

performances. This could be probably attributed to the reduction of feed consumption and/or malabsorption of nutrients induced by MD effects on the gastro-intestinal tract and/or inhibition of protein synthesis. MD exposure may account to reduced food intake seen in the MD-treated group. Hence, these findings were similar to the results published by Ogutcu et al. (2008) and Messarah et al. (2012) who reported that OPs exposure have significantly induced disturbances of the total body weight, absolute and relative liver weights of rats. In the present study, the exposure of rats to MD induced hematological changes (essentially RBC and Hb). Our results corroborated with the findings of Ojezele and Abatan (2009), who reported a reduction in RBC and Hb in MD treated group, which can be attributed to intravascular haemolysis, anemia, or to haemopoiesis depression. On the other hand, the Hb is found to be a major source of radical production when it interacts with redox xenobiotics (French et al., 1978; Rifkind and Nagababu, 2013).

Liver is the target organ of OPs toxicity (Mazumder, 2005), and the leakage of hepatic enzymes such as ALT, AST and ALP are commonly used as an indirect biochemical index of hepatocellular injury (Klaassen and Watkin, 1984). In the present finding, MD intoxication caused a significant increase in the status of the serum ALT, AST, ALP and LDH activities and that might be related to the release of these enzymes from the

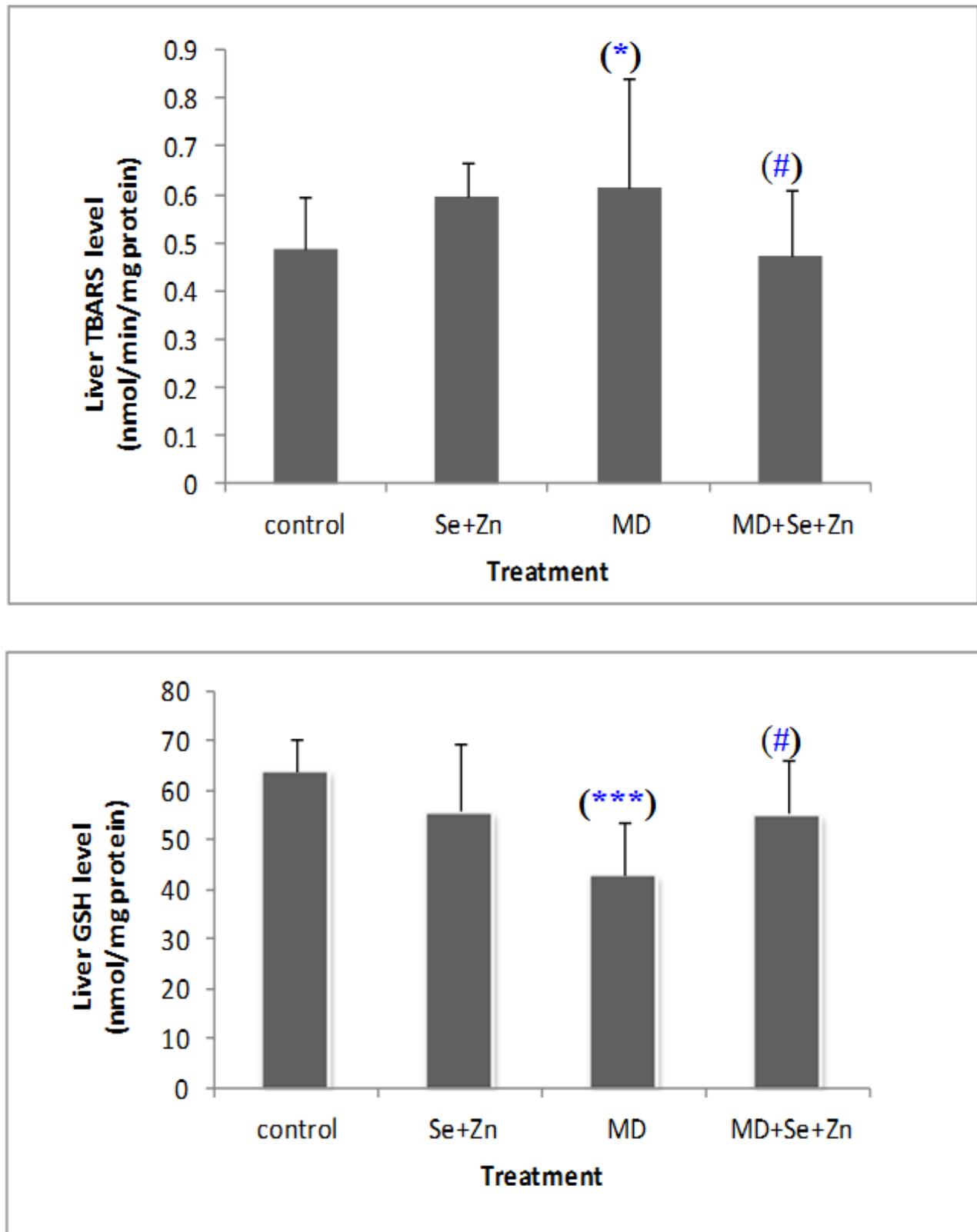


Figure 2. Liver malondialdehyde (nmol/mg prot.) and reduced glutathione (nmol/mg prot.) levels of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD plus Se+Zn) after 4-week treatment. Values are given as mean \pm SEM for group of 7 animals each. *MD and MD+Se+Zn groups compared with control group (* p \leq 0.05; *** p \leq 0.001). # MD+Se+Zn group compared with MD group (p \leq 0.05).

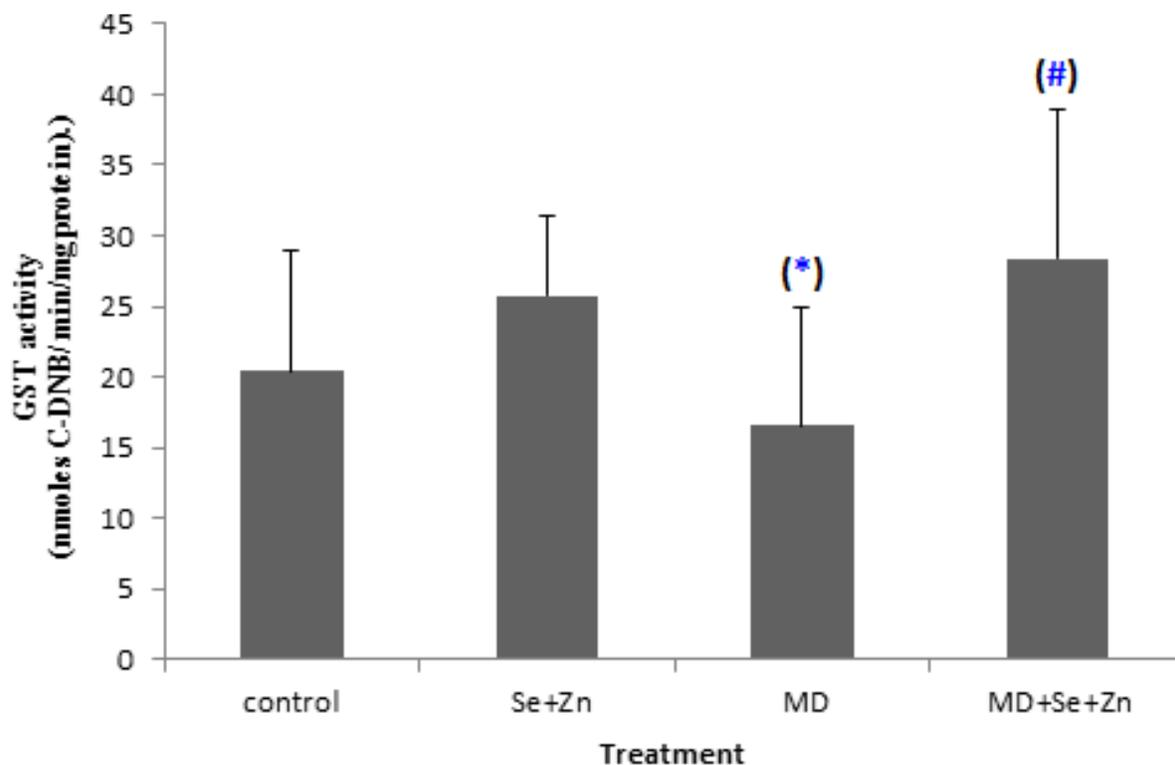


Figure 3. Liver glutathione-S-transferase (nmoles C-DNB conjugate formed/min/mg protein) activity of control and rats treated with selenium plus zinc (Se+Zn), methidathion (MD) or their combination (MD plus Se+Zn) after 4-week treatment. Values are given as mean \pm SEM for group of 7 animals each. * MD and MD+Se+Zn groups compared with control group (* p \leq 0.05). #MD+Se+Zn group compared with MD group (# p \leq 0.05).

cytoplasm into the blood stream rapidly after the rupture of plasma membranes and cellular damage caused by the free radicals produced during the MD metabolism. These results are consistent with the previous findings realized by some researchers who had found an association between OPs toxicity and the increased oxidative stress of rats (Sefi et al., 2011; Attia et al., 2012). Consequently, these biochemical changes could be correlated with histological alterations. In fact, the actual study suggests that MD produces various pathological perturbations in liver such as bleeding, mono nuclear cell infiltration, vascular congestion, dilation of sinusoids and necrosis. These observations possibly come from the toxic effects of generated reactive oxygen species which upset the liver membrane components. Similarly, OP-treated rats have experienced almost the same perturbations including inflammation, and hepatocellular degeneration (Gokalp et al., 2003; Kalender et al., 2010; Sutcu et al., 2006). However, the co-treatment of Se plus Zn has reduced the histological alterations induced by MD, which might be attributed to their antiradical/antioxidant roles. Moreover, these results are in good accordance with those obtained by other studies which have postulated the beneficial role of Se and Zn on histopathological and enzymatic changes of

rats (Goel et al., 2005; Ben Amara et al., 2010; Heikal et al., 2012). Though, the beneficial role of Se and Zn in reducing oxidative stress parameters in the present study might be related to its mild antioxidant potential against toxins induced hepatotoxicity. The observed increase of plasma cholesterol level is associated with the decline of plasma triglycerides; in MD exposed rats is probably a potential indicator for fatty acid metabolism, and implicitly of a possible membrane lipid peroxidation. To disclose the matter, MDA hepatic quantification was tested and found to be higher, indicating lipid peroxidation resulting from exposure to MD. Thus, it becomes conceivable that the observed alteration in circulating cholesterol and triglycerides levels may be a consequence of membrane lipid peroxidation and free radical release.

In the present investigation, significant decrease in plasma proteins and bilirubin concentration were recorded in MD treated rats. Similarly, it has been reported that the decrease in the protein concentration of MD-treated rats is likely originated from alterations in protein synthesis and/or metabolism (Chinoy and Memon, 2001). The administration of Se plus Zn with MD has certainly protected the liver function from MD intoxication as indicated by the significant restoration of plasma biochemical indicators such as cholesterol,

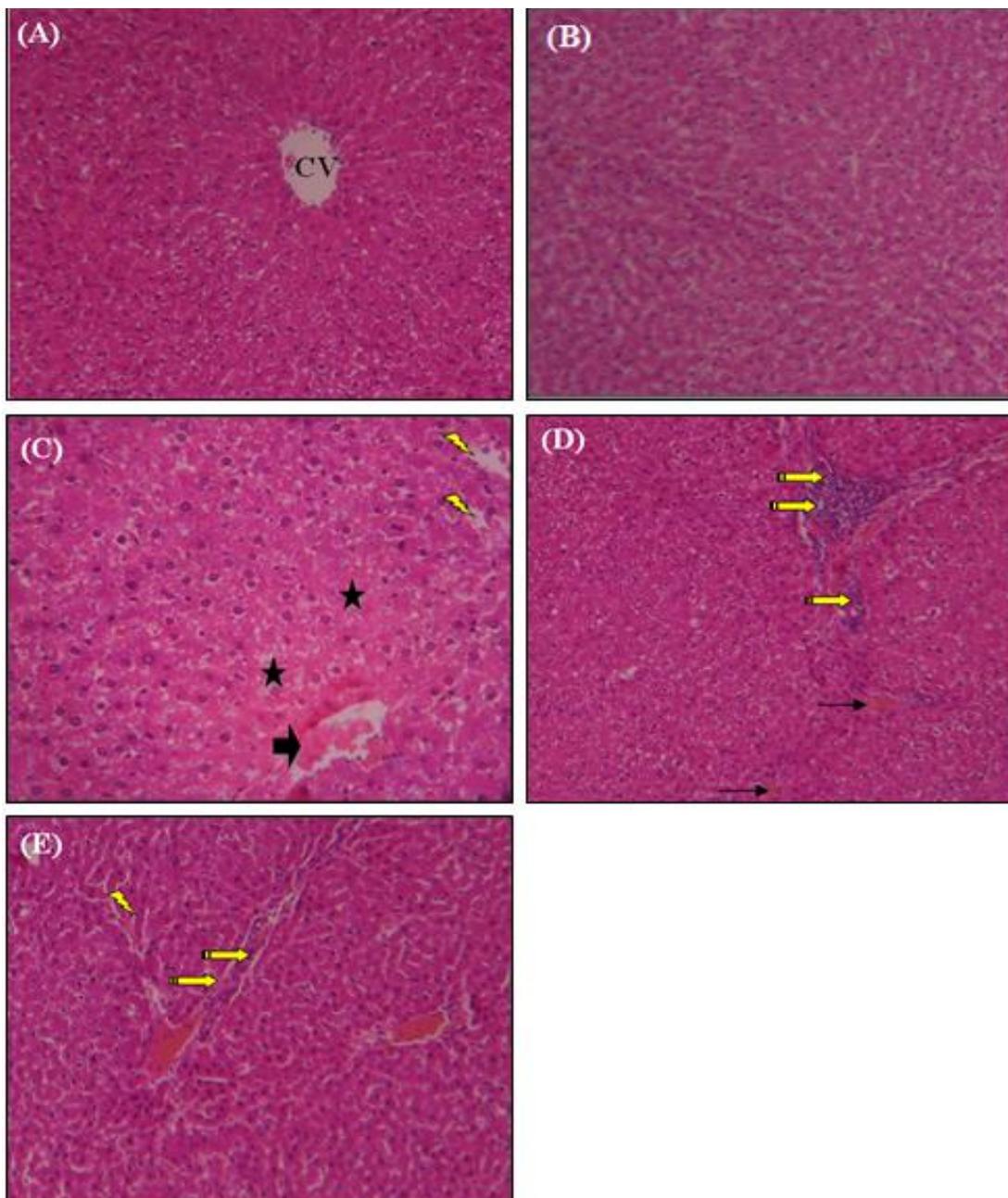


Figure 4. Effect of methidathion (MD) and selenium plus zinc (Se+Zn) co-administrated with MD on histopathological damages in the liver. Controls (A, x100), treated with Se+Zn (B, x100), MD (C and D, x250) and Se plus Zn co-administrated with MD (E, x100) after 4 weeks of treatment, as revealed by photomicrograph of H&E. CV: Central vein. Vascular congestion (↘), mononuclear infiltration (→), dilation of sinusoids (★), and hemorrhage (➤) and necrosis (➤).

triglycerides, proteins and bilirubin. OPIs induce reactive oxygen species and subsequent depletion of antioxidant cell defenses can result in disruption of the pro-oxidant/antioxidant balance in mammalian tissues (Valko et al., 2006). Also, OPIs generates reactive oxygen species (ROS) and causes organ toxicity. Consequently, ROS directly react with cell biomolecules, causing

damages to lipids, proteins and DNA, and hence leading to cell death (Halliwell and Gutteridge, 2002). However, lipid peroxidation is a basic cellular deteriorating process induced by oxidative stress and occurs readily in the tissues rich in highly oxidisable polyunsaturated fatty acids (Halliwell, 1994). MDA is a marker of membrane LPO resulting from the interaction of ROS and the cellular

membrane (Guneş et al., 2007a; 2007b). In our previous studies, pesticides such as diazinon and methomyl (Messarah et al., 2012; Djeflal et al., 2012) caused a significant increase in LPO in a manner similar to the present study and, in addition, treatment with a combination of Se and Zn after the administration of MD led to a significant decrease in LPO levels. The significant drop in GSH levels promoted by MD caused a reduction in the effectiveness of the antioxidant enzyme defense system, sensitizing the cells to ROS (Altuntas et al., 2002a). Thus, the decreased GSH levels have caused a decline in GPx activity, which leads to possible hepatic oxidative injury. It can be suggested that the reduction, especially in GPx activity seen in this study, is in accordance with these pathologic processes. This situation may be caused by the diminution in SOD and CAT activities, as a result of reduced GPx activity in the present investigation. Under these circumstances, the endogenous antioxidant enzymes are likely to be perturbed because of over-production of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately restock tissue antioxidants.

GST may play an important role in cellular detoxification of ionizing radiation in human (Movafagh et al., 2005). A significant decrease of GST activity in rats treated with MD was observed. This is in good accordance with the study of Attia et al. (2012), which correlate the significant reduction of GST by the decrease in GSH and glutathione dependent enzyme system. The actual results revealed that MD caused a statistically significant decrease of liver SOD activity. Thus, the supplementation of Se and zinc to MD treated groups has normalized the levels of SOD. These results agree with previous study on rat liver (Altuntas et al., 2002b) regarding the effect of MD *in vivo*. The decrease in the activity of superoxide dismutase in MD-intoxicated animals may be owed to the consumption of this enzyme in converting the O_2^- to H_2O . The dismutation of O_2^- to H_2O is catalyzed by SOD which contains both copper and zinc. The latter is known to induce the production of metallothionein, which is very rich in cysteine, and is an excellent scavenger of HO^\bullet (Mansour and Mossa, 2009). Furthermore, the NADPH oxidases are a group of plasma membrane associated enzymes, which catalyze the production of O_2^- from oxygen by using NADPH as the electron donor. Zinc is reported to be an inhibitor of this enzyme (Prasad, 2008).

Conclusion

In conclusion, this study demonstrates that exposure to MD provoked hepatotoxicity by inducing lipid peroxidation and depletion in antioxidant enzyme activities of rats. However, Se and Zn treatment could improve the histological alteration induced by MD which could be related to the antioxidant efficacy of the synergy effect given by

by selenium and zinc. Similarly Se and Zn could protect liver against MD toxicity by reducing MDA level and increasing the activities of antioxidant enzymes. Thus to cope with MD toxicity, more attention is needed to limit its use, in one hand, and to supplement food with antioxidants as that of Se and Zn, on the other hand.

Abbreviations: MD, Methidathion; ALT, alanine transaminase; AST, aspartate transaminase; ALP, Alkaline phosphatase; LDH, lactate dehydrogenase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; LPO, lipid peroxide; MDA, malondialdehyde; O_2^- , superoxide anion; $^\bullet OH$, hydroxyl radical; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, TBA reactive substances.

Conflict of interest

The authors declare that there are no conflicts of interest.

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