Dimethoate Induced Oxidative Damage and Histopathological Changes in lung of Adult rats: Modulatory Effects of Selenium and/or Vitamin E

Ibtissem Ben AMARA[1], Nejla SOUDANI[1,¥], Afef TROUDI[1,¥], Ahmed HAKIM[2],

Khaled Mounir ZEGHAL[2], Tahia BOUDAWARA[3], and Najiba ZEGHAL[1,#]

1. Animal Physiology Laboratory, Faculty of Science, BP1171, 3000 Sfax, University of Sfax, Tunisia;
2. Laboratory of Pharmacology, Faculty of Medicine, 3029 Sfax, University of Sfax Tunisi; 3. Anatomopathology Laboratory, CHU Habib Bourguiba 3029 Sfax, University of Sfax, Tunisia

Abstract

Objective   To determine the efficiency of selenium and/or vitamin E to alleviate lung oxidative damage induced by dimethoate, an organophosphorus compound.

Methods   Adult Wistar rats were exposed during 30 days either to dimethoate (0.2 g/L of drinking water), dimethoate+selenium (0.5 mg/kg of diet), dimethoate+vitamin E (100 mg/kg of diet), or dimethoate+selenium+vitamin E.

Results   Exposure to dimethoate caused oxidative stress in lung evidenced by an increase of malondialdehyde, protein carbonyl groups and advanced oxidation protein products. An increase in glutathione peroxidase, superoxide dismutase, catalase and a decrease in acetylcholinesterase and butyrylcholinesterase activities, glutathione, non-protein thiols and vitamins C levels were observed. Histopathological changes in lung tissue were noted as emphysema, hemorrhages and hemosiderin deposits. Co-administration of selenium or vitamin E to the diet of dimethoate treated rats ameliorated the biochemical parameters as well as histological impairments. The joint effect of these elements was more powerful in antagonizing dimethoate-induced lung oxidative damage.

Conclusion   We concluded that selenium and vitamin E ameliorated the toxic effects of this pesticide in lung tissue suggesting their role as potential antioxidants.

Key words: Dimethoate; Selenium; Vitamin E; Rats; Lung; Antioxidant status; Histopathological studies

INTRODUCTION

The application of pesticides to control of pests in land and water poses nowadays potential health hazards to wildlife and humans. Among them, organophosphates (OPs) have been used for almost five decades in agriculture, veterinary medicine and industry as lubricants, plasticizers and flame-retardants. Their uncontrolled use in agriculture and public health operations has increased the scope of ecological imbalance and many non-target organisms consequently become victims[1]. Dimethoate (DM), an important organophosphorus pesticide, is frequently used against a wide range of insects and mites and for indoor control of houseflies. It causes health problems to producers, workers and farm owners[2]. Generally, the majority of the population is chronically exposed to low doses of DM via food, contaminated drinking water, or by application of household insecticides containing DM[3-4]. According to Reuber[5], exposure to DM

causes several abnormalities like benign and malignant neoplasms of the liver, endocrine organs and lymphatic system, atrophy of the testes, chronic renal disease and parathyroid hyperplasia. Recently, the World Health Organisation[6] has declared that OPs including DM induce toxicity in organisms[7-9]. The lipophilic nature of OPs facilitates their interaction with the cell membrane and leads to perturbations in the phospholipids bilayer structure, enhancing the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues[10]. Some reports indicated that the respiratory tract and in particular the lungs are the main target of ROS generated by several toxicants[11-12]. In fact, the lung is the first vital organ to come in contact with inhaled[13] and ingested toxicants like paraquat herbicide [14], chlorpyrifos[15] and fenthion[16]. Oral ingestion of OP compounds can cause adverse effects on many organs, including the lung[17]. Additionally, some studies have reported that OP compounds cause pulmonary impairments in mice and rabbits, such as alveolar congestion, hemorrhage, neutrophil infiltration, emphysematous changes, and cellular aggregation in the vascular walls or air spaces[13,18-19].

More attentions have been paid to the protective effects of natural antioxidants against chemicals-induced lung toxicities, especially when ever free radical generations are involved[15,20]. Selenium (Se) or vitamin E is used as preventive elements in various health disorders with oxidative stress as factor in their pathophysiology. It has been proposed that Se, a cofactor of the glutathione peroxidase (GPx), may prevent lipid peroxidation in mammals[21]. The major function of GPx is to detoxify hydrogen peroxide (H_2O_2) and lipid hydroperoxides[22] and to maintain cellular redox state. In addition, GPx is involved in physiological events such as differentiation, signal transduction and regulation of pro-inflammatory cytokine production[23]. Vitamin E, a naturally occurring antioxidant nutrient, plays an important role in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors[24].

To date, most studies have focused on identifying protective antioxidant agents against oxidative stress induced by pesticides, particularly OP compounds. To our knowledge, there is little information available on pulmonary oxidative stress induced by dimethoate. Besides, the potential ability of vitamin E and/or Se to attenuate lung toxicity induced by DM has not yet been investigated. Therefore, the present study aims to determine the efficiency of these elements in antagonizing oxidative damage in adult rats exposed to DM.

MATERIALS AND METHODS

Chemicals

Sodium selenite (Na_2SeO_3), Vitamin E (α-tocopherol acetate) and all other chemicals, required for all biochemical assays, were obtained from Sigma Chemicals Co. (St. Louis; MO, USA). DM [O,O-dimethyl-$S(N$-methyl-carbomethyl) phosphor-odithioate] was delivered by BASF (Ludwigshafen, Germany) with 98% purity.

Animals and Treatment

Adult rats of Wistar strain, weighing 160±10 g, were purchased from the Central Pharmacy (SIPHAT, Tunisia). They were housed at ambient temperature 22±3 °C in a 12-hour light/dark cycle and a minimum relative humidity of 40%. The animals had free access to commercial pellet diet (SICO, Sfax, Tunisia) and water ad libitum. The general guidelines for the use and care of living animals in scientific investigations were followed[25]. The handling of the animals was approved by the Tunisian Ethical Committee for the Care and Use of Laboratory Animals.

One week after acclimatization to laboratory conditions, rats were randomly divided into seven groups of six each: Group I served as controls; Group II received in their drinking water dimethoate (0.2 g/L); Group III received both dimethoate (0.2 g/L) and selenium (0.5 mg/kg of diet); Group IV was treated with dimethoate (0.2 g/L) and vitamin E (100 mg/kg of diet); Group V received dimethoate (0.2 g/L) +selenium (0.5 Na_2SeO_3 mg/kg of diet)+vitamin E (100 mg α-tocopherol acetate/kg of diet) and groups VI and VII received either selenium (0.5 Na_2SeO_3 mg/kg of diet) or vitamin E (100 mg α-tocopherol acetate/kg of diet). Treatments were carried out over a period of 30 days. In our experiments, we have tested different doses of DM: no toxic effects and no oxidative stress were observed in adult rats treated with DM at doses used between 0.1 and 0.15 g/L. From 0.2 g/L, oxidative stress was identified in adult rats without lethal effects. But with doses over 0.2 g/L, DM provokes severe signs of toxicity and mortality. So, the present study was designed to investigate the toxicity of 0.2 g/L DM administered to adult rats via drinking water. This dose which corresponds to 1/4 of LD_{50} is consistent with previous studies[2,26-28]. The Se dose (0.5 mg/kg of diet) used in our experiment and in other findings gave high protection against stress

conditions in several tissues and improved the seleno-dependent glutathione peroxidase (GSH-Px) activity in groups co-administered with selenium[29-31]. Lower doses of selenium give less protection while higher doses are not much more effective [32]. The dose of vitamin E (100 mg/kg of diet) used by Chow[33] gave high protection against toxicity and could prevent or minimize the progression of lipid peroxidation as well as antioxidants alterations, as obtained in our experimental study.

The animals in the different groups were killed, at the end of treatments, by cervical decapitation to avoid stress. Lungs were dissected out, cleaned and weighed. Some lungs were homogenized (10% w/v) in a phosphate buffer (0.1 mol/L, pH 7.4) and centrifuged at 10.000×g for 20 min. The resulting supernatants were stored at −80 °C until biochemical assays. Other lungs were immediately removed, cleaned and fixed in 10% formalin solution and embedded in paraffin for histological studies.

Biochemical Estimations

Protein Quantification Lung protein contents were measured according to the method of Lowry et al.[34] by using bovine serum albumin as standard.

Malondialdehyde (MDA) Measurement The lung malondialdehyde concentrations, index of lipid peroxidation, were determined spectrophotometrically according to Draper and Hadley[35]. Briefly, an aliquot of the lung extract supernatant was mixed with 1 mL of 5% trichloroacetic acid and centrifuged at 2500 ×g for 10 min. An amount of 1 mL of thiobarbituric acid reagent (0.67%) was added to 500 µL of the supernatant and heated at 90 °C for 15 min. The mixture was then cooled and measured for absorbance at 532 nm by using a spectrophotometer (Jenway UV-6305, Essex, England). The malondialdehyde values were calculated by using 1,1,3,3-tetraethoxypropane as standard and expressed as nmoles of malondialdehyde/g of tissue.

Determination of Lung Advanced Oxidation Protein Products Levels Advanced oxidation protein products (AOPP) levels were determined according to the method of Kayali et al.[36]. Briefly, 0.4 mL of the lung extract was treated with 0.8 mL phosphate buffer (0.1 mol/L; pH 7.4). After 2 min, 0.1 mL of 1.16 mol/L potassium iodide (KI) was added to the tube followed by 0.2 mL of acetic acid. The absorbance of the reaction mixture was immediately recorded at 340 nm. The concentration of AOPP for each sample was calculated by using the extinction coefficient of

261 $cm^{-1} \cdot mmol/L^{-1}$ and the results were expressed as µmoles/mg protein.

Determination of Lung Protein Carbonyl Content Lung protein carbonyl (PCO) content was measured with the method of Reznick and Packer[37]. Briefly, 100 µL of the lung extract supernatant were placed in glass tubes. Then 500 µL of 10 mmol/L 2,4-dinitrophenylhydrazine (DNPH) in 2 mol/L HCl were added. Tubes were incubated for 1 h at room temperature. Samples were vortexed every 15 min. Then 500 µL of TCA (20%) were added and the tubes were left on ice for 5 min followed by centrifugation for 10 min. The protein precipitates were collected. The pellet was then washed twice with ethanol-ethyl acetate (v/v). The final precipitate was dissolved in 600 µL of 6 mol/L guanidine hydrochloride solution and incubated for 15 min at 37 °C. The absorbance of the sample was measured at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH (ε=2.2x10^4 $cm^{-1} \cdot mol/L^{-1}$) and expressed as µmoles/mg protein.

Determination of Lung Antioxidant Enzyme Activities 1)*Catalase (CAT).* activity was assayed by the method of Aebi[38]. Enzymatic reaction was initiated by adding an aliquot of 20 µL of the homogenized tissue and the substrate (H_2O_2) to a concentration of 0.5 mol/L in a medium containing 100 mmol/L phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of µmole H_2O_2 consumed/(min·mg) of protein. 2)*Superoxide dismutase (SOD).* activity was estimated according to Beauchamp and Fridovich[39]. The reaction mixture contained 50 mmol/L of tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mmol/L EDTA, 13 mmol/L l-methionine, 2 µmol/L riboflavin and 75 mmol/L nitro blue tetrazolium (NBT). The developed blue colour in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units/mg of protein. 3)*Glutathione peroxidase (GPx).* activity was measured according to Flohe and Gunzler[40]. GPx catalyzes the oxidation of reduced glutathione by cumene hydroperoxide. In the presence of reduced glutathione reductase and nicotinamide adenine dinucleotide phosphate reduced form (NADPH), the oxidized reduced glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH-NADP$^+$. The decrease in absorbance at 340 nm was measured. The enzyme activity was expressed as nmole of GSH oxidized/min/mg protein.

Lung Glutathione Levels   Glutathione (GSH) in the lung was determined by the method of Ellman[41] modified by Jollow et al.[42] The method is based on the development of a yellow colour when DTNB (5,5-dithiobtis-2 nitro benzoic acid) is added to compounds containing sulfhydryl groups. Five hundred microlitres of tissue homogenate in phosphate buffer were added to 3 mL of 4% sulfosalicylic acid. The mixture was centrifuged at 1600 ×g for 15 min. Five hundred millilitres of supernatants were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as μg/g of tissue.

Hydrophilic Antioxidants   Ascorbic acid (vitamin C) determination was performed as described by Jacques-Silva et al.[43] Protein in the lung homogenate extract was precipitated in 10 volumes of a cold 4% trichloroacetic acid solution. An aliquot of sample (300 μL) was adjusted with H_2O to a final volume of 1 mL and incubated at 38 °C for 3 h, and then 1mL of H_2SO_4 65% (v/v) was added to the medium. The reaction product was determined by using colour reagent containing 4.5 mg/mL dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/mL). The data were expressed as μg ascorbic acid/g tissue.

Lung non-protein Thiols Levels   Lung non-protein thiols (NPSH) levels were determined by the method of Ellman[41]. A 500 μL aliquot of the lung homogenate extract was mixed with 10% trichloroacetic acid (1v/1v). After centrifugation, the protein pellet was discarded and free-SH groups were determined in a clear supernatant. A 100 μL aliquot of the supernatant was added to 850 μL of 1 mol/L potassium phosphate buffer (pH=7.4) and to 50 μL of DTNB (10 mmol/L) 5, 5-dithio-bis (2-nitrobenzoic acid). Absorbance of colorimetric reaction was measured at 412 nm. Total NPSH content was expressed as μmoles/g of tissue.

Determination of Acetylcholinesterase (AchE) Activity in Lung   Acetylcholinesterase activity was measured immediately in the lung homogenates extract according to the method of Ellman et al.[44], by using acetylthiocholine iodide as a substrate. The reaction mixture was composed of as follows: phosphate buffer (0.1 mol/L; pH=8) and 0.01 mol/L DTNB. The hydrolysis rate of acetylthiocholine iodide is measured at 412 nm through the release of the thiol compound which, when reacted with DTNB, produces the colour-forming compound TNB. The reaction was initiated by adding 0.075 mol/L of acetylthiocholine iodide. Activities were expressed as micromole of substrate/min/mg protein.

Butyrylcholinesterase (BuchE) Activity in Lung Butyrylcholinesterase activity was determined by the method of Ellman et al.[44] with some modifications. The hydrolysis rate was measured at acetylthiocholine concentrations of 0.8 mmol/L in 1mL assay solutions with 100mmol/L phosphate buffer, pH 7.5, and 1.0 mmol/L DTNB. Fifty microliters of the lung homogenate supernatant was added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 2-3 min (intervals of 30 s) at 25 °C. All samples were run in duplicate.

Histological Studies

Some lung samples, intended for histological examination by light microscopy, were immediately fixed in 10% of formalin and processed in a series of graded ethanol solutions. They were then embedded in paraffin, serially sectioned at 3 μm and stained with hematoxylin-eosin. Six slides were prepared from each lung. All sections were evaluated for the degree of lung injury.

Statistical Analysis

The data were analyzed by using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analyses were performed by using one-way Analysis of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) test as a post hoc test for comparison between two groups. All values were expressed as means±SD. Differences were considered significant if $P<0.05$.

RESULTS

Effects of DM on General Health

During the experiment, rats in the control group and in the Se or vitamin E-treated group did not show any sign of toxicity. However, DM treated rats showed varying degrees of clinical signs including huddling, depression, conjunctivitis, mild tremor, piloerection, diarrhea and dyspnea. The observed signs were related to the cholinergic crisis. No other significant clinical manifestations were observed in the Se+DM, vitamin E+DM, and in the Se+vitamin E+DM-treated rats.

Estimation of Lipid Peroxidation in Lung

Our results revealed an increase of lipid peroxidation in the lung of the DM-treated group as evidenced by the enhanced malondialdehyde levels in

the lung homogenate extract of adult rats (+74%) when compared to the controls (Table 1). The supplementation of Se alleviated lipid peroxidation without reaching normal values, while vitamin E totally restored malondialdehyde levels. The co-administration of Se and vitamin E significantly decreased lung malondialdehyde levels to reach control values.

Table 1. Malonaldialdehyde, Advanced Oxidation Protein Products (AOPP) and Protein Carbonyls Levels in the Lung of Adult Rat Controls or Treated during 30 Days with DM, DM+Se, DM+Vit E, DM+Se+Vit E, Se, Vit E

Parameters & Treatments	Control	DM	DM+Se	DM+Vit E	DM+Se+Vit E	Se	Vit E
Malonaldialdehyde$^{\alpha}$	51.580± 4.957	89.885± 4.211***	64.770± 4.630^{**++}	52.184± 3.947^{+++}	49.899± 2.235^{+++}	47.457± 3.834	48.362± 4.847
AOPP$^{\beta}$	0.120± 0.035	0.332± 0.042***	0.138± 0.019^{+++}	0.117± 0.047^{+++}	0.093± 0.016^{*+++}	0.109± 0.021	0.113± 0.028
Protein carbonyls$^{\gamma}$	12.772± 1.584	23.803± 2.881***	15.863± 1.860^{*++}	15.456± 1.330^{*++}	12.264± 1.865^{+++}	9.476± 1.927*	11.518± 2.275

Note. $^{\alpha}$Malonaldialdehyde: nmoles of MDA/g tissue. $^{\beta}$AOPP: μmoles/mg protein. $^{\gamma}$Protein carbonyls: μmoles/mg protein. Values are expressed as means ± SD for six animals in each group. Comparisons are made between two groups: DM, (DM+Se), (DM+Vit E), (DM+Se+Vit E), Se, Vit E group vs control group: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, (DM+Se), (DM+Vit E), (DM+Se+Vit E) group *vs* DM group: $^{++}P<0.01$; $^{+++}P<0.001$.

Protein Oxidative Damage Markers in Lung

Table 1 shows the levels of advanced oxidation protein products (AOPP) and of protein carbonyls (PCO), markers of protein oxidative damage in the lung tissue of normal and experimental animals. In the DM group, a significant increase of AOPP and PCO levels in the lung tissue of adult rats (+177 and +86%) was observed when compared to the controls. Co-treatment of rats with Se and/or vitamin E resulted in a marked decrease in lung AOPP and PCO levels when compared to the DM group.

Enzymatic Antioxidant Status in the Lung

In the lung homogenates of DM-treated rats, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities increased significantly by 58%, 93%, and 82% in adult rats, when compared to the controls (Table 2). The increase of SOD, induced by DM treatment, was restored to the normal value either by Se or vitamin E, while the catalase activity remained high. Supplementation of Se in the diet of the (DM+Se)-group alleviated the glutathione peroxidase activity which was similar to that of controls, whereas vitamin E supplementation partially ameliorated the GPx activity when compared to the DM-group. The administration of both Se and vitamin E in the diet of the DM group improved catalase, superoxide dismutase and glutathione peroxidase activities by making them reach normal values.

Table 2. Enzymatic Antioxidant Activities (glutathione peroxidase, catalase and superoxide dismutase) in the Lung of Adult Rat Controls orTreated during 30 days with DM, DM+Se, DM+Vit E, DM+Se+Vit E, Se, Vit E

Parameters & Treatments	Control	DM	DM+Se	DM+Vit E	DM+Se+Vit E	Se	Vit E
Catalase$^{\alpha}$	92.149 ±9.540	145.691± 8.202***	115.585± 16.646^{*++}	114.025 ± 8.238 $^{*++}$	92.985± 6.127^{+++}	93.910± 10.920	91.879± 10.896
Superoxide dismutase$^{\beta}$	24.957 ±5.981	48.134± 6.872***	31.921± 2.140^{+++}	32.743± 1.469^{+++}	26.855± 1.781^{+++}	23.829 ± 3.040	22.284 ± 2.634
Glutathione peroxidase$^{\gamma}$	22.743 ± 6.135	41.412± 6.126***	25.483± 5.183^{+++}	30.838± 5.784^{*++}	24.735± 4.495^{+++}	24.351 ± 4.747	25.471± 5.032

Note. $^{\alpha}$Malonaldi. $^{\alpha}$Catalase: μmoles H_2O_2 degraded/min/mg protein. $^{\beta}$Superoxide dismutase: units/mg protein. $^{\gamma}$Glutathione peroxidase: nmoles of GSH/min/mg protein. Values are expressed as means±SD for six animals in each group. Comparisons are made between two groups: DM, (DM+Se), (DM+Vit E), (DM+Se+Vit E), Se, Vit E group vs control group: $^{*}P<0.05$; $^{***}P<0.001$, (DM+Se), (DM+Vit E), (DM+Se+Vit E) group *vs* DM group: $^{++}P<0.01$; $^{+++}P<0.001$.

Table 3. Non Enzymatic Antioxidant Levels (glutathione, non protein thiols, vitamin C and E) in the Lung of Adult Rat Controls or Treated during 30 Days with DM, DM+Se, DM+Vit E, DM+Se+Vit E, Se, Vit E

Parameters & Treatments	Control	DM	DM+Se	DM+Vit E	DM+Se+Vit E	Se	Vit E
Glutathione$^{\alpha}$	57.125± 0 .131	33.937± 4.556[***]	54.425± 0 .472[*++]	46.537± 5.108[*++]	58.637± 6.660[+++]	60.687± 2.644	57.225± 3.585
Non-protein thiols$^{\beta}$	1.821± 0.286	0.942± 0.190[***]	1.577± 0.154[+++]	1.278± 0.3279[+++]	1.778 ± 0.346[+++]	1.851± 0.198	1.701± 0.198
Vitamin C$^{\gamma}$	82.968± 9.815	48.721± 7.010[***]	67.808± 8.782[*++]	73.150± 7.091[+++]	81.278± 7.635[+++]	78.082± 12.404	80.593± 6.332

Note.$^{\alpha}$Glutathione : μg/g tissue. $^{\beta}$Non-protein thiols: μmoles/g tissue. $^{\gamma}$Vitamin C: μg/g tissue. Values are expressed as means±SD for six animals in each group. Comparisons are made between two groups: DM, (DM+Se), (DM+Vit E), (DM+Se+Vit E), Se, Vit E group vs control group: [*]$P<0.05$; [***]$P<0.001$, (DM+Se), (DM+Vit E), (DM+Se+Vit E) group *vs* DM group: [++]$P<0.01$;   [+++]$P<0.001$.

Hydrophilic Antioxidant (Vitamin C) Levels in Lung

The data presented in Table 3 showed the levels of vitamin C in the control and tested groups. The exposure of rats to DM caused a significant decrease in lung vitamin C levels (-41%) when compared to the controls. Treatment with Se or vitamin E ameliorated vitamin C levels when compared to the DM group. Supplementation of both Se and vitamin E in the diet of the DM-treated group restored vitamin C levels to normal values.

Glutathione (GSH) and non Protein Thiol (NPSH) Levels in the Lung

A significant decrease of glutathione (GSH) and non protein thiol (NPSH) levels in the lung was evident in the DM group (-41% and -48%, respectively) when compared to those of controls (Table 3). Supplementation of Se alone or combined with vitamin E in the diet of the DM group ameliorated GSH and NPSH levels when compared to the DM-group.

Lung Acetylcholinesterase (AchE) and Butyrylcholinesterase (BuchE) Activities

Exposure to DM pesticide was estimated by using AChE and BuChE. Adult rats treated with DM showed a significant inhibition of lung AChE(-59%) and BuChE(-23%) activities. The administration of Se and vitamin E in the DM group ameliorated these parameters without reaching normal values (Figure 1).

Histological Studies

Light microscopic examination of the lung in the controls indicated normal aspects of alveoli and interstitial tissue (Figure 2A). In the DM-treated rats, histological sections showed abnormalities (Figures 2B) when compared to the controls. In fact, lung exhibited hemorrhages with red blood cells and purple nuclei. Other material was pink and air spaces were white. Emphysema was also noted, showing some air sac damages. There were hemosiderin deposits observed under microscopic magnification X400 (Figure 3B). Co-administration of Se or vitamin E ameliorated lung histological pictures (Figures 2C and 3D). After addition of both Se and vitamin E in the diet of the DM group, lung damages significantly decreased (Figure 2E). The histological pattern was normal in rats treated only with Se or vitamin E (Figures 2F and 2G). The histopathological changes are graded and summarized in Table 4. Histological grading was made according to four severity grades: −(none); + (mild); ++ (moderate) and +++ (severe).

Table 4. Grading of the Histopathological Changes in the Lung Sections of Adult Rat Controls or Treated during 30 Days with DM+Se, DM+Vit E,   DM+Se+Vit E, Se, Vit E [scoring was done as follows: none(−), mild(+), moderate(++) and, severe(+++) damage]

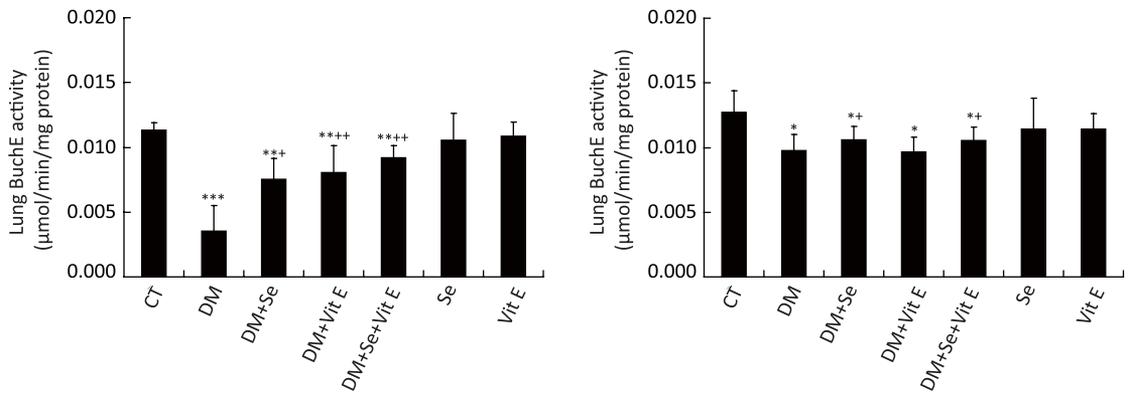| Groups	Hemorr-hage	Enlarged alveoli	Emphysema	Hemosiderin deposits
Controls	-	-	-	-
DM	++	+++	+++	+++
DM+Se	+	++	+	+
DM+Vit E	+	++	+	+
DM+Se+	-	+	-	-
Se	-	-	-	-
Vit E	-	-	-	-

Figure 1. Acetylcholinesterase (AchE) and butyrylcholinesterase (BuchE) activities in the lung of adult rat controls or treated during 30 days with dimethoate (DM), dimethoate+selenium (DM+Se), dimethoate+vitamin E (DM+Vit E),   dimethoate+selenium+vitamin E (DM+Se+Vit E), selenium (Se), vitamin E (Vit E). Values are expressed as means±SD. DM, (DM+Se), (DM+Vit E), (DM+Se+Vit E), Se, Vit E group *vs* control group: [*]P<0. 05; [**]P<0.01; [***]P<0.001, (DM+Se), (DM+Vit E), (DM+Se+Vit E) group *vs* DM group: [+]P<0.05; [++]P<0.01.
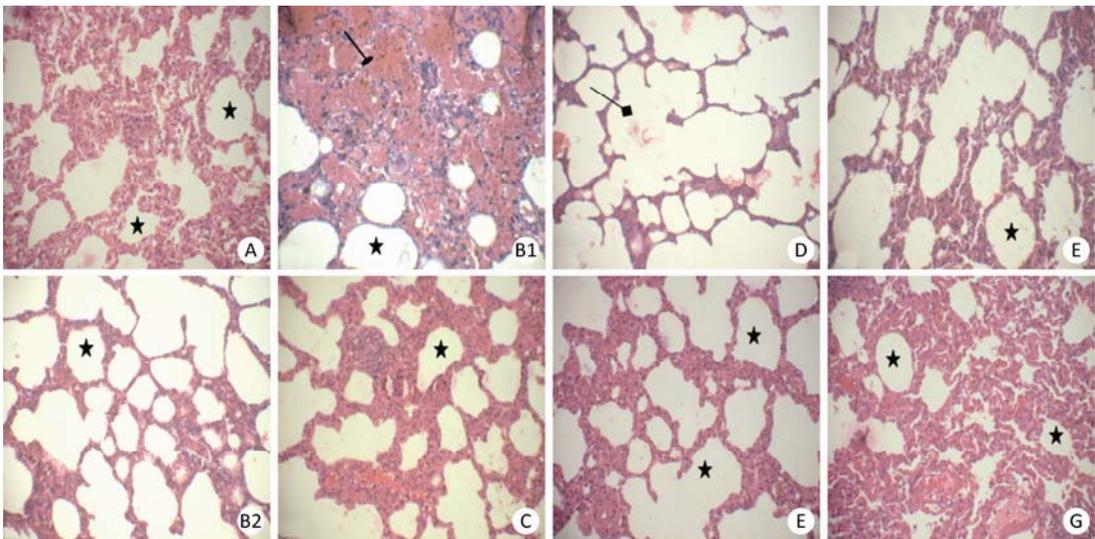
Figure 2. Histological findings in the lung tissue of adult rats from the seven experimental groups: (A) Control group—showed normal alveoli and interstitial tissue; (B₁) Dimethoate group—showed hemorrhages: red blood cells are red, nuclei are purple, other material is pink, and air spaces are white (indicated by arrows) ; (B₂) Dimethoate group—showed emphysema with damaged air sacs (indicated by arrows); (C) Dimethoate+selenium group—Histological damage decreased; (D) Dimethoate+vitamin E group—Histological picture showed a significant decrease in the lung damages; (E) Dimethoate+selenium+vitamin E group—Histological picture showed normal lung tissue; (F) Selenium or (G) vitamin E groups—showed normal lung tissue without any pathological changes. Hematoxylin-eosin, X200. Arrows indicate: ★:normal alveoli,   ➤◆:emphysema, ➤◗:haemorrhage.
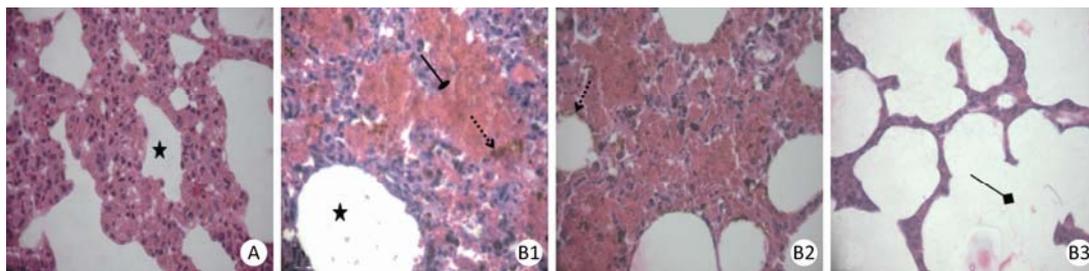
Figure 3. Histological findings in the lung tissue of adult rats from the seven experimental groups: (A) Control group—showed normal alveoli and interstitial tissue; (B₁) Dimethoate group—showed hemorrhages and hemosiderin deposits in interstitial tissue (indicated by arrows); (B₂) Dimethoate group—showed hemosiderin deposits in alveoli (indicated by arrows); (B₃) Dimethoate group— showed emphysema. Hematoxylin-eosin, 400. Arrows indicate: ★:normal alveoli, ◆:emphysema, ◀:haemorrhage, ⋯▶:hemosiderin.

DISCUSSION

Dimethoate is an organophosphate insecticide known to produce oxidative stress in human and animal cells. As a lipophilic molecule, it can easily pass through the cell membrane into the cytoplasm. Once inside the cell, dimethoate can induce a high level of damages in several tissues including lung. In the present study, exposure of rats to DM through drinking water resulted in a significant increase in lipid peroxidation and protein oxidation in lung tissue as indicated by the significant increase in MDA content, protein carbonyls and AOPP levels, suggesting that DM activated the formation of free radicals. Our results were in accordance with those obtained by Uzun et al.[15] and Cemek et al.[16], who found an induction of oxidative stress in lung tissue after oral ingestion of chlorpyrifos and fenthion, the organophosphorus compounds. According to some authors, the generation of reactive oxygen species (ROS) may be the result of organophosphates metabolism by cytochrome P450s, monooxygenases which catalyze oxidation by addition of molecular oxygen atom into a substrate (organophosphate) through an electron transport pathway[45-47]. Another way of ROS generation in OP toxicity is a high energy consumption coupled with an inhibition of oxidative phosphorylation[48]. Moreover, proteins can be modified by direct attack of ROS, giving rise to carbonyl group formation into side chains and/to sulfhydryl groups reduction in amino acids[49]. In our study, Se or vitamin E supplemented in the diet of the DM group alleviated lung injury by decreasing LPO and protein oxidation. This could be explained by the important role of vitamin E and Se in preventing hydroxyl radicals' formation and in protecting the integrity and the function of tissues[29,50-51]. These

elements could be therefore useful as free radical scavenger compounds against stress conditions in the lung. The latter has a rich network of antioxidant defenses to protect itself from oxidative stress[52]. Among them, antioxidant enzymes (GPx, SOD and CAT) are the line of defense in the lung against oxidants[53]. The present results showed an increase of GPx, catalase and SOD activities which could reflect an adaptation to oxidative conditions. Moreover, the enhancement of LPO, obtained by us and by Aggarwal et al.[54], indicated that free radicals production exceeded the capacity of detoxification mechanisms. Our observations concerning the increase in antioxidants activities were in accordance with some studies conducted on rats exposed to dimethoate and showing toxicity in liver, brain and serum[2,55-56]. Additionally, the current study showed that treatment with DM associated to nutritional supply of vitamin E or Se improved SOD, GPx and catalase activities as compared with the DM group. Yet, the joint effect of Se and vitamin E is more powerful than Se or vitamin E used alone in improving antioxidants activities against lung injury. Our results were in accordance with those of Chappuis and Poupon[57], who demonstrated that the protection of cells against oxidative stress, induced by xenobiotics, takes place with both vitamin E and Se involved in the capture of free radicals.

Among the antioxidant defense system of the respiratory tract, non protein thiols (NPSH), glutathione (GSH) and vitamin C play an important role against oxidative stress in lung tissue. In fact, NPSH is an intracellular antioxidant in some compartments such as the epithelial lining fluid of the lung[58]. This antioxidant has been implicated in various cellular events in pulmonary tissue such as inflammatory response, modulation of

redox-regulated signal transduction, regulation of cell proliferation, remodeling of extracellular matrix, maintenance of surfactant and antiprotease screen, apoptosis, immune modulation and mitochondrial respiration[59]. Likewise, GSH is considered to be an important scavenger of free radicals and as a cofactor of several detoxifying enzymes against oxidative stress, e.g., glutathione peroxidase, glutathione-S-transferase and others[58]. In our study, GSH and NPSH levels decreased in the lung extracts of the DM-treated group compared to those of control rats, reflecting their consumption through the oxidative stress. Our results were in accordance with Kaviarasan et al.[61], who showed that low levels of non enzymatic antioxidants may result from their increased utilization to scavenge free radicals and to protect other cellular constituents from oxidative damage. Moreover, the decreased concentration of vitamin C in the lung of DM-treated rats could be explained by the depletion of GSH since it is directly involved in recycling this vitamin as reported by Chen et al.[62]. When vitamin E was supplemented in the diet of the DM group, GSH and vitamin C levels were improved, demonstrating the potential protective effects of α-tocopherol. Both vitamin E and selenium are able to compensate or repair lung damages against the harmful effects of DM by improving the antioxidants defense system. In fact, the scavenging property and the ability of Se to inhibit the radical generation could further reduce the oxidative threat caused by DM. According to Saito and Takahashi[63], Se supplementation increased the activities of selenoproteins, perhaps by the high incorporation of selenocysteine in selenoproteins, which may decrease free radical-mediated LPO and regenerate glutathione.

The most common method to evaluate the biological impact of OPs on target and non-target species is the assessment of AChE inhibition[64]. This method has been widely used as a specific biomarker of anticholinesterase agents, like OPs[65]. AchE has been described to contribute to the integrity and permeability of the synaptic membrane during neurotransmission and conduction[17]. Recent evidence suggests that, besides AchE, BuChE catalyses the hydrolysis of the neurotransmitter, acetylcholine, and serves as a co-regulator of cholinergic transmission[66]. BuChE is a coregulator with AchE of acetylcholine levels in airways[67] and trachea[68]. BuChE is an excellent bioscavenger of OPs pesticides and is used to evaluate pulmonary function[69]. In our experimental study, a decrease of AchE and BuchE, in

the DM group, was noted as well as several clinical signs of toxicity which spoke in favour of muscarinic intoxication, as previously reported by Thabet et al.[70] and Jokanovic and Kosanovic[71]. On the other hand, the decrease of AchE is the main mechanism of OP induced oxidative stress. In fact, according to Abbas and Hayton[72] and Stenersen[73], OP inhibits AchE activity by targeting the serine hydroxyl group on the AchE active site, where they bind to and inactivate the enzyme. In addition, the decreasing AchE activity was also claimed to be attributed to the secondary structure damage of enzyme via chemicals bound to sulfhydryl group, besides serine amino acids in active enzyme region[74]. In the same context, Das and Mukherjee[75] have stated that the reason for decrease of AchE enzyme activity might be due to pollutant effects directly on the active part of enzyme or indirectly on the inhibition of enzyme synthesis. Moreover, it is known that the superoxide radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzyme and decrease AchE enzyme activity. Likewise, BuchE activity is reduced when the cells are more susceptible to free radicals actions, probably in consequence to changes of antioxidant enzyme activities in several tissues including lung[17,76]. Our results showed amelioration in AchE activity after vitamin E and/or Se supplies to the diet of the DM group. These elements probably protected AchE and BuchE activities via their antioxidant properties. Our hypothesis is in agreement with the previous findings of Tsakiris et al.[77], Melo et al.[78], and Delwing-de Lima et al.[76], showing that antioxidants are able to prevent AchE and BuchE alterations.

To substantiate the biochemical findings, lung histological examination was undertaken. DM treatment caused pulmonary impairment such as hemorrhage, hemosiderin deposits and emphysema in alveoli. The latters are the air spaces where oxygen is exchanged with carbon dioxide in the blood. After DM treatment, alveoli became enlarged, leading to emphysema, a respiratory disease. This enlargement caused break down of alveoli walls or rupture narrowing the airways that provoked difficulty in breathing oxygen and exhaling carbon dioxide. This could be due to reactive oxygen species or inflammatory cells, including activation of lung macrophages and infiltration of neutrophils, which might contribute to emphysematous changes found by us and by others[79-80]. In addition, massive and continuous oxidative stress may overwhelm the antioxidant capacity, thereby causing damage to the

lung tissue[81]. Therefore, it is possible that ROS accumulation occuring in lung tissue of the DM group could explain histopathological pictures in which emphysema may take place. Additionally, there were hemosiderin deposits in lung tissue of the DM group, suggesting that their formation may be related, according to Epstein et al.[82], to phagocytosis of red blood cells and hemoglobin following hemorrhage. Vitamin E and/or Se could serve as the potential protective agents against lung tissue damages induced by DM. As reported by Shirpoor et al.[83], vitamin E is able to compensate or repair tissue disturbances by abrogating apoptotic signals, suppressing LPO and protein oxidation and by improving the antioxidant defense system. Moreover, selenium protects tissue against damages induced by free radicals and also permits the regeneration of lipid molecules in membrane through reacylation[84].

In conclusion, the present data show that DM intoxication enhances lipid peroxidation in lung tissue, disturbs the antioxidant system and induces lung histopathological changes. The co-administration of Se and vitamin E with DM attenuates the toxicity of this pesticide in adult rats, objectified by biochemical and histological improvement. On the basis of this study, it should be taken into consideration that the nutritional supplementation of Se and vitamin E, during DM exposure, might act as a protective agent against lung injury induced by this organophosporus.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

REFERENCES

1. Das BK, Mukherjee SC. Chronic toxic effects of quinalphos on some biochemical parameters in Labeo rohita (Ham.). Toxicol Lett, 2000; 114, 8-11.
2. Sharma Y, Bashir S, Irshad M, et al. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicology, 2005; 206, 49-57.
3. WHO/IPCS. Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Environmental Health Criteria vol. 180. WHO, Geneva, 1996; 110-2.
4. WHO/IPCS. Classification of pesticides by hazard and guidelines to classification. Switzerland: Geneva, 2001.
5. Reuber MD. Carcinogenicity of dimethoate. Environ Res, 1984; 34, 193.
6. WHO. The WHP Recommended Classification of Pesticides by Hazard and Guidelines to Classification. World Health Organization Programme on Chemical Safety, Geneva, 2009.
7. Banerjee BD, Seth V, Ahmed RS. Pesticide-induced oxidative stress: perspectives and trends. Rev Environ Health, 2001; 16, 1-40.
8. Akhgari M, Abdollahi M, Kebryaeezadeh A, et al. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Hum Exp Toxicol, 2003; 22, 205-11.
9. Sivapiriya V, Jayanthisakthisekaran J, Venkatraman S. Effects of dimethoate (O,O,-dimethyl S-methyl carbamoylmethylphos-phorodithioate) and ethanol in antioxidant status of liver and kidney of experimental mice. Pest Biochem Physiol, 2006; 85, 115-21.
10. Videira RA, Antunes-Madeira MC, Lopes VI, et al. Changes induced by malathion, methylparathion and parathion, on membrane lipid physicochemical properties, correlate with their toxicity. Biochim Biophys Acta, 2001; 1511, 360-8.
11. Luchese C, Stangherlin EC, Gay BM, et al. Antioxidant effect of diphenyl diselenide on oxidative damage induced by smoke in rats: Involvement of glutathione. Ecotoxicol Environm Saf, 2009; 72, 248-54.
12. Pinton S, Luchese C, Stangherlin EC, et al. Acute exposure to diphenyl ditelluride causes oxidative damage in rat lungs. Ecotoxicol Environ. Saf. doi:10.1016/j.ecoenv.2010.07.031.
13. Morowati M. Inhalation toxicity studies of Thimet (Phorate) in the male swiss albino mouse, Mus musculus: II. Lung histopathology, pseudocholinesterase level and haematological studies. Environ Pollut, 1998; 103, 309-15.
14. Malekinejad H, Rahmani F, Hassan F. Influences of sub-acute exposure to paraquat on cytochrome P450 3A2 expression in rat liver and lungs. Pestic Biochem Physiol, 2010; 96, 149-54.
15. Uzun FG, Demir F, Kalender S, et al. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food Chem Toxicol, 2010; 48, 1714-20.
16. Cemek M, Büyükokuroğlu ME, Büyükben A, et al. Effects of vitamin E and selenium on tissue bio-element status in organophosphate toxicity of rats. Pestic Biochem Physiol, 2010; 98, 9-18.
17. Celik I, Isik I. Neurotoxic effects of subacute exposure of dichlorvos and methyl parathion at sublethal dosages in rats. Pestic Biochem Physiol, 2009; 94, 1-4.
18. Yurumez Y, Ikizceli I, Sozuer EM, et al. Effect of inteleukin-10 on tissue damage caused by organophosphate poisoning. Basic Clin Pharmacol Toxicol, 2007; 100, 323-7.
19. Yavuz Y, Kaya E, Yurumez Y, et al. Technetium-99m diethylenetriaminepentaacetic acid radioaerosol scintigraphy in organophosphate induced pulmonary toxicity: experimental study. Clin Toxicol, 2008; 46, 711-5.
20. Khan RA, Khan MR, Sahreen S. Protective effect of Sonchus asper extracts against experimentally induced lung injuries in rats: A novel study Exp. Toxicol. Pathol, 2011. doi:10.1016/j.etp.2011.01.007.
21. Crespo AMV, Lanca MJ, Vasconcelos S, et al. Effect of selenium supplementation on some blood biochemical parameters in male rats. Biol. Trace Element Res, 1995; 47, 343-7.
22. Pappas AC, Zoidis E, Surai PF, et al. Selenoproteins and maternal nutrition. Comp. Biochem. Physiol, 2008; 151, 361-72.

23. Moghadaszadeh B, Beggs AH. Selenoproteins and their impact on human health through diverse physiological pathways. Physiology, 2006; 21, 307-15.

24. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J Trace Elem Med Biol, 2004; 18, 113-21.

25. Council of European Communities. Council instructions about the protection of living animals used in scientific investigations. Official Journal of the European Communities, 1986, (JO 86/609/CEE) L358, 1-18.

26. Sharma Y, Bashir S, Irshad M, et al. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology, 2005b; 215, 173-81.

27. Kamath V, Rajini PS. Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. Toxicology, 2007; 231, 137-46.

28. Mahjoubi-Samet A, Fetoui H, Zeghal N. Nephrotoxicity induced by dimethoate in adult rats and their suckling pups. Pestic Biochem Physiol, 2008; 91, 96-103.

29. Ognjanovic BI, Markovic SD, Pavlovic SZ, et al. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. Physiol Res, 2008; 57, 403-11.

30. Ben Amara I, Troudi A, Garoui E, et al. Protective effects of selenium on methimazole nephrotoxicity in adult rats and their offspring. Exp. Toxicol Pathol, 2010. doi:10.1016/j.etp.2010.04.007.

31. Soudani N, Troudi A, Bouaziz H, et al. Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. Ecotoxicol EnvironSaf, 2011; 74, 513-20.

Hotz CS, Fitzpatrick DW, Trick KD, et al. Dietary iodine and selenium interact to affect thyroid hormone metabolism of rats. J Nutr, 1997; 127, 1214-8.

33. Chow CK. Effect of dietary vitamin E and selenium on rats: pyruvate kinase, glutathione peroxidase and oxidative damage. Nutr Res, 1990; 10, 183-94.

34. Lowry OH, Rosebrugh NJ, Farr AL, et al. Protein measuremernt with the Folin phenol reagent. J Biol Chem, 1951; 193, 265-75.

35. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol, 1990; 186, 421-31.

36. Kayali R, Cakatay U, Akcay T, et al. Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissues of ageing rat. Cell Biochem. Funct, 2006; 24, 79-85.

37. Reznick AZ, Packer L. Oxidative Damage to Proteins: Spectrophotometric Method for Carbonyl. Method Enzymol. Academic Press, New York, 1994, pp. 357.

38. Aebi H. Catalase in vitro. Method Enzymol, 1984; 105, 121-6.

39. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acryl amide gels. Anal Biochem, 1971; 44, 276-87.

40. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Method Enzymol, 1984; 105, 114-21.

41. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys, 1959; 82, 70-7.

42. Jollow DJ, Mitchell JR, Zampaglione N, et al. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzeneoxide as the hepatotoxic intermediate. Pharmacology, 1974; 11, 151-69.

43. Jacques-Silva MC, Nogueira CW, Broch LC. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharm. Toxicol, 2001; 88, 119-25.

44. Ellman GL, Courtney KD, Andres V, et al. A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochem Pharmacol. 1961;7, 88-95.

45. Jakoby WB, Ziegler DM. The enzymes of detoxication. J Biol Chem, 1990; 265, 29715-8.

46. White RE. The involvement of free radicals in the mechanisms of monooxygenases. Pharmacol Ther, 1991; 49, 21-42.

47. Chambers JE, Carr RL, Boone S, et al. The metabolism of organophosphorus insecticides, Handbook of Pesticide Toxicology, Ed. II, Academic Press, 2001; USA 2, 919-27.

48. Milatovic D, Gupta R C, Aschner M (2006). Anticholinesterase toxicity, oxidative stress. Sci World J, 2006; 6, 295-310.

49. Halliwell B, Gutteridge JMC. Oxidative stress: adaptation, damage, repair and death. In: Free Radicals in Biology and Medicine, Oxford University Press, Oxford, 2001, pp. 246-350.

50. Pascoe G, Olafsdottir K, Read DJ. Vitamin E protection against chemical induced cell injury. I. Maintenance of cellular protein thiols as a cytoprotective mechanism. Arch Biochem Biophys, 1987; 256, 150-8.

51. Li X, Hill KE, Burk RF, et al. Selenium spares ascorbate and K-tocopherol incultured liver cell lines under oxidant stress. FEBS Lett, 2001; 508, 489-92.

52. Morcillo EJ, Estrela J, Cortijo J. Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants. Pharmacol Res, 1999; 40, 393-404.

53. Van der Vliet A, Cross CE. Oxidants, nitrosants, and the lung. Am J Med, 2000; 109, 398-421.

54. Aggarwal M, Naraharisetti SB, Sarkar SN, et al. Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: A biochemical study. Arch Environ Contam Toxicol, 2009; 56, 139-48.

55. Sayim F. Dimethoate-induced biochemical and histopathological changes in the liver of rats. Exp Toxicol Pathol, 2007; 59, 237-43.

56. Attia AM, Nasr H. Dimethoate-induced changes in biochemical parameters of experimental rat serum its neutralization by black seed (Nigella Sativa L.) Oil Slovak J Anim Sci, 2009; 42, 87-94.

57. Chappuis P, Poupon J. Le rôle de sélénium dans la défense du stress oxydatif. Cah Nutr Diet, 1991; 26, 295-7.

58. Moss M, Guidot DM, Wong-Lambertina M, et al. The effects of chronic alcohol abuse on pulmonary glutathione homeostasis. Am J Respir Crit Care Med, 2000; 161, 414-9.

59. Rahman I, MacNee W. Oxidative stress and regulation of glutathione in lung inflammation. Eur Respir J, 2000; 16, 534-54.

60. Masella R, DiBenedetto R, Vari R, et al. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem, 2005; 16, 577-86.

61. Kaviarasan S, Sundarapandiyan R, Anuradha CV. Protective action of fenugreek (Trigonella foenum graecum) seed polyphenols against alcohol-induced protein and lipid damage in rat liver. Cell Biol Toxicol, 2008; 24, 391-400

62. Chen Z, Young TE, Ling J, et al. Increasing vitamin C content of plants through enhanced ascorbate recycling. Proc Natl Acad Sci USA, 2003; 100, 3525-30.

63. Saito Y, Takahashi K. Characterization of selenoprotein P as a selenium supply protein. Eur J Biochem, 2002; 269, 5746-51.

64. Ferda DEA, Ugur S. The effect of Dichlorvos on acetylcholinesterase activity in some tissues in rats. Acta Vet, 2010; 60, 123-31.

65. Galgani F, Bocquené G. Molecular biomarkers of exposure to marine organisms to organophosphorus pesticides and carbamates, in: L. Lagadic, J.-C. Amiard (Eds.), Use of

biomarkers for environmental quality assessment. CBC Press, 2000, pp. 120-1.

66. Geula C, Darvesh S. Butyrylcholinesterase, cholinergic neurotransmission and the pathology of Alzheimer's disease. Drugs Today, 2004; 40, 711-21.

67. Norel X, Angrisani M, Labat C, et al. Degradation of acetylcholine in human airways: role of butyrylcholinesterase. Br J Pharmacol, 1993; 108, 914-9.

68. Adler M, Filbert MG. Role of butyrylcholinesterase in canine tracheal smooth muscle function. FEBS Lett, 1990; 267, 107-10.

69. Manoharan I, Boopathy R, Darvesh S, et al. A medical health report on individuals with silent butyrylcholinesterase in the Vysya community of India. Clin Chim Acta, 2007; 378, 128-35.

70. Thabet H, Brahmi N, Kouraïchi N, et al. Intoxications par les pesticides organophosphorés: nouveaux concepts. Organophosphorus poisoning: New concepts. Réanimation, 2009; 18, 633-9.

71. Jokanovića, M, Kosanović M. Neurotoxic effects in patients poisoned with organophosphorus pesticides. Environmental Toxicology and Pharmacology, 2010; 29, 195-201.

72. Abbas R, Hayton WL. A physiologically based pharmacokinetic and pharmacodynamic model for paraoxon in rainbow trout. Toxicol Appl Pharmacol, 1997; 145, 192-201.

73. Stenersen J. Chemical pesticides: Mode of action and toxicology. CRC Press Baca Ration, Florida, 2004.

74. Szabo A, Nemcsok J. The effect of pesticides on carp (Cyprinus carpio). Acetylcholinesterase and its biochemical characterization. Ecotoxicol Environ Saf, 1992; 23, 39-45.

75. Das BK, Mukherjee SC. Chronic toxic effects of quinalphos on some biochemical parameters in Labeo rohita (Ham). Toxicol Lett, 2000; 114, 8-11.

76. Delwing-de Lima D, Wollinger LF, Casagrande AC, et al. Guanidino compounds inhibit acetylcholinesterase and butyrylcholinesterase activities: Effect neuroprotector of vitamins E plus C. Int J Dev Neurosc, 2010; 28, 465-73.

77. Tsakiris S, Angelogianni P, Schulpis KH, et al. Protective effect of l-phenylalanine on rat brain acetylcholinesterase inhibiton induced by free radicals. Clin Biochem, 2000; 33, 103-6.

78. Melo JB, Agostinho P, Oliveira CR. Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. Neurosci Res, 2003; 45, 117-27.

79. Andreadis AA, Hazen SL, Comhair SA, et al. Oxidative and nitrosative events in asthma. Free Radic Biol Med, 2003; 35, 213-25.

80. Carnevali S, Petruzzelli S, Longoni B, et al. Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. Am J Physiol Lung Cell Mol Physiol, 2003; 284, L955-63.

81. Lee JH, Lee DS, Kim EK, et al. Simvastatin inhibits cigarette smoking-induced emphysema and pulmonary hypertension in rat lungs. Am J Respir Crit Care Med, 2005; 172, 987-93.

82. Epstein CE, Elidemir O, Colasurdo GN, et al. Time course of hemosiderin production by alveolar macrophages in a murine model. Chest Dec, 2001; 120, 2013-20.

83. Shirpoor A, Salami S, Khadem-Ansari MH, et al. Cardioprotective effect of vitamin E: rescues of diabetes-induced cardiac malfunction, oxidative stress, and apoptosis in rat. J Diabetes Complications, 2009; 23, 310-6.

84. McPherson A. Selenium vitamin E and biological oxidation. In: Cole DJ, Garnsworthy PJ (eds) Recent advances in animal nutrition. Oxford: Butterworth and Heinemann's, 1994; p.3-30.