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Dimethoate-induced Oxidative Stress and Morphological Changes in the Liver of Guinea Pig and the Protective Effect of Vitamin C and E

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ABSTRACT

Dimethoate (DM), an organophosphate insecticide, has been used worldwide in agriculture and domestic for several years which has led to a variety of negative effects in non target species including humans. Therefore, the present study investigated the ameliorative properties of vitamin C (vitC) and E (vitE) on DM toxicity of guinea pigs. The animal groups were orally administered with either vehicle, vitC (200 mg kg^{-1} body weight) and vitE (200 mg kg^{-1} body weight), 1/50 LD_{50} of DM (7 mg kg^{-1} b.w.) and 1/50 LD_{50} of DM + vitC and vitE daily for 28 days. Administration of DM resulted in a significant increase in the levels of various serum marker enzymes (AST ALT and ALP). Similarly, significant increase in Lipid Peroxidation (LPO) level while induced significant decreases in the activities of liver Catalase (CAT) and Glutathione-S-Transferase (GST). In contrast, co-administration of vitC and vitE to DM-treated animals restored most of these biochemical parameters to nearly normal levels. Also, DM induced histopathological alterations such as cytoplasmic vacuolization and degeneration in nuclei, congestion, an enlargement of the blood vessels and lymphocytes infiltration in the liver. These changes were ameliorated by vitamins co-administration. The results showed that co-treatment of vitE and vitC protected guinea pigs from DM-induced biochemical and histopathological changes.

Key words: Dimethoate, guinea pigs, oxidative stress, vitamins

INTRODUCTION

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors (Prakasam *et al*., 2001). The use of pesticides causes severe environmental and health hazards to organisms (Abdollahi *et al*., 2004; Tuzmen *et al*., 2008). Organophosphate (OP) compounds are widely used and include some of the most toxic chemical agents. Due to their high insecticidal activity, low environmental persistence and moderate toxicity, the OP compounds are the most favored insecticides. They are widely used in agriculture, medicine, industry and have caused severe environmental pollution (Al-Saleh, 1994; Storm *et al*., 2000). Recently, more than 100 different OP compounds have been synthesized and are extensively used worldwide (Buyukokuroglu *et al*., 2008). These pesticides may reach the marine environment through rivers and the atmosphere (UNEP, 1991). OP pesticides are known to cause inhibition of

Acetylcholinesterase (AChE) activity in the target tissues (Jayaratnam and Maroni, 1994) which accumulates acetylcholine and prevents the smooth transmission of nerve functions leading to convulsions and death. Exposure to OP pesticides is associated with toxic effects on humans and animals (De-Bleecker *et al*., 1993; Betrosian *et al*., 1995; Tsatsakis *et al*., 1998; Hagar *et al*., 2002). Toxicity of OP pesticides results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (Reuber, 1984; Aly and El-Gendy, 2000). Dimethoate (DM) [O,O-dimethyl-S(N-methylcarbomethyl) phosphorodithioate] which is one of the most important OP pesticides, is frequently used in agriculture against a wide range of insects and mites as both a systemic and a contact pesticide. It is also used for indoor control of houseflies. The residue of DM and its analog are also found in foods, including cow's milk (Srivastava and Raizada, 1996). Previous studies indicate that DM intoxication causes cellular injury and oxidative stress which leads to lipid peroxidation and free radical production (Maiti *et al*., 1996; Maiti and Kar, 1997; Singh *et al*., 2004, 2006; Sharma *et al*., 2005a, b). Recent studies have shown that acute and subchronic exposure to DM alters the antioxidant status and the histology of liver, brain and testes of rats (Sayim, 2007b; Astiz *et al*., 2009; Saafi *et al*., 2011) and human erythrocytes (Gargouri *et al*., 2011). The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals and drugs. Hepatotoxicity is therefore an important endpoint in the evaluation of the effect of a particular xenobiotic. Clinical chemistry and histopathological evaluations are commonly used methods for detecting organ-specific effects related to chemical exposure (Travlos *et al*., 1996). Vitamin C (vitC) is a well-known low molecular weight antioxidant that protects the cellular compartment from water-soluble oxygen nitrogen radicals (Jurczuk *et al*., 2007). Vitamin E (vitE) has long been recognized as being the major lipid-soluble, chain breaking antioxidant that prevents free radicals from initiating peroxidative tissue damage (Verma *et al*., 2007). Several experimental studies have shown that vitamins C and E could ameliorate pesticide toxicity (Altuntas *et al*., 2002; Yavuz *et al*., 2004; Uzunhisarcikli *et al*., 2007). It have also shown that, vitE inhibits free radical formation by scavenging lipid peroxyl radicals and is converted into α-tocopheroxyl radical as a consequence (Arita *et al*., 1998; Kalender *et al*., 2004, 2005b) and may effectively minimize lipid peroxidation in biological systems (Kalender *et al*., 2002). Synergistic effect of antioxidants is most powerful in reducing storage and toxicity of reactive oxygen species (Schwenke and Behr, 1998; Aslam *et al*., 2010). In fact, several studies demonstrated that the cellular antioxidant activity is reinforced by the presence of dietary antioxidants (Prior and Cao, 2000; Pincemail *et al*., 2002; Kiefer *et al*., 2004). Accordingly, interest has recently grown in the used of antioxidants to prevent oxidative damage as a factor in the pathophysiology of various health disorders (Kalender *et al*., 2005a; Koechlin-Ramonatxo, 2006; Kasdallah-Grissa *et al*., 2007; Mehmetc-ik *et al*., 2008; Shireen *et al*., 2008). In this regard, studies on vitamin C and E are promising, mainly due to their antiradical activity, indicating that they could provide an important dietary source of antioxidants.

The present study was undertaken to investigate some of the biochemical and histopathological alterations which might occur as a result of DM intoxication. In addition, to study the protective effect of vitC and vitE supplementation on DM induced liver injury in guinea pigs.

MATERIALS AND METHODS

Chemicals: Dimethoate 40 EC was applied as a commercial emulsifiable concentrate formulation containing 40% active ingredient. Vitamin C (Shaphar, Shanghai pharmaceutical Co. Ltd., China) and vitamin E (Pharco Pharmaceutical, Alexandria, Egypt) were used for this study. Both the DM and vitE were reconstituted appropriately in olive oil for the final concentration immediately prior to use.

Animals and treatment schedule: Adult male guinea pigs (weighing 550-700 g) were obtained from the animal house of Biology department, Ibb University-Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions with free access to diet and water *ad libitum*. Guinea pigs have been used because they, like humans, are incapable of synthesizing ascorbic acid; also, some metabolic characteristics in guinea pigs are similar to those in humans (Stith and Das, 1982). Animals were randomly divided into four groups with five animals each. Animals of the 1st group were served as control and given the vehicle. Animals of the 2nd group were orally given a combined dose of vitC and vitE at a dose of 200 mg kg^{-1} b.w. day^{-1}. VitC and vitE were dissolved in water and olive oil, respectively. Animals of 3rd group were orally given DM (7 mg kg^{-1} b.w. per day; 1/50 of the LD_{50}) dissolved in olive oil. Animals of 4th group were administrated with DM preceding by 30 min with vitC and vitE at the same previous dose of all. The regime schedule were selected according to previous studies (Kalender *et al*., 2010; Mansour and Mossa, 2010). All the previous administrations were repeated daily for 28 days. At the end of the 4th week (28 days) of treatment, the animals were sacrificed and dissected. Blood and tissue samples were taken for biochemical and light microscope investigations. Care and treatment of animals was approved and practices were performed according to approval of ethics regulation at the Ibb University.

Estimation of liver function: The activities of cellular enzymes (AST, ALT) were determined by the methods of Tietz (1970). While, the activity of ALP was determined by the methods of King (1965). The enzymes activity was expressed as U L^{-1}.

Measurement of lipid peroxidation: Lipid Peroxidation (LPO) was determined based on that of Ohkawa *et al*. (1979). A 10 (w/v) tissue homogenate from the liver was required for this assay (this homogenate contained 1%, v/v, dimethyl sulfoxide to prevent further oxidation). To 0.2 mL Aliquots of tissue homogenate was added 0.2 mL 8.1% (w/v) sodium dodecyl sulfate solution, 1.5 mL 20% (v/v) acetic acid solution (pH 3.5) and 1.5 mL 0.8% (w/v) thiobarbituric acid solution. The mixture was made up to 4.0 mL with distilled water and heated to 95°C for 1 h. The samples were cooled and centrifuged at 2000 xg for 10 min and absorbance measured at 532 nm. Results were expressed as nmol malondialdehyde formation/mg protein.

Estimation of antioxidant enzymes: Catalase (CAT) activity was measured by the method of Aebi (1984). The reaction mixture was consisted of 0.5 mL phosphate buffer (50 mM, pH 7.0), 0.1 mL of sample, 0.5 mL of 30 mM 1mL H_2O_2 and distilled water to make a total volume of 1.5 mL. Change in absorbance was recorded at 240 nm. Catalase activity was calculated in terms of µmols H_2O_2 consumed/min/mg protein.

Glutathione-S-Transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al*. (1974) using S-2, 4-dinitrophenyl glutathione (CDNB) as a substrate. The principle of the method is based on measurement of the conjugation of CDNB with reduced glutathione. The formation of adduct of CDNB, S-2,4-dinitrophenyl glutathione was monitored by measuring the net increase in absorbance at 340 nm against the blank. The activity of GST was expressed in terms of µmol/min/mg protein.

The total protein content of liver homogenate was determined by the method of Lowry *et al*. (1951).

Histopathological examination: Control and experimental animals were put under light ether anaesthesia, dissected as quickly as possible and then livers were removed. Small pieces were fixed in 10% neutral formalin for 24 h, then washed by the running tap water and stored in 70% ethyl alcohol, until further processing. Blocks of about 5×5 mm size were dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and stained with haematoxylin and eosin.

Statistical analysis: The quantitative values obtained were expressed as Mean±SD. Total variation, present in a set of data was estimated by one-way Analysis of Variance (ANOVA). Differences with a p-value of <0.05 were considered as statistically significant. Post hoc analysis of group differences was performed by LSD test. The treated groups were compared both with each other and with untreated control groups.

RESULTS

Results of liver function: The administration of DM stimulated the activity of AST by 64% versus those of control animals. Statistically, the stimulation was significant ($p<0.01$). Co-treatment with vitC and vitE to the DM-administrated animals significantly inhibited ($p<0.05$) the activity of AST and the inhibition was 28%.

The activity of ALT was stimulated by 37% after DM-administration compared with controls. The statistical analysis showed that the stimulation was significant ($p<0.01$). When vitC and vitE administered to DM-given animals, the activity of ALT was significantly inhibited ($p<0.01$) by 21%. There was a significant stimulation ($p<0.01$) by 59% in the activity of ALP resulted from the administration of DM versus those of control animals. In contrast, there was a significant inhibition ($p<0.01$) by 24% to vitC and vitE co-treatment versus those of DM-administered animals (Table 1).

Results of lipid peroxidation: Levels of LPO were increased significantly ($p<0.01$) by 84% in the liver homogenates of DM-treated guinea pigs as compared to control group. However, it was observed that the LPO levels were decreased by 37% in the group which received DM along with vitC and vitE. The decrease in LPO levels was significant ($p<0.01$) as shown in Table 2.

Results of antioxidant enzymes: CAT activity was also found to be inhibited significantly ($p<0.01$) by 41% in DM treated group, as compared to the control group. However, the activity of CAT was significantly elevated ($p<0.001$) by 120% in animals which received DM along with the vitamins (C and E) as compared to the DM treated group (Table 2).

Table 1: The activities of AST, ALT and ALP enzymes (Means±SD), stimulation (S%) and inhibition (I%) in control and different treated adult male guinea pigs

Measurements groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})
Control	18.80±2.48^{a}	25.60±3.36^{a}	49.60±04^{a}
VitC and vitE	20.60±3.77^{a}	27.25±3.30^{a}	52.25±4.27^{a}
DM	30.80±3.96^{b}	35.00±4.08^{b}	79.00±8.25^{b}
S% vs. control	64	39	59
VitC and E + DM	22.20±3.27^{ca}	27.80±2.38^{ca}	60.40±7.09^{ca}
I% vs. DM	28	21	24

Means in the same columns assigned with the same letter show insignificant differences

Table 2: The activities of LPO, CAT and GST enzymes (Mean±SD), stimulation (S%) and inhibition (I%) in the liver of control and different treated adult male guinea pigs

Measurements groups	LPO (nmol mg^{-1} protein)	CAT (µmol min mg^{-1} protein)	GST (µmol min mg^{-1} protein)
Control	1.71±0.32[a]	6.73±1.99[a]	37.09±12.69[a]
VitC and vitE	1.87±0.25[a]	8.62±1.56[a]	36.62±7.95[a]
DM	3.15±0.65[b]	3.99±1.12[b]	21.68±6.39[b]
S% or I% vs. control	S = 84	I = 41	I = 42
VitC and E + DM	1.99±0.27[ac]	8.79±1.09[c]	39.28±11.35[ac]
S% or I% vs. DM	I = 37	S = 120	S = 81

Means in the same columns assigned with the same letter show insignificant differences

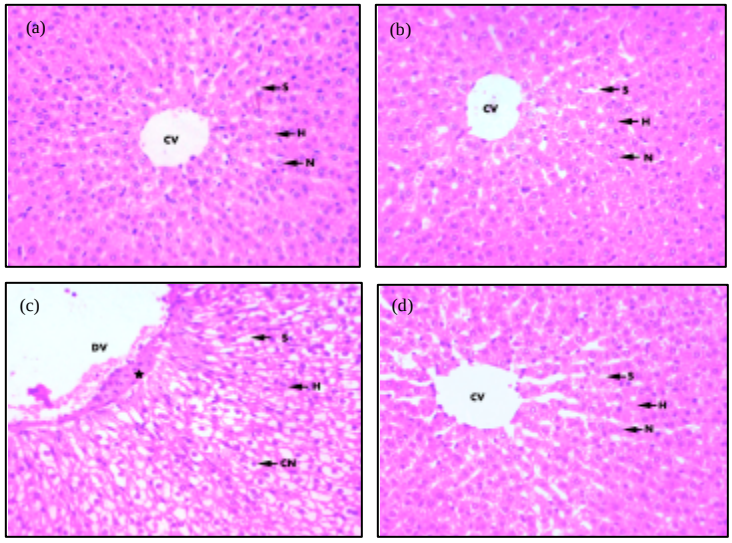

Fig. 1(a-d): Hepatoprotective effect of vitC and vitE against dimethoate-induced hepatotoxicity in guinea pigs. Liver sections were stained with H and E: (a) normal; (b) vitC and vitE (100 mg kg^{-1} body w.t.) (c) DM-treated animals; (d) vitC and vitE (100 mg kg^{-1} body w.t.) + DM; magnification 400 X. (H) hepatocytes, (CV) central vein, (DV) dilated vessel, (N) nucleus, (S) sinusodial space, (CN) condensed nucleus, (*) proliferative epithelia

The activity of GST was significantly ($p<0.01$) inhibited by 42% in DM administered group, as compared to the control group. However, the activity of GST in animals that received DM administration along with the vitC and vitE, was significantly stimulated ($p<0.001$) by 81% as compared to the controls (Table 2).

There were no significant differences among the activities of the studied hepatic enzymes (AST, ALT and ALP), the level of oxidized lipid and the activities of the antioxidative enzymes (CAT and GST) in control and vitC and vitE treated animals.

Results of liver morphological changes: After 4 weeks of DM administration, many histopathological changes were observed in the liver sections (Fig. 1c) compared with those of controls (Fig. 1a). The parenchymatous cells appeared large-sized with cytoplasmic vacuolization

and condensed nuclei. Also, disruption of hepatic architecture, dilated congested blood vessels with proliferative lining epithelia and lymphocytes infiltration were observed. However, co-treatment of DM administrated animals with vitamins (C and E) showed little pathological alterations when compared with those of DM alone (Fig. 1d). Again, administration of vitC and vitE to the animals did not induce any pathological changes and the liver tissue appears like the control (Fig. 1b).

DISCUSSION

For several decades, the extensive use of different pesticides in agriculture and for public health purposes, has led to drastic effects in many non-target species including man (WHO/PCS, 1996). Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Ahmed *et al*., 2000). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction.

In fact, available data on the hepatotoxicity action of DM were limited for adult guinea pigs. In the present study, oral administration of DM to guinea pigs caused a significant hepatic damage, as observed from the elevation of hepatospecific enzyme activities, as well as severe alterations in different liver parameters. The DM treated animals had significantly higher AST, ALT and ALP levels than the controls. When the liver cell membrane is damaged, several enzymes located in the hepatocyte cytosol, including AST, ALT and ALP are secreted into the blood (Ncibi *et al*., 2008). Consequently, these serum enzymes are markers of liver damage (Gokcimen *et al*., 2007; Eraslan *et al*., 2009). It has been shown that, OP insecticides can elevate the enzymatic activities of ALP, ALT and AST (Kalender *et al*., 2005b; Ogutcu *et al*., 2008; Ncibi *et al*., 2008). Recent studies of Saafi *et al*. (2011) and Ben Amara *et al*. (2011) reported that, DM raises the ALT AST and ALP levels in rats. This elevation of liver marker enzymes was consistent with the damage to the hepatic tissues in the DM-treated guinea pigs seen by light microscopy.

Different mechanisms have been postulated to explain DM induced liver injury, such as lipid peroxidation and interaction with membrane components resulting from free radicals' attack on biological structure (Stosh and Bagchi, 1995). In the current study, the lipid peroxidation levels in liver were increased significantly in the DM-treated animals. In fact, the involvement of oxidative stress following exposure to OP has been reported by Banerjee *et al*. (2001), Akhgari *et al*. (2003) and Sivapiriya *et al*. (2006). Antioxidants constitute the primary defense system that limits the toxicity associated with free radicals (Pincemail *et al*., 2002). In this study, DM induced oxidative damage by producing reactive oxygen species and decreasing the biological activities of some liver antioxidant enzymes, such as CAT and GST. Present results were in consistence with previous studies which have shown that acute and subchronic exposure to DM generates lipid peroxidation and alters the antioxidant status of several tissues in rats (Sharma *et al*., 2005a, b; Sayim, 2007a, b).

In the present study, the morphology of the liver seemed to be mostly affected by DM treatment alone. The changes were large-sized parenchymatous cells, cytoplasmic vacuolization and condensed nuclei, disruption of hepatic architecture, dilated congested blood vessels with proliferative lining epithelia, an increase in the number of Kupffer cells and lymphocytes infiltration. Accordingly, its role in metabolic conversions is its susceptibility to chemical injury (Shakoori *et al*., 1990). OP insecticides are known to induce various histopathological changes in the liver tissues (Goel *et al*., 2005; Gokcimen *et al*., 2007; Sayim, 2007a). Such as hemorrhage, inflammatory cell infiltration (Morowati, 1997; Elhalwagy *et al*., 2008), tissue damage and necrosis

(Kalender *et al.*, 2006). Also, OP insecticides have been found to affect the cytochrome P450 system or the mitochondrial membrane transport system of hepatocytes (Gokcimen *et al.*, 2007). In support of our finding DM produced enzymatic changes in liver of dams associated with mild pathomorphological changes in liver and brain (Salem, 2005; Srivastava and Raizada, 1996).

Antioxidant vitamins have a number of biological activities, including immune stimulation and altering the metabolic activities of carcinogens. These vitamins can also prevent genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites (Verma *et al.*, 2007). The co-administration of vitamins with DM to guinea pigs resulted in marked improvement of the liver enzymes activities when compared to that which received DM alone. One of the possible explanations for the observed recovery of various enzyme activities involved in the detoxification following vitC and vitE treatment could be because these materials exert their hepatoprotective influenceby acting as antioxidants (Nagababu *et al.*, 1995; Ramadan *et al.*, 2002). Supporting our finding, Verma *et al.* (2007) demonstrate that vitamins (C and E) in efficiently inhibits *in vitro* lipid peroxidation in chlorpyrifos induced oxidative stress. Moreover, our light microscopic analyses revealed that the DM-treated animals which received vitamins co-administration did not exhibit the hepatic morphological changes seen in the livers of the DM-treated group. Thus, vitC and vitE could ameliorate the liver damage induced by DM intoxication. There are several reports supported the role of antioxidant in attenuating the histopathology of some pesticides and toxins in experimental animals for example, ascorbic acid supplementation prevents the testicular damage induced by DM intoxication (El-Elaimy and Gabr, 1990). Also, Sutcu *et al.* (2006) revealed a hsistopathological changes in liver tissue of rats treated with methidathion and the severity of these lesions was reduced by administration of a combination of vitC and vitE.

In conclusion, this study may constitute the first attempt to evaluate the effects of vitamins (C and E) on DM-induced hepatotoxicity in adult guinea pigs. The binding of the present study illustrated that administration of vitamins (C and E) is capable of reversing the oxidative toxic effects of DM. These data suggest that vitamins, by preventing hepatic toxicity, may enhance the selectivity of these vitamins in the patients who occupationally exposed to DM.

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