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Effect of Exposure to Mixture of Four Organophosphate Insecticides at No Observed Adverse Effect Level Dose on Rat Liver: The Protective Role of Vitamin C

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ABSTRACT

The present study was designed to evaluate the effect of exposure to mixture of four Organophosphate insecticides (OPIs) at dose equaled to No Observed Adverse Effect Level (NOAEL) for 28 day on liver of male rats and their attenuation by vitamin C (V.C). Rats were divided into four groups of six each: control, OPIs, OPIs+V.C and V.C group. The activities of serum aspartate aminotransferase (AST), Alanine Transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were statistically ($p \leq 0.01$) increased, while the activity of cholinesterase (ChE) was decreased in rats exposed to OPIs. Supplementation of V.C was mitigating the adverse effects of OPIs but the increased were still significantly ($p \leq 0.05$). Body weight and total protein concentration were statistically ($p \leq 0.05$) decreased, while relative liver weight was statistically ($p \leq 0.05$) increased in OPIs-treated group. In addition, administration of OPIs resulted in damage of liver structures. Combination therapy with V.C significantly ($p \leq 0.05$) restored these alterations to within the normal limits and prevents disruptions of liver structures. According to these results, it is suggested that exposure to multi-chemical with a common mechanism of toxicity might cause hazardous effects at NOAEL levels to non-target organisms, including humans.

Key words: Organophosphorus insecticides, hepatotoxicity, ascorbic acid, NOAEL, common mechanism of toxicity, rats

INTRODUCTION

Organophosphorus insecticides (OPIs) form a largest and most diverse group of insecticides. The wide application of OPIs in public health and agricultural programs was accompanied by potentially hazardous impact on humans, animals, plants and environment (water, air, soil and food) and causes severe acute and chronic poisoning (Abdollahi *et al.*, 1999). OPIs compounds are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE), leading to accumulation of acetylcholine and subsequent activation of cholinergic, muscarinic and nicotinic receptors (Ecobichon, 1996). Acute mammalian toxicity (cholinergic crisis) of phosphorothioate pesticides depends on Mixed Function Oxidase (MFO) catalysed activation to their corresponding oxygen analogs, which are direct inhibitors of AChE (Maroni *et al.*, 2000).

Liver plays a central role in the detoxification process and faces the threat of maximum exposure to xenobiotics and their metabolic by-products. The susceptibility of liver tissues to this

stress due to exposure to pesticides is a function of the overall balance between the degree of oxidative stress and the antioxidant capability (Khan *et al.*, 2005). However, Reactive Oxygen Species (ROS) have been implicated in hepato and neurotoxicity induced by several OPIs (Bagchi *et al.*, 1995; Mansour and Mossa, 2010a, b, 2011) and is associated with lipid peroxidation and phospholipids degradation (Mansour and Mossa, 2009).

In fact, the toxicity of OPIs results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (Kossmann *et al.*, 1997; Nagymajtenyi *et al.*, 1998; Gomes *et al.*, 1999; Aly and El-Gendy, 2000; Mansour and Mossa, 2011). Previous studies are indicating that OPIs exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues (Samanta and Chainy, 1995) with simultaneous generation of reactive oxygen species (Sharma *et al.*, 2005). However, the Food Quality Protection Act (passed in August 1996) requires EPA to consider the cumulative risk to multiple chemicals with a common mechanism of toxicity when setting allowable residues on crops. EPA may conclude that organophosphorus insecticides exert their neurotoxic effects through a common mechanism of toxicity (Mileson *et al.*, 1998). A mechanism of toxicity is described as the major steps leading to an adverse health effect following interaction of a pesticide with biological targets (US EPA, 1997). It has been reported that some of OPIs (e.g., chlorpyrifos) exposures exceed No Observable Adverse Effect Level (NOAEL) for pregnant women and children, even in scenarios of common use (Davis and Ahmed, 1998).

Vitamin C, a water-soluble vitamin, participates in a large number of cell functions. In addition to the antioxidant-protective role in cell injury (Majewska and Bell, 1990; Martin and Frei, 1997; Blasiak and Kowalik, 2001) vitamin C accelerates the degradation of intra and extracellular proteins targeted to the lysosomal lumen by autophagic and heterophagic pathways, relevant for the removal of abnormal proteins that accumulate with aging (Martin *et al.*, 2002).

The present study was designed to evaluate the effect of exposure to mixture of four OPIs at dose equaled to No Observed Adverse Effect Level (NOAEL) on liver of rats and the protective effects of vitamin C against liver damage induced by these insecticides.

MATERIALS AND METHODS

Chemicals and reagent: Chlorpyrifos (Pestban® 48% EC) was obtained from Agrochem, Alwatneia Co., Alex., Egypt; profenofos (Curacron® 72% EC) from Ciba-Geigy AG; diazinon (Nasr-Cidol® 60% EC) and malathion (Nassrlathion® 57% EC) from El-Nasr Mediate Chemical Co., Egypt.

Kit of lactate dehydrogenase (LDH) was obtained from Spinreact (Santa Coloma, Spain) alanine aminotransferase (ALT), cholinesterase (ChE), total protein, albumin and lipid peroxidation were obtained from Biodiagnostic (Egypt). Kit of aspartate aminotransferase (AST) was obtained from ELITech Group Co., SEPPIM S.A.S. Zone industriellement 61500 SEES France and alkaline phosphatase (ALP) from Analyticon® Biotechnologies AG. Am Mühlenberg 10, 35104 Lichtenfels/Germany. All other chemicals were of reagent grades and obtained from the local scientific distributors in Egypt.

Animals and groups: Male rats of the Wistar strain (*Rattus norvegicus*) weighing 204±8 g were obtained from Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt. Animals were kept in clean plastic cages with free access to food (standard pellet diet) and tap water *ad-libitum*, under standardized housing conditions (12 h light/dark cycle, temperature was 22±1°C and a minimum relative humidity of 40%) in the laboratory animal room. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to four groups, each consisting of six

rats, as follows: First group (control), second group (OPIs), third group (OPIs+V.C.) and fourth group (V.C.).

All OPIs and V.C. were dissolved in distilled water and given via oral route for 28 consecutive days. Dosages of each administered were daily freshly prepared and adjusted weekly for body weight changes. OPIs were administered at a dose equal to No Observed Adverse Effect Level (NOAEL) diazinon ($0.06 \text{ mg kg}^{-1} \text{ b.wt.}$), chlorpyrifos ($1 \text{ mg kg}^{-1} \text{ b.wt.}$), malathion ($29 \text{ mg kg}^{-1} \text{ b.wt.}$) and profenofos ($0.3 \text{ mg kg}^{-1} \text{ b.wt.}$) (Tomlin, 2004). The selected dose of V.C ($200 \text{ mg kg}^{-1} \text{ b.w.}$) was based on previous study by Aly *et al.* (2010). The control group received an equivalent volume of distilled water (0.5 mL/rat).

The experimental protocols and procedures were approved by the Local Ethics Committee at the National Research Centre (NRC), Dokki, Cairo, Egypt.

Blood samples and body weight: In all groups, body weights were weekly recorded. At the end of exposure period, the blood samples were drawn from all rats under ether anesthesia by puncturing the retroorbital venous plexus of the animals with a fine sterilized glass capillary and collected in glass tubes to separate the sera. Within 20 min of blood collection, the sera samples were drawn from blood after centrifugation at 3500 rpm (600 g) for 10 min at 4°C , using Hereaeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany. The sera was kept in a deep freezer (-20°C) until analyzed.

Liver/body weight ratio: After blood collection, the rats were sacrificed by cervical dislocation. Liver of rats was quickly removed and weighted individually, Then, the liver/the body weight ratio were calculated.

Liver dysfunction biomarkers: All serum biomarkers were determined using a commercial kit in accordance with manufacturers' instructions. The activity of cellular enzymes such as aspartate aminotransferase and alkaline phosphatase (Reitman and Frankel, 1957) alanine transaminase (Sherwin, 1996) were determined in sera. While, the activity of serum cholinesterase (ChE) and the concentration of albumin and total protein were determined by the methods of Ellman *et al.* (1961), Westgard and Poquette (1972) and Gornall *et al.* (1949), respectively.

Calculation of globulin in serum: Serum proteins comprise of albumin and globulin (alpha, beta and gamma). Therefore, concentration of globulins can be calculated as follows:

Globulins (g dL^{-1}) = total protein (g dL^{-1}) - albumin (g dL^{-1}) and thus Albumin/Globulin ratio (A/G) could be estimated.

Lipid peroxidation: Malondialdehyde (MDA), as a marker for lipid peroxidation (LPO) was determined in serum by the method of Draper and Hadley (1990) and expressed in nmol mL^{-1} .

Histopathological studies: Liver samples were dissected and fixed in formalin, dehydrated and imbedded in paraffin wax. Then, the sections were stained by haematoxylin and eosin (H and E). Two slides were prepared for each rat, each slide contained two sections and examined for histopathological changes under light microscope.

Spectrophotometric measurements: The Spectrophotometric measurements were performed by using a Shimadzu UV-VIS Recording 2401 PC (Japan).

Statistical analysis: The data were analyzed by using SPSS (version 17.0) for Windows and expressed as Means±S.D. Paired samples t-test was used to compare the data of the control and those of treatments.

RESULTS

Body and relative liver weights: As shown in Fig. 1, body weight significantly ($p \leq 0.05$) decreased in OPIs-treated group in comparison to control. In contrast, V.C significantly ($p \leq 0.05$) restored OPIs-induced decrease in body weight. At the end of treatment periods (28 day), body weight accounted to 276.80 ± 13.92 g in control and 217.80 ± 20.17 g, 271.76 ± 21.94 g and 274.43 ± 13.75 g in OPIs, OPIs+V.C and V.C-treatment, respectively. The reduction in body weight accounted to -21.32% in OPIs-treatment and was improved to -1.82% in OPIs+V.C-treatment (Fig. 1). Present results showed significant ($p \leq 0.05$) increase in the relative liver weight in OPIs-treatment and this effect was restored in OPIs+V.C-treatment (Fig. 2). These results showed that the use of V.C was mitigating the adverse effects of exposure to OPIs.

Liver dysfunction markers and lipid peroxidation (LPO): As show in Table 1, OPIs significantly ($p \leq 0.05$) reduced serum total protein (6.88 ± 0.16 g dL⁻¹) in comparison to controls (7.59 ± 0.35 g dL⁻¹). Combination therapy with V.C significantly ($p \leq 0.05$) restored OPIs effect to within the normal limits. In the other hand, no significant changes were observed in albumin,

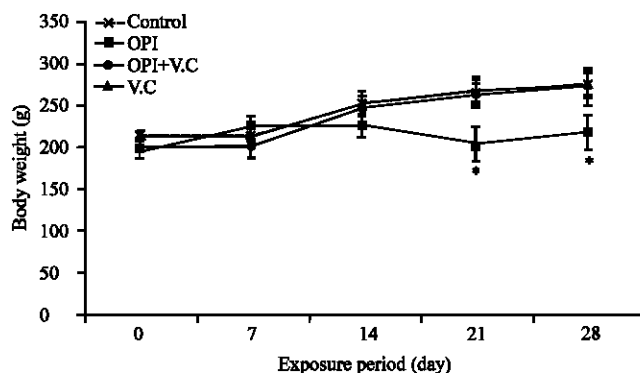


Fig. 1: Body weight of rats exposed to mixture of four OPIs for 28 day and the protective effect of vitamin C. Each Values are Means±SD; n = 6; statistical difference from the control: *Significant at $p \leq 0.05$. OPIs: Organophosphate insecticides and V.C: Vitamin C

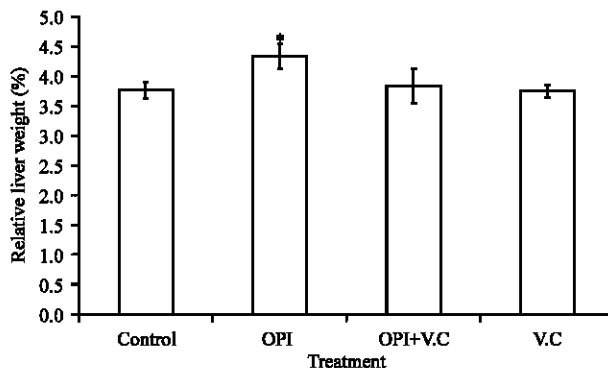


Fig. 2: Relative liver weight of rats exposed to mixture of four OPIs for 28 day and the protective effect of vitamin C. Each Values are Means±SD; n = 6; statistical difference from the control: *Significant at $p \leq 0.05$. OPIs: Organophosphate insecticides and V.C: Vitamin C

globulin concentration and Albumin/Globulin (A/G) ratio of rats exposed to different treatments and the values were found comparable to controls (Table 1).

In OPIs-treated group, the activity of serum ALT, AST, ALP and LDH were significantly ($p \leq 0.01$) increased compared to control value (Table 2). The activities of ALT, AST, ALP and LDH were accounted to 78.20, 57.33, 232.80 and 239.02 U L⁻¹ in OPIs-treated group, respectively.

Supplementation of V.C was mitigating the adverse effects of exposure to OPIs but the increase in the enzyme activities were still significantly ($p \leq 0.05$) compared to the control. The activities of ALT, AST, ALP and LDH were accounted to 61.40, 49.68, 164.60 and 166.10 U L⁻¹ in OPIs+V.C-treated group, respectively. Also, ChE activity was significantly ($p \leq 0.01$) inhibited by OPIs treatment (Table 2) while restored ($p \leq 0.05$) by combination therapy of V.C.

As shown in Fig. 3, malondialdehyde (MDA), as a marker for lipid peroxidation (LPO) significantly ($p \leq 0.05$) increased in OPIs-treated group in comparison to control. While, V.C significantly ($p \leq 0.05$) restored OPIs-induced increase in LPO.

Table 1: Serum total protein, albumin, globulin concentration and A/G ratio in rats exposed to mixture of four OPIs for 28 day and the protective effect of vitamin C

Treatment	Total protein (g dL ⁻¹)	Albumin (g dL ⁻¹)	Globulin (g dL ⁻¹)	Albumin/globulin (A/G) ratio
Control	7.59±0.35	3.22±0.17	4.37±0.45	0.75±0.11
OPI	6.88±0.16*	2.99±0.15	3.97±0.38	0.76±0.10
OPI+V.C.	7.22±0.32	3.13±0.04	4.09±0.34	0.77±0.07
V.C.	7.56±0.38	3.17±0.13	4.39±0.43	0.73±0.10

OPI: Organophosphate insecticides, V.C: Vitamin C, Values are Means±SD; n = 6; Statistical difference from the control: *Significant at $p \leq 0.05$ and **Highly significant at $p \leq 0.01$

Table 2: ALT, AST, ALP, LDH and ChE activities in the sera of rats exposed to mixture of four OPIs for 28 day and the protective effect of vitamin C

Treatment	ALT (U L ⁻¹)	AST (U L ⁻¹)	ALP (U L ⁻¹)	LDH (U L ⁻¹)	ChE (U L ⁻¹)
Control	48.40±3.65	33.65±1.19	151.00±3.16	970.55±4.64	333.15±10.78
OPI	78.20±5.36**	57.33±0.80**	232.80±10.08**	239.02±12.97**	268.67±23.86**
OPI+V.C.	61.40±6.77*	49.68±2.23*	164.60±5.55*	166.10±9.15*	301.73±18.48*
V.C.	50.40±4.16	32.89±2.08	153.60±6.73	980.05±7.81	326.53±22.64

OPI: Organophosphate insecticides, V.C: Vitamin C, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, ChE: Cholinesterase, ALP: Alkaline phosphatase, Values are Means±SD; n = 6; Statistical difference from the control: *Significant at $p \leq 0.05$ and **Highly significant at $p \leq 0.01$

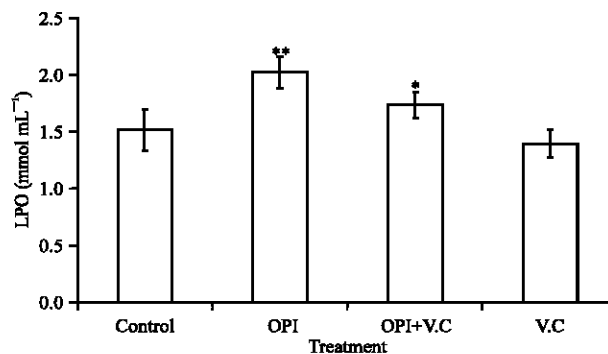


Fig. 3: Lipid peroxidation in the sera of rats exposed to mixture of four OPIs for 28 day and the protective effect of vitamin C. Each Values are Means±SD; n = 6; statistical difference from the control: *Significant at $p \leq 0.05$ and **Highly significant at $p \leq 0.01$. OPIs: Organophosphate insecticides and V.C: Vitamin C

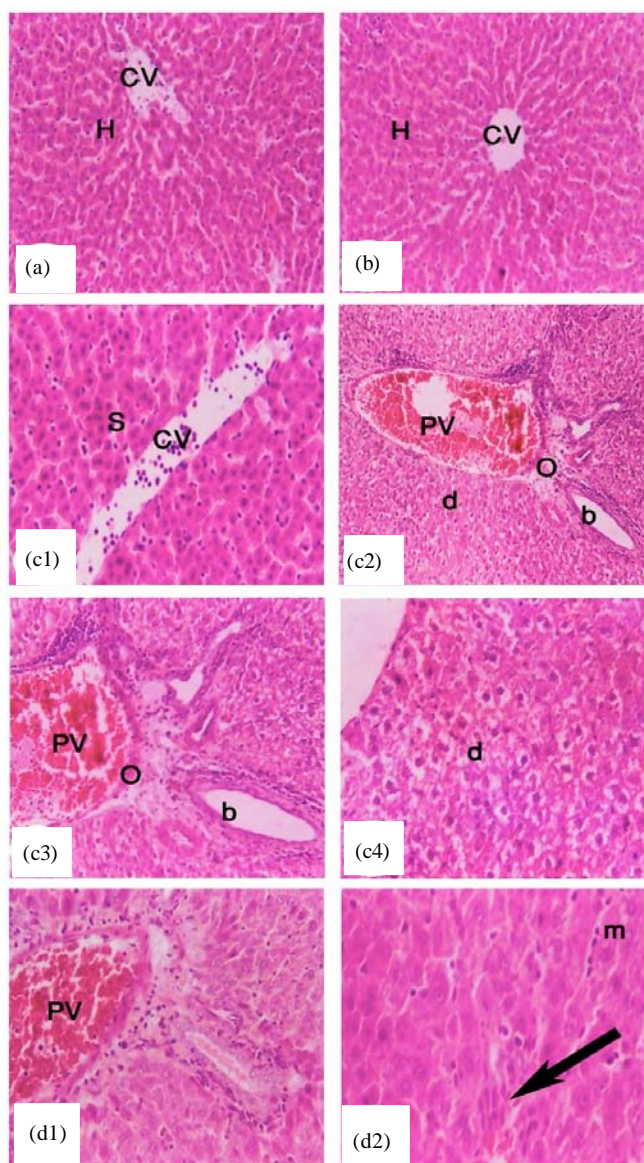


Fig. 4: Liver paraffin sections stained by haematoxylin and eosin (H and E) for histopathological changes. Control group (A) and V.C group (B) showing; intact histological structure of Central Vein (CV) and hepatocytes (H) (x 64). OPIs group showing: (c1) dilatation and congestion of Central Vein (CV) and Sinusoids (S) and impacted by leucocytes (x 80), (c2) sever congestion of Portal Veins (PV) with dilated cystic bile duct (b) and oedema portal area (O) as well as degeneration in hepatocytes (d) (x 64), (c3) congested Portal Vein (PV), oedema in portal area (O) and dilated cystic bile duct (b) (x 80) and (c4) degenerated hepatocytes (d) (x 160). V.C.-group showing: (d1) Congestion in the Portal Vein (PV) and (d2) kupffer cells proliferation (arrow) and inflammatory cell infiltration (m) in between the hepatocytes (x 160)

Histopathological finding: In light microscopic examinations, histopathological changes were observed in liver of OPIs and OPIs+V.C-treated groups compared to control group. These changes were more frequent in OPIs-treated groups. As shown in Fig. 4, the liver sections of control [A] and V.C-treated groups [B] showed normal liver structures. With respect to the hepatic histoarchitecture of the OPIs-treated group, dilatation and congestion in the central veins sinusoids and impacted by leucocytes [C1], sever congestion of portal vein with dilated cystic bile duct, oedema and degradation in hepatocyte [C2], oedema in portal area and dilated cystic bile duct [C3] and degeneration hepatocytes [C4] were observed. In OPIs+V.C-treated group, congestion in the portal vein [D1] and kupffer cells proliferation and inflammatory cells infiltration in between hepatocytes [D2] were observed.

DISCUSSION

For several decades, the extensive use of different OPIs in agriculture and for public health purposes, has led to drastic effects in many non-target species including human (Cantelli-Forti *et al.*, 1993; WHO/PCS, 1996). As concluded by EPA, OPIs are exerted their neurotoxic effects through a common mechanism of toxicity (Mileson *et al.*, 1998) and the mechanism of toxicity is described as the major steps leading to an adverse health effect following interaction of a pesticide with biological targets (US EPA, 1997).

For the toxicologist, the consequences of hepatic enzyme induction are known to form lack of any obvious effect to substantial anatomical or metabolic/physiological changes. Also, organ and relative organ weights are important criteria for evaluation of organ toxicity. Changes in the body weight after insecticide dosing have been used as a valuable index of insecticide-related organ damage (Mansour and Mossa, 2010a, b). In the present study, oral administration of OPIs to male rats resulted in significant ($p \leq 0.05$) reduction in body weights and significant ($p \leq 0.05$) increase in the relative liver weights. Results showed that the use of V.C was restored the body weights and relative liver weights of OPIs-treated group to within the normal range. However, the body weight decrease as a result of exposure to tested-OPIs was considered to be the result of direct toxicity of tested compounds and/or indirect toxicity related to the liver damage. During the exposure period, we did not detect any significant alteration in the diet consumption of the rats following OPIs-intoxication. Therefore, the reduction in rats body weights gains may be due to the overall increased degradation of lipids and proteins as a result of the direct effects of organophosphate compound (Goel *et al.*, 2005; Mansour and Mossa, 2011). Other studies showed that OPIs (e.g., CPF) cause decrease in body weight in rats (Woolliams *et al.*, 1983) and mice (Ambali *et al.*, 2007). Vitamin C administration to OPIs-treated rats showed a better body weight gain compared to OPIs-treated rats. The protective effects of V.C have been reported previously by other investigators (Khan and Sinha, 1994; Gultekin *et al.*, 2001; Hong *et al.*, 2002; Aly *et al.*, 2010).

The liver, the key organ involved in numerous metabolic functions and plays a central role in the detoxification process and faces the threat of maximum exposure to xenobiotics and their metabolic by-products (Meyer and Kulkarni, 2001). There is no doubt that reactive oxygen species play an important role in pathological changes in the liver, particularly in the cases of alcoholic and toxic liver diseases (Poli and Parola, 1997). The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of the overall balance between the degree of oxidative stress and the antioxidant capability (Khan *et al.*, 2005; Mansour and Mossa, 2010a, b). In fact, lipid peroxidation has been suggested as one of the molecular mechanisms involved in organophosphorus pesticides-induced toxicity (Kehrer, 1993). Levels of MDA, a major oxidation

product of peroxidized polyunsaturated fatty acids, have been considered as an important indicator of lipid peroxidation (Kalender *et al.*, 2004). In our study, malondialdehyde (MDA), as a marker for lipid peroxidation (LPO) was significant ($p \leq 0.05$) increased in OPIs-treated group. It has been used as a measure of these xenobiotics-induced oxidative stresses, which may be defined as the disequilibrium between the peroxidants and antioxidants in biological systems (Kelly *et al.*, 1998).

Serum enzymes including ALT, AST, ALP and LDH are mainly used in the evaluation of hepatic damage. Results of the present study revealed that OPIs-treatment caused an increase in the activities of AST, ALT, ALP and LDH in serum of male rats. Awad *et al.* (1998) found that cell damage exhibited good correlation with the enzyme leakage. Therefore, the increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place (Meyer and Kulkarni, 2001; Khan *et al.*, 2005). The increase in serum LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream (Wang and Zhai, 1988). It has been previously reported that during liver damage there was an observed decrease in antioxidant defenses in the liver (Seven *et al.*, 2004).

Decreases in serum total protein and slightly decreases in albumin were observed in the present study following OPIs treatment. The decrease in total protein in OPIs-treated group may be due to the liver dysfunctions and disturbance in the biosynthesis of protein. Previous studies showed that protein content was changed in human (Nabila *et al.*, 1990); rats (Enan *et al.*, 1982; El-Bakary, 1993; Mansour and Mossa, 2010a, b, 2011) and *Rana tigrina* (Khan and Tabassum, 2003) as a result of insecticide exposure.

Cholinesterase (ChE, EC 3.1.1.8) activity has traditionally been monitored as a biomarker of organophosphate exposure and decrease in cholinesterase activities in rats exposed to OPIs, showed the effect of OPIs on esterases enzymes. It has already been mentioned that ChE is synthesized mainly in hepatocytes and secreted into the blood stream (Brown *et al.*, 1981). The activity is reduced in liver dysfunction due to reduced synthesis in contrary to other serum enzymes whose activities increase as a result of cell membrane damage (Moss and Henderson, 1999). According to the present results, OPIs-treatment caused a statistical ($p \leq 0.01$) significant decrease in serum ChE activity compared to the control group, while restored ($p \leq 0.05$) by combination therapy of V.C. According to Anderson and Borlak (2007), the development of toxic liver injury follows a two-staged course, the first phase of which is characterized by initiation of the injury and may involve direct interaction with a toxicant, which may exhibit dose-dependency (Mehendale, 2005). In contrast, the second phase is characterized by progress of the injury in a toxicant-independent fashion that is dominated by secondary events. Among the mechanisms contributing to phase two of toxic liver injury are the following 3 proposed: A) contribution of inflammatory cells (Piguet *et al.*, 1990; Czaja *et al.*, 1994; Laskin and Pendino, 1995), B) oxidative stress and lipid peroxidation (Slater, 1984; Poli, 1993) and C) leakage of degrading enzymes (Poli *et al.*, 1987; Mehendale, 2005).

The histopathological results of the current study demonstrated that 28-day exposure of male rats to a formulation grade of OPIs (diazinon = 0.06 mg kg⁻¹ b.wt.; chlorpyrifos = 1 mg kg⁻¹ b.wt. malathion = 29 mg kg⁻¹ b.w.t. and profenofos = 0.3 mg kg⁻¹ b.wt.) resulted in degenerative changes in hepatocyte, oedema in portal area, dilatation and congestion in the central veins of the liver. Previous work has reported dilatation of central vein, degradation, congestion, oedema, hyalinosis, fibrosis and necrosis in the liver of suckling rats or mothers exposed to 1.00 mg kg⁻¹ b.wt and 1.35 mg kg⁻¹ b.wt. of chlorpyrifos during lactation period (Mansour and Mossa, 2010a, 2011). Also, hepatocellular degeneration and necrosis was recorded in rat treated with profenofos (Mansour *et al.*, 2008) malathion and diazinon (Lox and Davis, 1983).

The hepatic function tests incorporated the histopathological lesions observed in the present study. These observations indicated marker changes in the overall histoarchitecture of liver in response to OPIs, which could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membrane components of the cell. OPIs-exposure promotes the oxidative damage of liver cells by enhancing peroxidation of membrane lipids (Banerjee *et al.*, 1999). This oxidative damage might ultimately enhance apoptosis unless the deleterious effects of the oxidative stress are counteracted by the endogenous cellular defense mechanisms that include enzymatic and non enzymatic free radicals scavenger (Ranjbar *et al.*, 2002). The ability of V.C to improved liver dysfunction biomarkers may be due to its antiperoxidative properties. V.C, as an antioxidant agent, may have inhibited the chain reactions of OPIs-generated free radicals or scavenged the reactive free radicals before reaching their hepatic targets. Both animal (Odigie *et al.*, 2007) and human (Dogun and Ajala, 2005) studies have shown V.C to be a potent antioxidant which mediates its antioxidant effect by scavenging free Reactive Oxygen Species (ROS). Other studies have equally shown the protection of V.C and other vitamins in hepatic oxidative damage (Barja *et al.*, 1994; Appenroth *et al.*, 1997). Therefore, results of the present study suggests V.C ameliorating effects to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity. Also, V.C is playing the important role in V.C-dependent metabolic reactions that influences essential physiological processes and the activation of biological defense mechanisms (Arrigori and De Tullio, 2002). In conclusion, the results of the current study indicate that mixture of four OPIs (diazinon, chlorpyrifos, malathion and profenofos) at dose equal to NOAEL induces biochemical and histopathological changes in the liver of exposed rats. According to these results, it is suggested that exposure to multi-chemical with a common mechanism of toxicity might cause hazardous effects at NOAEL levels to non-target organisms, including humans. Moreover, the adverse effects in liver of rats could be ameliorated by antioxidant (e.g., vitamin C).

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