Journal of Hazardous Materials 182 (2010) 273–278

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats

H.M. Abdou*, R.H. El Mazoudy

Zoology Department, Faculty of Science, Alexandria University, Egypt

ARTICLE INFO

Article history:
Received 26 March 2010
Received in revised form 5 June 2010
Accepted 8 June 2010
Available online 15 June 2010

Keywords:
Diazinon
Rats
Lipid peroxidation
Antioxidant enzymes
Biochemical parameters

ABSTRACT

Diazinon (Dz) is used in ectoparasiticide formulations for external parasitic control, resulting in environmental deleterious effects on biological systems. Thus we aimed to investigate the effects of different doses of diazinon on some biochemical parameters and histological alterations in female rats. The rats were divided into two groups. The first group was used as control. The second group was divided into four subgroups that were treated with 8, 10, 12 and 20 mg/kg BW of diazinon, respectively. The results showed that treatment with Dz induced significant ($p < 0.05$) increases in the level of serum malondialdehyde (MDA) and the activity of lactate dehydrogenase (LDH). The results revealed a significant ($p < 0.05$) decreases in the activities of serum acetylcholinesterase (AChE), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Meanwhile, the results showed significant ($p < 0.05$) increases in serum total lipids, total cholesterol, triglycerides, high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in Dz-treated subgroups, compared to the control group. The histological analysis of cardiac and skeletal muscle fibers demonstrated large areas of degenerating muscle fibers with evident loss of transverse striations and wide interfascicular spaces. In conclusion, Dz induced varying degrees of oxidative damage and histological alterations according to its dose.

1. Introduction

Pesticides are ubiquitous in the environment and have significant economic, environmental and public health impact. Their usage helps to improve human nutrition through greater availability, longer storage life and lower costs of food. Pesticides also reduce human labor requirements and attendant risk of work-related injury. These agents actively assist in the control of food-borne and vector-borne diseases [1]. Exposure to pesticides may involve large segments of population which include agriculture workers and their families, besides the general population who may be exposed through home application of pesticides or via residues on food [2]. Diazinon (0,0-diethyl-0-[2-isopropyl-6-methyl-4-pyramidinyl] phosphorothioate) is an organophosphorus compound with an anticholinesterase mode of action. It is used extensively to control flies, lice, insect pests of ornamental plants and food crops, as well as nematodes and soil insects in lawns and croplands [3]. Diazinon degrades rapidly in the environment, with half-time persistence usually less than 14 days while it may remain biologically active in soils for 6 months or longer. However, the mechanisms by which pesticides cause damage involve multiple reaction pathways [4]. Several studies of varying duration of exposure with organophosphorus or pyrethroid pesticides have postulated a possible role for the generation of free radicals during the toxic process [5]. Oxidative damage is characterized by the production of reactive oxygen species, which include free radicals and peroxides. The targets for these highly reactive free radicals include proteins, lipids, carbohydrates and nucleic acids. The myriad reactions can be categorized as peroxidation reactions resulting in the oxidation of polyunsaturated fatty acids that form components of membrane lipids as well as sulfur-oxidation reactions that modify the reactivity of sulfur containing amino acids by inactivating proteins and enzymes [6]. Some studies have demonstrated also that the administration of subacute dichlorvos and methyl parathion increase the concentration of malondialdehyde (MDA; lipid peroxidation end product) and cause changes in the antioxidative systems in the various tissues of rats [7,8]. Also, collective results of Isik and Celik [9] have demonstrated that exposure of fish to pesticides induces an increase in MDA joined with fluctuated antioxidative systems. This study was initiated to (1) assess the effect of different doses of diazinon (Dz) on lipid peroxidation; and (2) investigate the histological alterations in heart and skeletal muscles in female rats.

2. Materials and methods

Diazinon was applied as a commercial emulsified concentrate formulation containing 60% active ingredient from Egychem for

* Corresponding author. Tel.: +20 3 3925875/5441801; fax: +20 3 3911794.
E-mail address: dr.heba_abdou3000@yahoo.com (H.M. Abdou).

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doi:10.1016/j.jhazmat.2010.06.026

Table 1
Changes in the levels of MDA and the activities of GPx, SOD, LDH and AChE in serum samples of female rats treated with different doses of Diazinon.

Parameters	Experimental groups				
	Control	Dz_{-8}	Dz_{-10}	Dz_{-12}	Dz_{-20}
MDA[a]	5.79 ± 0.24^{A}	5.99 ± 0.40^{B}	6.56 ± 0.38^{B}	8.31 ± 0.29^{C}	10.23 ± 0.29^{D}
GPx[b] (U/L)	44.77 ± 4.43^{A}	41.90 ± 3.56^{B}	36.80 ± 2.43^{B}	25.50 ± 4.06^{C}	15.20 ± 3.78^{D}
SOD[c] (U/L)	68.37 ± 7.05^{A}	65.34 ± 3.60^{B}	59.97 ± 3.95^{B}	41.30 ± 4.32^{C}	32.33 ± 3.35^{D}
LDH[d] (U/L)	295.76 ± 2.56^{A}	317.94 ± 4.46^{B}	463.14 ± 3.92^{C}	591.03 ± 3.32^{D}	711.10 ± 4.12^{E}
AChE[e] (U/L)	74.15 ± 2.69^{A}	71.90 ± 3.90^{B}	67.11 ± 4.98^{B}	55.46 ± 3.49^{C}	42.13 ± 3.89^{D}

Values are expressed as means ± SD; $n = 7$ for each experimental group. Mean values within a row not sharing a common superscript letter (A–E) were significantly different, $p < 0.05$.

[a] MDA: as malondialdehyde (mmol/L).
[b] GPx: glutathione peroxidase (U/L).
[c] SOD: superoxide dismutase (U/L).
[d] LDH: Lactate dehydrogenase (U/L).
[e] AChE: acetylcholinesterase (U/L).

Chemicals Company, Egypt. It was diluted with saline for preparing the required toxicant concentrations.

Thirty-five female Sprague-Dawley rats (180–220 g) were obtained from the animal house of Faculty of Medicine, Alexandria University, Egypt. Animals were cadged in groups of 7 rats and provided with pelleted food and water adlibitum. After 1 week of acclimatization, animals were divided into two groups. The first group (7 female rats) was used as controls and received 0.2 ml saline by gavage as vehicle. The second group, 28 female rats (diazinon-treated group), was divided into four equal subgroups. Each subgroup included seven animals. Rats in diazinon treated subgroups were orally received 8, 10, 12 and 20 mg/kg body weight of diazinon, respectively. The oral L.D50 = 300 mg/kg for female rats. Rats were orally administrated 0.2 ml of constituted diazinon by gavage. The treatments were continued for 3 weeks (6 times/week). At the end of the experimental period, rats were sacrificed by cervical decapitation and blood samples were collected from the heart and left in a refrigerator for 30 min before centrifugation. The clear non-hemolyzed sera were stored at −20 °C till measurements. Also, the heart and skeletal muscles from the hind leg were immediately removed, and washed with saline solution. Three rats from each group were used for the histological examinations. The serum lipid peroxidation end product; MDA was measured as thiobarbituric acid reactive substance [10]. The total activity of glutathione peroxidase (GPx, EC. 1.1.1.9) was determined by the method of Puglia and Valentine [11]; one unit of GPx activity was defined as the utilization of 1 mole of substrate per min at 37 °C. Superoxide dismutase (SOD, EC.1.15.1.1) activity was measured in serum samples according to Misra and Fridovich [12]. Also, estimation of the serum activity of lactate dehydrogenase (LDH, EC.1.1.1.2.7) was carried out as described previously [13]. Acetylcholinesterase (AChE, EC.3.1.1.7) activity was assayed by the method of Ellman et al. [14]. Sera were assayed also for total lipids, cholesterol and triglycerides (TG) [15]. High density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) were determined according to the method of Warnick et al. [16].

2.1. Histopathology

Heart and skeletal muscles from the experimental groups were immediately fixed in 10% formalin, then treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4–6 μm thickness. The sections were stained with Hematoxylin and Eosin (H&E) stain for studying the histopathological changes [17].

2.2. Statistical analysis

Data were analyzed as a completely randomized design [18] using the linear model procedure of SAS (1986). Means were statistically compared using least significant difference (LSD) test at $p \leq 0.05$ significance level.

3. Results

The effect of different doses of diazinon on serum biochemical parameters is summarized in Table 1. The results showed that treatment with Dz induced significant ($p < 0.05$) increase in the level of MDA in the diazinon-treated groups compared to the control group. Concurrent with this result, a significant elevation in the activity of LDH was recorded in all treated groups, compared with the control one. On the other hand, the results in Table 1 revealed a significant ($p < 0.05$) decrease in the activity of GPx after Dz treatment in the diazinon-treated groups, compared to the control one. Also, the activity of SOD was significantly reduced ($p < 0.05$) with varying degrees, according to the dose of Dz, in the diazinon-treated rats in comparison with the control group (Table 1). Furthermore, diazinon treatment caused significant ($p < 0.05$) decrease in the activity of serum AChE in all Dz-treated groups compared to the control group (Table 1). Meanwhile, the results in Table 2 showed that treatment of rats with different doses of diazinon induced significant ($p < 0.05$) increase in serum total lipids, total cholesterol, triglycerides, HDL-C and LDL-C compared to the control group. The

Table 2
The effect of different doses of Diazinon on Serum lipids and lipoproteins contents of female rats.

Lipid (mg/dl)	Experimental groups				
	Control	Dz_{-8}	Dz_{-10}	Dz_{-12}	Dz_{-20}
TL	459.13 ± 3.08^{A}	493.27 ± 2.18^{B}	561.16 ± 2.39^{C}	721.42 ± 2.43^{D}	1355.23 ± 3.18^{E}
TG	102.65 ± 2.69^{A}	121.03 ± 2.50^{B}	132.44 ± 2.43^{C}	146.45 ± 2.32^{D}	193.77 ± 1.48^{E}
Cholesterol	61.29 ± 3.35^{A}	71.76 ± 3.66^{B}	78.46 ± 3.98^{C}	90.76 ± 4.00^{D}	109.49 ± 4.52^{E}
HDL-C	34.34 ± 2.85^{A}	47.64 ± 3.10^{B}	57.18 ± 2.97^{C}	63.45 ± 3.38^{D}	83.85 ± 2.76^{E}
LDL-C	24.07 ± 3.15^{A}	36.24 ± 2.86^{B}	42.28 ± 2.39^{C}	50.81 ± 4.40^{D}	61.60 ± 4.09^{E}

Values are expressed as means ± SD; $n = 7$ for each experimental group, Mean values within a row not sharing a common superscript letter (A–E) were significantly different, $p < 0.01$ TL: total lipids; TG: triglycerides; HDL-C: high density lipoprotein; and LDL-C: low density lipoprotein.

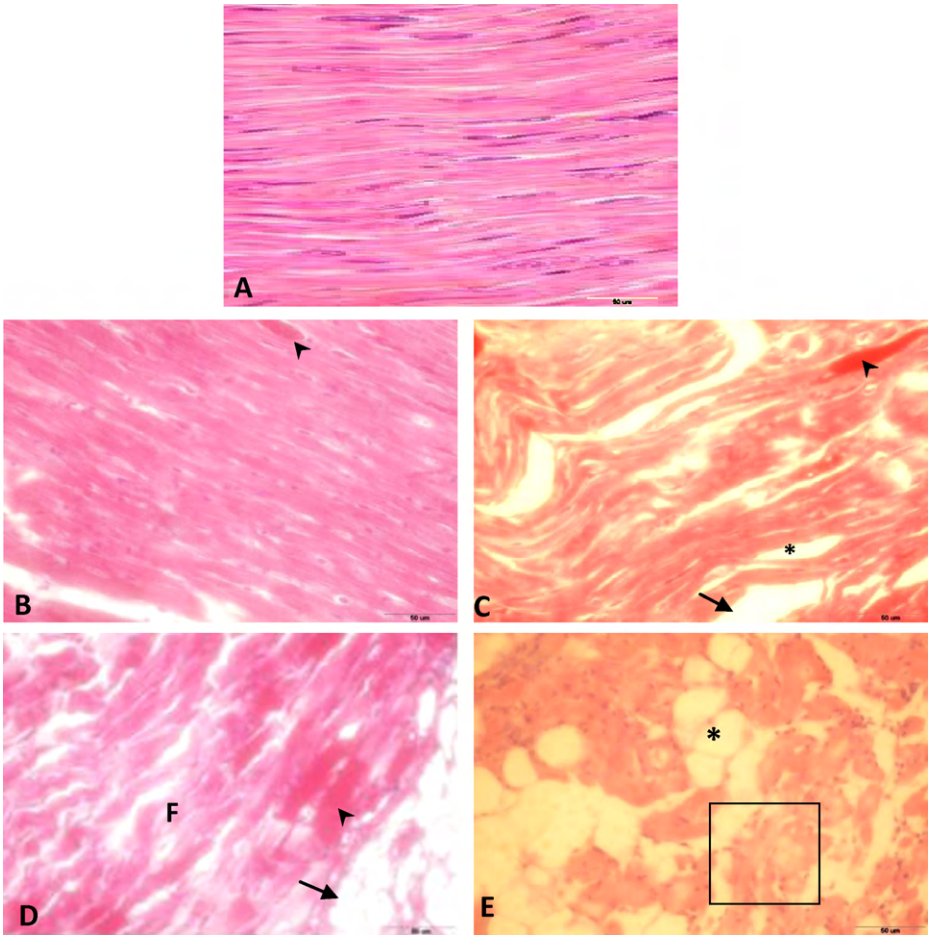

Fig. 1. Light micrograph sections of the heart tissues of rats as follows: control (A) and diazinon (Dz) treated rats (B–E), 8, 10, 12 and 20 mg/kg.
Note: distinct appearance of cardiac muscle fibers with stained nuclei and intramuscular connective tissue. Mild myocyte vacuolization (*), haemorrhages (➤), interstitial edema (→) myofibril disintegration (□), fibrosis (F) in (D, E) while less vacuolization, and disintegration in (B, C) compared to control myocytes. H&E stain (400× for B–E; 200× for A).

histological analysis of rat's heart and skeletal muscles are represented in Figs. 1 and 2. The heart tissues demonstrated normal myofibrils in the control group. While, in diazinon-treated groups, there were different degrees of degeneration ranged from slight to severe degrees according to the dose of Dz. The histological analysis manifested that diazinon treatment produced extensive subendocardial fibrosis in heart tissues (Fig. 1). Furthermore, Fig. 2 showed large areas of degenerating muscle fibers. Also, loss of transverse striations and wide interfascicular spaces were shown in muscle fibers with different degrees according to the dose.

4. Discussion

It is estimated that organophosphates are essential for human life. So, their frequent and widespread use is unavoidable. Diazinon is not only used in control of vegetables and fruits but also in ectoparasiticide formulations for sheep and cattle and in collars and washes for external parasitic control, resulting in distribution of diazinon in the environmental deleterious effects on biological systems [19]. The effects of organophosphates on humans are not diagnosed routinely because a direct cause–effect cannot be established by physicians. Our results showed that diazinon treatment caused significant ($p < 0.05$) decrease in the activity of serum AChE in all Dz-treated groups compared to the control group (Table 1). This is consistent with the study of Sarabia et al. [20]. They reported that animals intoxicated with Dz for 21 days revealed significant inhibition in the activity of plasma acetylcholinesterase. Also, in fishes, exposure to diazinon in sublethal doses was reported to affect the nervous system by inhibition of acetylcholinesterase activity [21]. Furthermore, the observations of Celik and Isik [22] suggested that the administration of methyl parathione and dichlorvos at sublethal concentrations inhibits AChE and butylcholinesterase (BChE) activities in rats. They also reported that inhibition of AChE may be a better biomarker for the assessment of neurotoxic effects in the living. Organophosphates toxicants generally elicit their effects by inhibition of acetylcholinesterase, which lead to accumulation of the neurotransmitter acetylcholine in synapses; in the neuromuscular junction, over stimulation of postsynaptic cholinergic receptors leads to muscle fasciculation and eventual paralysis [23]. Like other organophosphates, Dz requires cytochrome P-450-mediated metabolic activation to its respective "oxon" for its cholinergic toxicity [17]. Furthermore, an increase in the level of serum LDH was observed in our results. It has been suggested that when liver cells containing LDH are damaged or destroyed due to oxidative effect of diazinon, the integrity of cell membrane gets disturbed and it might become more porous and permeable or may rupture resulting in the leakage of this enzyme [24].

Our results showed a significant increase in the level of serum MDA indicating a noticeable increase in lipid peroxidation biomarker. These findings are in agreement with the results of Amirkabirian et al. [25] who reported increases in lipid peroxidation biomarker after acute exposure to different doses of Dz. Also, our results are consistent with the study of Isik and Celik [9] that have demonstrated that the applied dosages of MP and

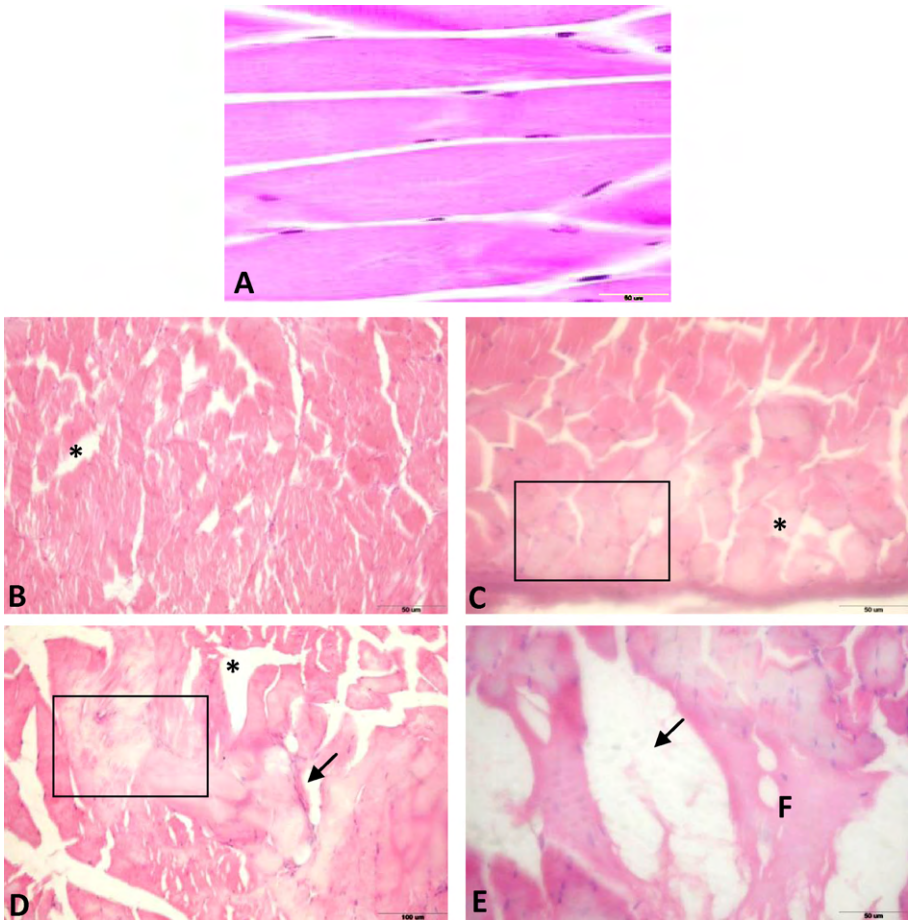

Fig. 2. Light micrograph sections of the skeletal muscle (hind leg) from (A) control rats and diazinon (Dz)-treated rats as follows: Group (B); 8 mg/kg. Group (C); 10 mg/kg. Group (D); 12 mg/kg and Group (E); 20 mg/kg. *Note*: (A) the normal configuration of muscle fibers with pronounced peripheral nucleus and intramuscular connective tissue. Large areas of degenerating muscle fibers (□) and muscle fibers vacuolization (*). Fibrosis (F). Pale stained with evident loss of transverse striations and wide interfascicular spaces were present (→) in diazinon-treated rats with different degradation at all doses. H&E stain (400× for B–E; 200× for A).

diazinon could have affected the antioxidant defense systems and MDA concentration in the rainbowtrout. Likewise; other studies recorded a significant increase in lipid peroxidation in liver and plasma of rats intoxicated with different doses of deltamethrin for 16 weeks and 30 days, respectively [26]. Lipid peroxidation is a process, which is determined by the extent of the peroxide-deforming free radical mechanism on the polyunsaturated fatty acids. It is plausible to speculate from our results that diazinon treatment may result in peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids and ultimately result in cellular deterioration [27]. Accumulation of lipid peroxide is believed to be a major contributor to the loss of cell function under oxidative stress conditions. Various methods were used to show the degree of lipid peroxidation in the organism. MDA is important and the most commonly used indicator of lipid peroxidation. Its level increases in tissues when they are exposed to oxidative stress. The increase in MDA level might be an indicator of decrease in enzymatic and non-enzymatic antioxidants of defense mechanisms. Previous studies reported that MDA is significantly increased in tissues of rats exposed to organophosphate compounds [28]. The results presented in these studies indicated that there was an increase in lipid peroxidation. Xenobiotics produce oxidative stress in some tissues homogenates by an enhanced lipid peroxidation, an inhibition of superoxide dismutase activity and a decrease of glutathione peroxidase activity [29]. Our study showed significant decreases in the activities of SOD and GPx in Dz intoxicated rats. Reports of Manna et al. [30] and Tuzmen et al. [5] supported our findings. They noticed a significant decrease in SOD activity when rats were intoxicated with high and low doses of deltamethrin for 31 days and 16 weeks, respectively. In addition, Altuntas et al. [28] found that the activity of SOD was respectively decreased with increasing Dz concentrations. They suggested that the inhibition of SOD is not directly mediated only by Dz but also due to increased generation of reactive oxygen species (ROS). On the other hand, Buyukokuroglu et al. [31] showed a significant elevation in SOD activity during intoxication with diazinon. Isik and Celik [9] suggested that the chemicals might be interacting primarily with the tissues, resulting in fluctuated enzymes activities by the way of increased reactive oxygen radicals as result of stress condition in the fishes. Furthermore, in the present study, diazinon caused significant increase in the plasma level of triglycerides. Previous studies demonstrated increases in the concentration of serum triglycerides in the experimental animals that were treated with different insecticides, including the organophosphate, dichlorvos [32] and carbamate furadan [33]. This elevation of serum or plasma triglycerides has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins [34]. Also, our study showed that oral treatment with different doses of Dz caused significant increases in serum LDL-C and HDL-C. HDL-C is mainly synthesized in the liver and intestinal cells. It plays an important role in cholesterol efflux from tissues and carries it back to the liver for removal as bile acids [35]. It has been established that the elevated serum or plasma HDL-C levels are antiatherogenic [36], whereas the reduced levels are associated with an increased risk for coronary artery dis-

ease [37]. Also, an increase of serum HDL-C was observed in the rats that were treated with dichlorvos [32]. In addition, the elevated cholesterol level in the present study due to diazinon treatment is consistent with the finding of Ashgar et al. [38], who reported that total cholesterol was elevated in male rabbits treated by methyl-parathion. The increase in the level of serum cholesterol may be due to an increased synthesis of cholesterol in the liver [39]. The present study is in agreement with other investigations which reported that organophosphate insecticides including diazinon cause increases of total cholesterol and total lipid levels [40]. The induced increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of liver cell membrane [41]. Also, the increase in serum total cholesterol level may be attributed to the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum [42]. An increase in cholesterol level may be a sign of liver damage. However, some pesticides cause decrease total cholesterol level [43]. Furthermore, our results showed wide spread disorder in the myocardial structure and subendocardial necrosis with capillary dilation in the heart muscles of diazinon-treated groups. In addition, there were different degrees of degenerative changes in myocardium and myofibrils in the same tissues. Also, there were mild degeneration, vacuolization and fasciculation of the myofibrils in both the heart and skeletal muscles. The present investigation clearly indicated the myocardiotoxicity of diazinon which could bring degenerative and inflammatory reaction on the heart. These results were in accordance with the findings of Dorval et al. [44] who reported that such changes could be due to the leakage of cellular contents caused by diazinon-mediated oxidative damage to the sarcolemmal structures handling cellular homeostasis. In addition, several studies indicated that diazinon could cause oxidative stress [45]. Concerning diazinon-induced cardiotoxicity, it is of value to mention that the tissue of the heart is very sensitive to free radical damage, because of its highly oxidative metabolism and its weaker antioxidant defense, compared to other organs, such as the liver [46]. This is also in accordance with our results concerning some biochemical markers in serum samples [47]. Also, other studies reported that organophosphates produce typical sings of toxicity such as skeletal muscles fasciculation associated with muscle fiber damage in mammals [48]. Furthermore, Buyukokuroglu et al. [49,50] have found that organophosphates caused muscle injury and apoptosis in male rats. Other findings indicated that toxic manifestations induced by organophosphate insecticides may be associated with an enhanced production of ROS [51]. Among ROS, superoxide anions, hydroxyl radicals, and hydrogen peroxide. ROS enhance the oxidative process and induce lipid peroxidation (LPO) damage in cell membranes. LPO is an autocatalytic process that is caused by free radicals. The heart tissue may be susceptible to oxidative damage due to the presence of polyunsaturated fatty acids (PUFAs), and oxygen, which may produce oxidative changes in myocytes [52]. In addition, Altuntas et al. [28] have also shown that single-dose treatment with diazinon increased LPO in erythrocytes. Also, diazinon caused increase of LPO levels in rat erythrocytes and pancreas [53]. In conclusion, Dz induced varying degrees of oxidative damage and histological alterations in cardiac and skeletal muscles, varied according to the different doses of Dz.

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