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Effects of atropine and pralidoxime pretreatment on serum and cardiac oxidative stress parameters in acute dichlorvos toxicity in rats

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ABSTRACT

Recent several studies have reported that oxidative stress could be an important component of the mechanism of cardiotoxicity due to organophosphate-induced toxicity. The aim of this study is to evaluate the oxidative and antioxidative parameters in cardiac toxicity of organophosphate poisoning, and determine the effects of atropine and pralidoxime on this parameters. The experimental groups were randomly divided into five groups as control (corn oil), dichlorvos (30 mg/kg), atropine (10 mg/kg), pralidoxime (40 mg/kg), and atropine (10 mg/kg) + pralidoxime (40 mg/kg) groups. Serum cholinesterase levels were suppressed with dichlorvos, and these reductions were inhibited with atropine and/or pralidoxime pretreatment. Serum, but not cardiac, total free sulfhydryl groups and paraoxonase activities were significantly increased in the pralidoxime group when compared to the control group. Serum arylesterase activities were elevated in the dichlorvos, atropine, pralidoxime, and atropine + pralidoxime groups when compared to the control group ($P < 0.05$). Total oxidant status, oxidative stress index, malondialdehyde and catalase activities in serum and cardiac tissues were not markedly different between the groups. No significant changes were also observed with cardiac myeloperoxidase and serum ceruloplasmin activities. In conclusion, these results showed that acute dichlorvos administration did not cause marked cardiac damage, and oxidative stress probably does not play a major role in dichlorvos-induced poisoning. On the other hand, especially pralidoxime treatment markedly increased the serum total free sulfhydryl groups, paraoxonase and arylesterase activities. However, the underlying mechanisms for these changes are not exactly known.

1. Introduction

Organophosphate (OP) compounds are widely used in different applications including agriculture. Dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) is one of the most widely used OP insecticides all over the world and is one of the most common OP compounds exposed in our country [1]. The widespread use of OP insecticides, however, brings high risks of severe health problems. Besides occupational poisoning in industrial production and agricultural application, instances of acute organophosphate poisoning (OPP) also include suicide, homicide, and accidental overdose [2,3]. The mechanism of toxicity is due to the inhibition of acetylcholinesterase (AChE), resulting in an accumulation of the neurotransmitter acetylcholine and the continued stimulation of acetylcholine receptors. Excess acetylcholine produces initial muscarinic and nicotinic receptor stimulation, followed by receptor paralysis. Signs and symptoms of toxicity include muscarinic, nicotinic, and central nervous system effects [4]. The standard treatment of OPP consists of reactivation of the inhibited AChE with an oxime antidote and reversal of the biochemical effects of acetylcholine with atropine [4]. So far, atropine and pralidoxime (PAM) are the most effective treatment used for the OPP. Nevertheless, the effects of these drugs on cardiac oxidative stress and antioxidative parameters are not exactly known.

Some studies showed that OP insecticides induced biochemical and histopathological changes in different tissues such as liver, uterus, retina, vascular wall and kidney [5–8]. The current body of knowledge about the cardiac toxicity of OPP largely consists of limited studies and case reports [2,9], but its pathogenesis and underlying mechanisms are not known. Cardiac effects (including various arrhythmias and conduction disturbances) and sudden death in OPP may take place after the poisoning. In addition, hypertension–hypotension, noncardiogenic pulmonary oedema,

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and myocardial damage in a few cases have also been described [2,10]. The mechanism by which OP induce cardiotoxicity has not been elucidated thus far, and it is difficult to pinpoint one mechanism as being the cause of cardiac toxicity related to OP.

Excessive reactive oxygen species (ROS) and lipid peroxidation (LPO) generation have been found to be involved in many diseases [11,12]. It is possible that the heart is particularly sensitive to this peroxidative damage due to its limited capacity in antioxidant defence systems that can enzymatically detoxify drug-related free radicals [13]. The heart, like other tissues, has enzyme and non-enzyme systems to neutralize free radicals. In general, the amount of antioxidants is sufficient to protect the heart from any oxidant production that might occur under normal circumstances. However, the antioxidant reserves can be inadequate under pathological situations such as ischaemia and reperfusion, inflammation, and administration of cardiotoxic drugs [14]. If the antioxidants present in the body are unable to overwhelm the free radicals produced, free radical activity can lead to cell damage, known as oxidative stress [15].

OP insecticides could induce oxidative stress, which might play a crucial role in OP-induced poisoning, suggested from *in vivo* and *in vitro* studies over the past several years [3,16]. In previous studies have been suggested that the subchronic and chronic toxicity of OP insecticides such as chlorpyrifos-ethyl, methidathion, fenthion, phosalone, phosphomidon, and trichlorfon may affect on LPO and some antioxidant enzyme status in different tissues [17–19]. The effects the subchronic of methidathion and diazinon on LPO and limited antioxidant enzymes in rat heart tissue was also studied in previous studies [17,20]. Studies have suggested that a decreased level of antioxidants and increased oxidants are associated with cardiovascular diseases [21]. However, the relationship between the cardiac damage in acute dichlorvos poisoning and the level of oxidant and antioxidants markers is not well known. Although most studies have stated that some antioxidant activity was decreased, and oxidant markers were increased in OP toxicity [17,20], the studies on the relationship between oxidant and antioxidant status and the cardiac damage in acute dichlorvos poisoning are very limited [22,23]. In our previous study [23], we showed that serum levels of creatine kinase, creatine kinase-MB, cardiac troponin I, myoglobin, N-terminal probrain natriuretic peptide, malondialdehyde, and glutathione were not affected with dichlorvos poisoning in rats. However, serum nitric oxide levels suppressed with dichlorvos and cardiac nitric oxide levels elevated with atropine + pralidoxime treatment [23]. Total oxidant status, oxidative stress index, total free sulfhydryl groups, catalase, paraoxonase, arylesterase, myeloperoxidase, and ceruloplasmin activities were not previously determined in dichlorvos poisoning. Therefore, we aimed to investigate oxidative parameters and antioxidative status in the cardiac damage in acute dichlorvos poisoning and their relationships with the treatment of atropine PAM.

2. Materials and methods

2.1. Chemical and drugs

Dichlorvos (30 mg/kg, prepared daily solution in corn oil, i.p., DDPV; Tarim-Veteriner Ilac Sanayi Ltd. Sti, Istanbul, Turkey), atropine (10 mg/kg, stock solution was dissolved in ethanol, prepared daily solution in saline, i.p.; Sigma–Aldrich Chemical Co., St. Louis, USA), PAM methylsulfate (40 mg/kg i.p., Contrathion 2%; Laboratories SERB, Paris, France), and thiopental sodium (120 mg/kg, dissolved in saline, i.p., Pental Sodyum; I.E. Ulagay, Istanbul, Turkey) were used in the present study. Control injections were made with corn oil as appropriate volume. Each animal received a total volume of 3 ml/kg of compounds tested or appropriate solvents at three different injection sites. All the other materials used were of analytical grade and all stock solutions were prepared in non-pyrogenic saline (0.9% NaCl; Eczacıbası-Baxter, Istanbul, Turkey) immediately before use.

2.2. Animals and treatment

Male Wistar rats, weighing 200–400 g, were used in this study. Animals were kept in colony rooms with 12 h light/dark cycles at a room temperature of 18–22 °C, and supplied with standard laboratory diet and tap water ad libitum. The investigation conforms with the Guide for the Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study was approved by the Local Ethics Committee.

Fifty-one rats were randomly divided into five groups:

Group 1 (control group, $n = 8$) received 1 ml/kg of corn oil [24].
Group 2 (dichlorvos group, $n = 14$) received 30 mg/kg of dichlorvos [25].
Group 3 ($n = 9$) received 10 mg/kg of atropine 5 min before 30 mg/kg of dichlorvos [26].
Group 4 ($n = 10$) received 40 mg/kg of PAM 5 min before 30 mg/kg of dichlorvos [27].
Group 5 ($n = 10$) received 10 mg/kg of atropine and 40 mg/kg of PAM before 30 mg/kg of dichlorvos. All drugs and vehicle were administered intraperitoneally (i.p.).

2.3. Observation of muscle fasciculation and toxic signs

Rats were observed 6 h after the administration of dichlorvos. Muscle fasciculation was assessed according to the following qualitative staging system as previously described by Zhou and colleagues [28]. Stage 0, no abnormal muscle fasciculation; stage 1, gentle tremor of the face and limbs without movement of the joints; stage 2, light muscle fasciculation of limbs and horizontal movement of the joints; stage 3, muscle fasciculation of limbs and vertical movement of the joints. This part of experiments was performed by one of the authors who was blinded to the protocol.

2.4. Serum and tissue samples

At the end of the experiment (after 6 h of dichlorvos or corn oil injection), venous blood samples were collected by direct heart puncture under thiopental (120 mg/kg) anaesthesia. Then samples were promptly centrifuged at 2500*g*, 4 °C, for 15 min, the serum removed and stored at −40 °C until assayed. The hearts were removed and weighed, washed twice with cold saline solution, placed into tubes and stored in a deep freeze (−20 °C) until processing. Tissues were homogenized in four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using an ultrasonic homogenizer (Branson sonifier 150, Dnabury, CT, USA) after cutting of the heart into small pieces with scissors. The homogenates were then centrifuged (Eppendorf, Centrifuge 5810R, Germany) at 7000*g* for 10 min at 4 °C and the pellets discarded. The supernatant was used for the biochemical analysis. All biochemical parameters were measured by investigators who were blind to the experimental protocol.

2.4.1. Serum cholinesterase (ChE) assay

Serum cholinesterase levels were measured by a colorimetric Ellman procedure (cholinesterase-ChEBi Kit, Spinreact, Spain) using a Prestige 24i autoanalyser (Prestige 24i, Tokyo Boeki Medical System, Japan) at a wavelength of 405 nm [29].

2.4.2. Serum ceruloplasmin (CP) activity assay

The activity of ceruloplasmin was measured according to Erel's method [30] in which ferrous ion is oxidized to ferric ions via ceruloplasmin ferroxidase activity. The results were expressed as units per litre (U/l).

2.4.3. Measurement of total antioxidant capacity (TAC)

TAC levels were determined using a novel automated colorimetric measurement method developed by Erel [31]. In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colourless substrate *O*-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in colour. Upon the addition of sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the sample, preventing the colour change and thereby providing an effective measure of the total antioxidant capacity of the sample. The assay has excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equivalents/l for serum, or mmol Trolox equivalent/g protein for cardiac tissue samples.

2.4.4. Measurement of total oxidant status (TOS)

TOS was determined using a novel automated measurement method, developed by Erel [32]. Oxidants present in the sample oxidize the ferrous ion–*O*-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of mmol H_2O_2 equivalent/l for serum, or mmol H_2O_2 equivalent/g protein for cardiac tissue samples.

2.4.5. Oxidative stress index (OSI)

The percentage ratio of TOS to TAC yields the OSI, an indicator of the degree of oxidative stress [33]. OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent/l)/TAC (mmol Trolox equivalent/l). The OSI value for the cardiac samples was also calculated as OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent/g protein)/TAC (mmol Trolox equivalent/g protein).

2.4.6. Malondialdehyde (MDA) assay

Lipid peroxidation was evaluated by a fluorometric method based on the reaction between MDA and thiobarbituric acid (TBA) [34]. Briefly, 50 μl of serum or supernatant of tissues was added to 1 ml of 10 mM diethylthiobarbituric acid (DETBA) reagent in a phosphate buffer (0.1 M, pH 3). The mixture was mixed for 5 s and incubated for 60 min at 95 °C. Samples were placed on ice for 5 min, and then 5 ml of butanol was added. The mixture was shaken for 1 min to extract the DETBA-MDA adduct and then centrifuged at 1500*g* for 10 min at 4 °C. Fluorescence of the butanol extract was measured at an excitation wavelength of 539 and emission wavelength of 553. 1,1,3,3,Tetraethoxypropane (obtained from Sigma) was used as the standard solution, and the values were presented as μmol/l for serum, or μmol/g protein for cardiac samples.

2.4.7. Measurement of total free sulfhydryl (SH) groups

Free sulfhydryl groups of samples were assayed according to the method of Ellman [35] as modified by Hu et al. [36]. Briefly, 1 ml of buffer containing 0.1 M Tris, 10 mM EDTA, pH 8.2, and 50 μl serum or sample were added to cuvettes, followed by 50 μl of 10 mM DTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as a standard and the result was expressed as millimolars for serum, or millimols per mg of protein (mmol/mg protein) for cardiac samples.

2.4.8. Paraoxonase (PON) and arylesterase (ARE) activities assay

Paraoxonase activity was measured in the absence of 1 M NaCl (basal paraoxonase). The rate of paraoxon hydrolysis (diethyl-*p*-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm at 37 °C. The amount of generated *p*-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which is 17,000 M^{-1} cm^{-1} [37]. Paraoxonase activity was expressed as U/l for serum or U/g protein for cardiac samples. Phenylacetate was used as a substrate to measure the arylesterase activity by monitoring the increase of absorbance at 270 nm at 37 °C. Enzyme activity was calculated from the molar absorptive coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as U/l serum [38].

2.4.9. Catalase (CAT) activity assay

Catalase activity was assayed by a method described by Goth [39]. Serum or homogenate (0.2 ml) was incubated in 1.0 ml substrate (65 μmol per H_2O_2 in 60 mM sodium–potassium phosphate buffer, pH 7.4) at 37 °C for 60 s. The enzyme reaction was stopped with 1.0 ml of 32.4 mM ammonium molybdate, and the yellow complex of molybdate and H_2O_2 was measured at 405 nm against a blank. CAT activity was linear up to 100 kU/l. If the CAT activity exceeded 100 kU/l, the homogenate was diluted with phosphate buffer (2- to 10-fold), and the assay was repeated. One unit of CAT decomposes 1 μmol of H_2O_2 min^{-1} under these conditions. Results were expressed as 10^3 U/l for serum, or 10^3 U/g protein for cardiac samples, which was calculated as follows:

$$\mathrm{CAT} = [\mathrm{A(Sample)} - \mathrm{A(Blank\ 1)}/\mathrm{A(Blank\ 2)} - \mathrm{A(Blank\ 3)}] \times 271$$

Blank 1 contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml hemolysate; blank 2 contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml buffer; blank 3 contained 1.0 ml buffer, 1.0 ml molybdate, and 0.2 ml buffer.

2.4.10. Tissue myeloperoxidase (MPO) activity assay

The method described by Wei et al. [40] was used for the tissue MPO activity assay, which is a lysosomal oxidative enzyme that is found in white blood cells, and data are expressed as U/g protein.

2.4.11. Protein content assay

Protein level was measured by the Lowry method [41].

2.5. Statistical analysis

All data are expressed as means ± SD or the percentage incidence unless otherwise indicated. Statistical analysis was performed using GraphPad Instat (version 3.05). Statistical comparison of more than two groups was performed by ANOVA followed by a Student–Newman–Keuls multiple comparisons test. Fisher's exact test was used to detect significant differences in the mortality incidence. The intensity of muscle fasciculation and toxic signs were compared with the Mann–Whitney *U* test. In all tests, *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Experimental findings

All the rats receiving acute 30 mg/kg doses of dichlorvos developed cholinergic signs (fatigue, tremor, cyanosis, excess of secretions, fasciculations, convulsion, and respiratory distress and arrest) within 3–5 min. These findings (fatigue, tremor, cyanosis, excess of secretions, and fasciculations) continued approximately one half to 1 h after dichlorvos administration in rats. Mortality and survival rates for all groups were determined within the first 6 h. Seven of 14 rats (with prolonged convulsion and respiratory distress and arrest) were dead at the end of the experiment (the mortality rate was 50%). There were no cholinergic findings and deaths in the control, atropine, PAM, and atropine + PAM groups during the first 6 h (Table 1). Mortality and survival rates were found to be statistically significant between the groups ($P < 0.05$).

3.2. Biochemical findings

Serum ChE and oxidative–antioxidative parameters of the groups are listed in Table 1. Mean serum ChE activities were 81.1 ± 26.8 U/l in the dichlorvos group compared with 190.9 ± 46.8 U/l in the control group ($P < 0.05$; Table 1). There were no significant differences in serum ChE activities in the atropine, PAM, and atropine + PAM groups when compared with the control group ($P > 0.05$, Table 1).

Cardiac oxidative and antioxidative activities of the groups is shown in Table 2. Total oxidant status, oxidative stress index, MDA levels, total antioxidant capacities, catalase activities in serum, and cardiac tissue were found to be statistically insignificant between the groups ($P > 0.05$, Tables 1 and 2). Additionally, serum ceruloplasmin activities showed no marked changes between the groups ($P > 0.05$, Table 1). No significant changes were also observed with cardiac myeloperoxidase activities ($P > 0.05$, Table 2).

Serum total free sulfhydryl groups were markedly elevated in the PAM group compared with the control group ($P < 0.05$; Fig. 1A). No significant differences in serum SH levels were recorded in the control, atropine and atropine + PAM groups when compared with the dichlorvos group ($P > 0.05$, Fig. 1A). There were no significant changes in cardiac tissue SH activities between all the groups ($P > 0.05$, Fig. 1B). Although serum PON activities were increased in the PAM group when compared with the control group ($P < 0.05$, Fig. 2A), no significant differences were found between the other groups ($P > 0.05$, Fig. 2A). Cardiac PON levels showed an insignificant changes between the groups ($P > 0.05$, Fig. 2B). Serum ARE activities were elevated in the dichlorvos, atropine, PAM, and atropine + PAM groups when compared with the control group ($P < 0.05$, Fig. 3). This elevation was not significant in the atropine, PAM, and atropine + PAM groups when compared to the dichlorvos group ($P > 0.05$, Fig. 3).

4. Discussion

The continued stimulation and eventual paralysis of the acetylcholine receptors account for the clinical signs and symptoms of organophosphate poisoning, including muscarinic, nicotinic, and central nervous system effects [4]. Acute OP can lead to a potentially lethal state characterized by muscular paralysis, autonomic overstimulation, and cardiorespiratory failure. Besides, cardiac complications accompany poisoning with these compounds, which may be serious and often fatal [2,10]. Although rhythm disturbances as cardiac effects of OP have been documented, it is not known whether the acute toxicity of OP causes damage in heart tissue. Povoa et al. [42] have reported that OP-induced acute poisoning with myocardial necrosis. Although this is the first case in the literature with histological confirmation of myocardial necrosis from OP intoxication, biochemical parameters of cardiac damage have not been examined. Worldwide OP report of mortality rates ranges from 3% to 30%. The mortality rate for poisoned patients who require ventilation is as high as 50% [1]. Up to 70% of patients with OPP have a high incidence of respiratory failure and a prolonged QT interval corrected for heart rate [1,2,43]. It has been reported that the administrations of subacute dichlorvos inhibited AChE and butyrylcholinesterase activities in the various tissues of rats [44]. The results of the present study showed that serum

Table 1
Some measured ChE and other parameters in serum for the groups, and toxic signs in rats.

Groups/parameters	Control ($n = 8$)	Dichlorvos ($n = 7$)	Atropine ($n = 9$)	PAM ($n = 10$)	Atropine + PAM ($n = 10$)
ChE (U/l)	190.9 ± 46.8	81.1 ± 26.8[+]	155.2 ± 74.5	156.8 ± 79.8	151.4 ± 60.8
TAC (mmol Trolox equivalent/l)	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.05	1.2 ± 0.1	1.1 ± 0.1
TOS (mmol H_2O_2 (equivalent/l)	10.0 ± 1.8	12.2 ± 3.9	11.1 ± 2.6	1.4 ± 5.4	8.6 ± 0.8
OSI (arbitrary units)	8.4 ± 2	10.9 ± 3.5	9.7 ± 2.5	8.1 ± 2.8	7.8 ± 0.9
MDA (μmol/l)	5.5 ± 1.3	7 ± 2.2	6.1 ± 1.6	5.96 ± 1.9	5.1 ± 0.3
CAT (10^3/l)	16.7 ± 2.6	17.3 ± 1.6	17.7 ± 1	16 ± 2.8	16.8 ± 1
CP (U/l)	628 ± 177.4	624.2 ± 133.8	606.2 ± 135.3	562.9 ± 49.2	492.2 ± 26.1
Total score of poisoning signs[a]	1 ± 0.4	18 ± 0.5[*]	1 ± 0.3	1 ± 0.3	1 ± 0.3

Values are means ± SD.
[a] Data are expressed as sum ± SD.
[+] $P < 0.05$, significant decrease in ChE activities when compared with the control group.
[*] $P < 0.05$, significantly different from other groups.

Table 2
Cardiac tissue parameters.

Groups/parameters	Control ($n = 8$)	Dichlorvos ($n = 7$)	Atropine ($n = 9$)	PAM ($n = 10$)	Atropine + PAM ($n = 10$)
TAC (mmol Trolox equivalent/g protein)	3 ± 0.3	2.7 ± 0.1	2.9 ± 0.27	3 ± 0.2	2.9 ± 0.4
TOS (mmol H_2O_2 (equivalent/g protein)	42.1 ± 9.3	37.2 ± 5	37 ± 5.25	38.3 ± 7.1	36.1 ± 4.5
OSI (arbitrary units)	14.3 ± 3.3	13.6 ± 1.5	12.6 ± 1.9	12.8 ± 2.4	13 ± 1.3
MDA (μmol/g protein)	33.7 ± 5.8	30.3 ± 4.7	32.2 ± 4.6	30.3 ± 5.3	30.7 ± 4.9
CAT (10^3 U/g protein)	13 ± 8.5	12.1 ± 5.5	6 ± 4.9	17.9 ± 12.7	11.4 ± 6.4
MPO (U/g protein)	2057.8 ± 722.8	1987.0 ± 480.6	2011.2 ± 334.4	1949.4 ± 609	1885 ± 446.9

Values are means ± SD.

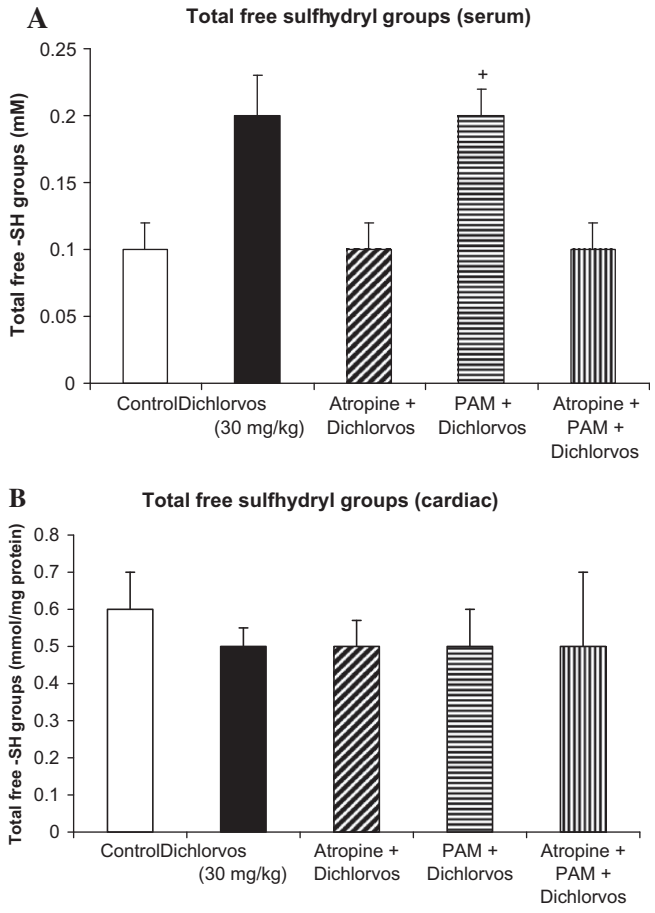

Fig. 1. Serum (A) and cardiac (B) SH groups activities for the groups. $^{+}P < 0.05$ when compared with the control group. Values are expressed as means ± SD.

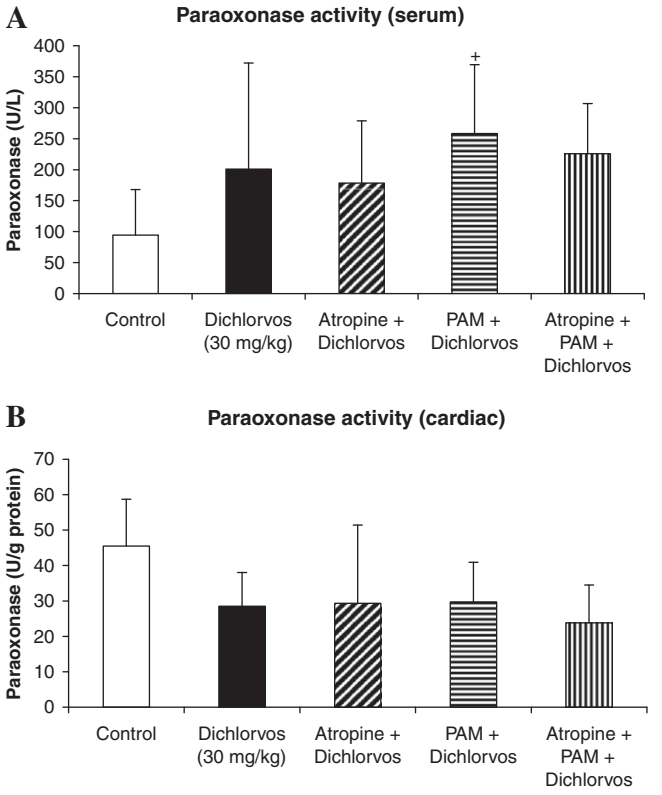

Fig. 2. Serum (A) and cardiac (B) PON activities for the groups. $^{+}P < 0.05$ when compared with the control group. Values are expressed as means ± SD.

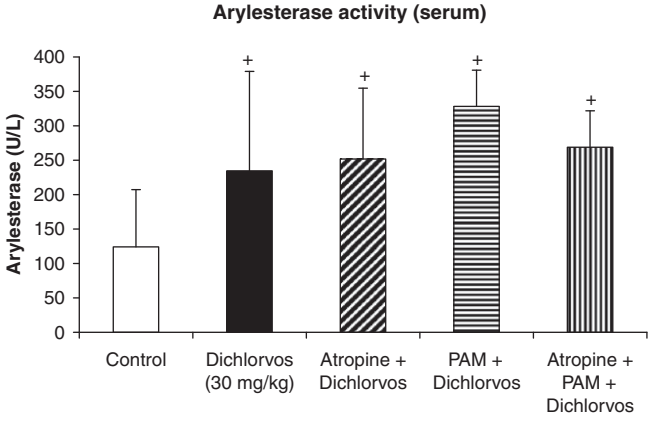

Fig. 3. Serum ARE activities for the groups. $^{+}P < 0.05$ when compared with the control group. Values are expressed as means ± SD.

ChE levels were decreased with dichlorvos, and these reductions were inhibited with atropine, PAM, or atropine + PAM pretreatments. Our results showed that pralidoxime did not markedly affect the serum ChE activities. These data support the results of one of the meta-analysis of clinical trials which showed that oximes are not effective in the management of organophosphate-poisoned patients and, surprisingly, they can be dangerous and worsen the patient's clinical situation [45]. All the rats receiving dichlorvos developed cholinergic signs and showed 50% mortality rate. There were no cholinergic findings and deaths in the control, atropine, PAM, and atropine + PAM groups during experimental period. Our data showed that atropine, PAM, or atropine + PAM pretreatments markedly reduced mortality.

The heart tissue may be susceptible to oxidative damage due to the presence of polyunsaturated fatty acids and oxygen, which may produce oxidative changes in myocytes. Recent studies point that oxidative stress could be an important part of the molecular mechanism of OP-induced toxicity [6,16]. In previous studies have indicated that various OP insecticides can affect the antioxidant enzyme status at different levels, and generally cause the LPO levels to increase. It was also shown that these OP insecticides significantly altered the activities of the main antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and CAT. In these studies, LPO has been suggested as one of the molecular mechanisms involved in OP-induced toxicity [16,18,19]. It has been reported that lipid peroxidation accompanied with decreased levels of total antioxidant capacity, total thiols, and cholinesterase activity in acute OPP patients [46]. Furthermore, a significant correlation between cholinesterase depression and reduced total antioxidant capacity has also been established [46]. There is also evidence that glutathione reductase significantly decreased in the erythrocytes, and SOD and glutathione-*S*-transferase activities were significantly increased with dichlorvos in rats [47]. It has been shown that the administrations of subacute dichlorvos increased significantly the levels of serum marker enzyme activities (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase) and leukocytosis [48]. Yavuz et al. [17] have shown that subchronic of methidathion administration increased MDA in rat heart tissue. In addition, Akturk et al. [20] reported that diazinon caused an increase on MDA levels and changes the activities of some antioxidant enzymes (SOD, GSH-Px, and CAT) in rat heart. However, in these studies, oxidative stress markers, PON, ARE, CP, MPO and SH

groups activities have not been investigated. On the contrary of these studies, in our present study, we did not observe an important changes in the OSI, TOC, MDA levels as oxidative stress parameters. Also, there were no statistically significant changes in TOS and CAT as an antioxidant parameters.

Ceruloplasmin, which is produced by hepatocytes and associated with copper metabolism, has antioxidant characteristics as an acute phase protein [49,50]. Previous studies have shown that CP is a powerful serum antioxidant, when iron-stimulated reactions are involved and this has mainly been ascribed to its ferroxidase activity. It has been reported that the levels and the activity of CP increase in inflammatory diseases, but its activity is inhibited via ROS [51,52]. Human CP has recently been ascribed a thiol-linked peroxidase activity which can remove H_2O_2 and LOOHs [50,53]. MPO is a heme-containing enzyme found in azurophilic granules of human neutrophils [54]. Previous studies reported that MPO has antioxidative effect and it may have a significant relationship with cardiovascular events [55]. In the light of this information, we investigated whether CP and MPO have any relationship with OPP-related cardiotoxicity. In our study, no significant changes were observed in serum and cardiac levels either in CP and MPO acute dichlorvos poisoning.

Paraoxonase-1 (PON1) is enzyme with three activities which are paraoxonase, arylesterase, and dyazoxonase. PON1 hydrolyses organophosphates, such as paraoxon, aromatic esters, for instance, phenyl acetate, and also lipid peroxidation products, and reduces the accumulation of them [56]. PON and ARE which are located on high dansite lipoprotein (HDL) are antioxidant, anti-inflammatory properties and calcium dependent esterase enzymes [57]. It has been shown both experimentally and clinically that the levels of these enzymes decrease in many diseases under oxidative stress [57,58]. PON1, which is a part of HDL cholesterol, is a strong antioxidant enzyme that is believed to have a protective role in the atherosclerotic process, by both contributing to HDL's protective effect against atherosclerosis and preventing lipoprotein peroxidation and oxidation of LDL cholesterol [57,58]. Recent articles indicated that PON1 and ARE reduce oxidative stress in serum and tissues, thus protecting against cardiovascular disease [57,58]. The study from Akgur et al. [59] on 28 patients with acute organophosphate poisoning revealed a correlation between PON1 levels and acute poisonings, a correlation which was shown to be lower than chronic poisonings. In another study Sozmen et al. [60] found that PON1 levels and alloenzymes play a significant role in organophosphate poisonings. However, in that study, oxidative stress markers and arylesterase activity were not investigated. In our study, dichlorvos poisoning caused a decrease in serum and cardiac PON activities; yet, this decrease was not statistically significant. Additionally, serum and cardiac PON levels remained the same in groups receiving atropine and atropine + PAM. However, in the group receiving PAM, there was a considerable increase in serum PON activity when compared to the control group, whilst no decrease was observed in their cardiac PON levels. Moreover, in the present study, we observed that dichlorvos intoxication increased of serum arylesterase activity. Similarly, this increase was also observed other all in the groups. There is only limited information available to date to show an approximate association between directly measured paraoxonase, arylesterase activities, and cardiotoxicity in OPP.

Total free sulfhydryl groups act as important cellular scavengers of peroxides and so help to protect cells from damage by these molecules. Sulfhydryl groups of intracellular and extracellular compartments are critical in the determination of protein structure, regulation of enzyme activity, and control of transcription factor activity and binding. Decrease in SH level not only impairs cells' response to oxidants, but also changes the functions of inflammatory cells [61]. In this study, although serum SH levels increased in the groups administered of dichlorvos and PAM, this increase was only statistically significant in the PAM group. However; there was no marked change in cardiac tissue free SH groups in the present study. Both the PON, ARE and SH activities were independently correlated with the MDA levels in our study.

As demonstrated by this study, (a) serum and cardiac oxidative stress and antioxidant parameters were not markedly affected by dichlorvos poisoning; (b) atropine and PAM did not cause a positive change in oxidative stress and some antioxidative parameters (CP, MPO, CAT, etc.); (c) a positive finding of this study is that PAM treatment could be argued to cause a marked increase in serum PON, ARE and SH levels. The mechanism behind this association is unknown. Yet, PAM might enhance antioxidative effect. To the best of our knowledge, there are no published data on the relationship between in these parameters with atropine and PAM treatment in the acute OPP. Hence, this is the first study evaluating the oxidative–antioxidative status of atropine + PAM administration in OP intoxication.

One of the limitations of this study could be attributed to the fact that acute (single dose) dichlorvos was administered, and experimental parameters were examined in a short time span (i.e. 6 h). Toxicity of dichlorvos is dependent on the dose and exposure period. Although Parveen and Kumar [62] have reported that the LD_{50} of dichlorvos for rats is 21.4 mg/kg body weight, our study showed 50% mortality with 30 mg/kg body weight dichlorvos dose. Nevertheless, our study has demonstrated that serum and cardiac oxidative stress parameters (OSI, TOC, and MDA) were not affected in acute dichlorvos poisoning. It was also found that administration of atropine and PAM did not have a pronounced effect on oxidative and antioxidant activity, except for PON, ARE and SH groups. Notwithstanding, larger controlled studies and long-term data in this regard would be especially intriguing. Therefore, we believe that there is a need for numerous further studies on the subject concerning oxidative and antioxidative parameters in both acute and chronic toxicity.

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