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Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity

Ajay Goel [a,b,*], Vijayta Dani [a], D.K. Dhawan [a,**]

[a] *Department of Biophysics, Panjab University, Sector 14, Chandigarh 160014, India*
[b] *Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Dallas, TX 75246, USA*

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Abstract

The present study investigated the hepatoprotective role of zinc in attenuating the toxicity induced by chlorpyrifos in rat liver. Male Sprague–Dawley (SD) rats received either oral chlorpyrifos (13.5 mg/kg body weight), zinc alone (227 mg/l in drinking water) or combined chlorpyrifos plus zinc treatment for a total duration of 8 weeks. The effects of these treatments were studied on various parameters in rat liver, including lipid peroxidation, antioxidant enzymes, levels of metallothionein (MT) and hepatic histoarchitecture. Chlorpyrifos treatment resulted in a significant increase in hepatic lipid peroxidation and activities of superoxide dismutase (SOD), glutathione peroxidase (G-Px) and glutathione reductase (GR). On the contrary, chlorpyrifos intoxication caused a significant inhibition in the levels of reduced glutathione (GSH), catalase (CAT) and glutathione-*S*-transferase (GST) activities. However, zinc treatment to chlorpyrifos-intoxicated animals normalized the otherwise raised levels of lipid peroxidation to within normal limits. Moreover, zinc treatment to these animals resulted in an elevation in the levels of GSH, catalase and GST, as well as a significant decrease in the levels of SOD. Levels of MT were also found to be depressed in chlorpyrifos-treated animals, but tended to increase following co-administration of zinc. Additionally, chlorpyrifos-treated animals demonstrated increased vacuolization, necrosis and ballooning of the hepatocytes and dilatation of sinusoids as well as increase in the number of binucleated cells. However, zinc administration to chlorpyrifos-treated animals resulted in overall improvement in the hepatic histoarchitecture, emphasizing the protective potential of zinc. Hence, the present study suggests the protective potential of zinc in alleviating the hepatic toxicity induced by chlorpyrifos.

Keywords: Zinc; Chlorpyrifos; Antioxidant enzymes; Liver histology; Metallothionein

1. Introduction

Chlorpyrifos belongs to the phosphorothioate class of organophosphorus insecticides. Since its development, this compound has been widely used for a variety of agricultural and public health applications [1]. Chlorpyrifos is activated to the corresponding oxygen analog, which in turn is responsible for its mammalian toxicity through acetylcholinesterase (AChE) inhibition. It has been demonstrated that the activation of chlorpyrifos to the

Abbreviations: AChE, acetylcholinesterase; CAT, catalase; G-Px, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione reduced; GST, glutathione-*S*-transferase; MT, metallothionein; SOD, superoxide dismutase

* Corresponding author. Tel.: +1 214 820 2692; fax: +1 214 818 9292.
** Corresponding author. Tel.: +91 172 2534121; fax: +91 172 2534118.
E-mail addresses: ajayg@baylorhealth.edu (A. Goel), dhawan@pu.ac.in (D.K. Dhawan).

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corresponding chlorpyrifos-oxon, is achieved through oxidative desulfuration by cytochrome P_{450}-dependent enzymes [2,3]. It has also been shown that repeated doses of chlorpyrifos caused significant hepatic atrophy [4]. Earlier reports from our laboratory have revealed that profile of liver marker enzymes and essential trace element were found to be adversely affected in rats subjected to chlorpyrifos treatment [5,6]. More recently, it was shown that short-term whole body exposure of chlorpyrifos in rats caused significant inhibition of AChE activity in different tissues including liver, kidney and spleen [7]. Chlorpyrifos treatment in these studies resulted in increased oxidative stress in the body, as evidenced by enhanced levels of thiobarbituric acid reactive substances (TBARS), accompanied by concomitant decrease in the levels of superoxide scavenging enzymes SOD, catalase and G-Px in liver, kidney and spleen [7].

Certain enzyme reactivators, such as oximes, constitute the most important means of preventive treatment following exposures to organophosphorus insecticides in humans [8]. However, the possible protective roles of safer preventative compounds, offering least amount of side effects are warranted to be explored. A number of studies have suggested zinc as a beneficial agent during peroxidative damage [9–11]. Moreover, recent studies from our group have demonstrated the efficacy of zinc in regulating the liver functions in nickel- and lead-intoxicated animals [12–14]. However, no studies have been performed to data to study if zinc may have beneficial effects in organophosphate-induced toxicity in experimental models of liver injury, which may have implications in managing humans with accidental exposures to such compounds.

Thus, in the current study, we have attempted to explore the possible hepatoprotection afforded by zinc with regard to its antioxidative potential in restoring the altered biochemical enzyme activities and liver histoarchitecture in chlorpyrifos-induced toxicity.

2. Materials and methods

2.1. Evaluation of chlorpyrifos purity

Before the initiation of various treatments, the purity of chlorpyrifos procured from Montari Agro Industries, Bombay, was evaluated using a VG 70S-11–250J + gas chromatographic–mass spectrometer at the Regional Sophisticated Centre, Panjab University, India. The mass/charge (m/z) ratios were determined for chlorpyrifos and the specific peaks obtained were compared with the available databases to identify the structure and purity of the compound.

2.2. Experimental design

2.2.1. Grouping of animals

Male Sprague–Dawley rats weighing 145 ± 20 g were procured from the central animal house, Punjab University, Chandigarh. The animals were housed in polypropylene cages in the departmental animal house under hygienic conditions and were acclimatized for at least 1 week before putting them on different treatments. The animals were maintained on the standard laboratory feed and water ad libitum, throughout the period of experimentation.

Animals were segregated into four different groups. Group 1 (G-1) served as normal controls and were fed with normal diet and water. Group 2 (G-2) animals were given an oral chlorpyrifos treatment at a dose level of 13.5 mg/kg body weight (oral LD_{50}; 135/kg body weight) in corn oil, every alternate day. Animals in Group 3 (G-3) served as normal zinc controls for the Group 4 (G-4) animals and were supplemented with zinc in the form of $ZnSO_4 \cdot 7H_2O$, at a dose level of 227 mg/L, added to the drinking water. Group 4 animals were given a combined treatment with chlorpyrifos as well as zinc in a similar manner, as was given to Groups 2 and 3 animals, respectively. All the treatments continued for a period of 8 weeks. Before sacrificing the animals, they were fasted overnight and euthanized under light ether anesthesia. Livers were removed immediately and one lobe was preserved in formalin for light microscopic studies and the other lobe was processed for various biochemical estimations.

2.3. Biochemical estimations

2.3.1. Protein

Protein assay was done by the method of Lowry et al. [15]. Briefly, the samples were diluted with 100 mM phosphate buffer (pH 7.5) to a volume of 0.5 ml. The reactions were diluted with 0.5 ml of 1.0N sodium hydroxide followed by the addition of 5.0 ml of reagent C (containing 48 ml of 2% sodium carbonate, 1.0 ml of 1% copper sulfate and 1.0 ml of 2% sodium potassium tartarate). Following 10 min incubation at room temperature the color was developed by addition of 1.0N Folin's phenol reagent and absorbance was measured spectrophotometrically at 750 nm.

2.3.2. Antioxidant defense system enzymes and lipid peroxidation

2.3.2.1. Preparation of 10,000 × g supernatant. The livers were removed and homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 0.15 M

KCl to obtain 25% homogenate, using a mechanically driven Teflon-fitted Potter Elvejhem homogenizer. The homogenates were centrifuged at 10,000 × *g* for 20 min. The 10,000 × *g* supernatant was re-centrifuged at 100,000 × *g* for 60 min. The microsomal pellet was suspended in 100 mM potassium phosphate buffer containing 20% glycerol (v/v). Aliquots of microsomes were stored at −20 °C till further use.

2.3.2.2. NADPH-dependent lipid peroxidation (LPO). Lipid peroxidation in the microsomal fraction was estimated by the formation of thiobarbituric acid reactive substances in the presence of enhancer (ADP–$FeCl_3$) [16]. NADPH-dependent formation of peroxidases leads to the breakdown of polyunsaturated fatty acids to malondialdehyde (MDA). The MDA thus formed reacts with thiobarbituric acid (TBA) to form a pink colored chromophore, which absorbs maximum at 535 nm. 2.0 ml incubation mixture for the formation of lipid peroxides contained 50 mM Tris–HCl buffer (pH 7.5), 0.1 mM NADPH, 1.7 mM $FeCl_3$, 0.1 mM adenosine diphosphate (ADP) and appropriate amount of microsomes. Blanks were run simultaneously with each reaction lacking NADPH. Following incubation at 37 °C for 10 min, 2.0 ml of TCA–TBA–HCl (15%–0.375%–0.25N, v/v) solution was added to all the tubes. All reaction tubes were heated for 15 min in a boiling water bath, followed by centrifugation at 1200 rpm for 10 min. The absorbance was measured at 535 nm on a spectrophotometer and MDA concentrations were calculated and expressed as nmol MDA formed/(min mg) protein.

2.3.2.3. Catalase. The method of Luck [17] was used for the estimation of catalase in 10,000 × *g* supernatant. Briefly, the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1.25×10^{-2} M H_2O_2 and appropriate amount of sample. Every sample was run along with appropriate blanks lacking H_2O_2. Decrease in the absorbance was measured at 240 nm and the enzyme activity was expressed as nmol H_2O_2 decomposed/(min mg) protein.

2.3.2.4. Superoxide dismutase (SOD). The activity of SOD in 10,000 × *g* supernatant was estimated by using the method of Kono [18]. The method is based on the principle of the inhibitory effect of SOD on reduction of nitroblue tetrazolium (NBT) dye by superoxide anions, which are generated by the photo-oxidation of hydroxylamine hydrochloride. The reaction mixture contained 1.3 ml of 50 mM sodium carbonate solution, 0.1 mM EDTA, 0.5 ml of 96 μM NBT and 0.1 ml of 0.6% Triton X-100. Reaction was initiated by the addition of 0.1 ml of 20 mM hydroxylamine hydrochloride (pH 6.0) and appropriate amount of 10,000 × *g* supernatant and the rate of NBT reduction was measured using a spectrophotometer.

2.3.2.5. Reduced glutathione (GSH). Estimation of GSH was performed in the tissue homogenates of liver by the method of Moron et al. [19]. Briefly, 0.1 ml of 25% trichloroacetic acid (TCA) was added to 0.5 ml (125 mg tissue equivalent) of the tissue homogenate. Following protein precipitation by TCA, the samples were centrifuged to obtain the supernatant. 0.1 ml of supernatant was incubated with 2.0 ml of freshly prepared 0.6 mM 5,5′-dithiobis (2-nitrobenzoic acid). Optical density of the yellow colored complex was measured at 412 nm against a reference lacking tissue homogenate. For each set of assays, a standard curve was obtained for GSH and utilized to calculate GSH content in liver homogenates from various treatment groups.

2.3.2.6. Glutathione reductase (GR). The enzyme was assayed by the method of Williams et al. [20]. For these measurements, 1.0 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.5), 3.0 mM EDTA, 1.0 mg bovine serum albumin, 0.1 mM NADPH, 3.3 mM GSSG and an appropriate amount of 10,000 × *g* supernatant. Blanks were run with each set of reactions lacking GSSG. The decrease in absorbance was monitored at 340 nm on a double beam spectrophotometer and the enzyme activity was expressed as μmol NADPH oxidized/(min mg) protein.

2.3.2.7. Glutathione-S-transferase (GST). This enzyme was assayed in the 10,000 × *g* microsomal fraction by the method of Habig et al. [21] using 1, chloro-2, 4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) and ethacrynic acid (EA) as substrates for GST. The increase in absorbance due to the formation of glutathione conjugates by GST with different substrates was measured in a double beam spectrophotometer. The blanks in all cases constituted of non-enzymatic reaction of GSH with respect to the substrates.

2.3.2.8. Glutathione peroxidase (GPx). Glutathione peroxidase was assayed by the method of Flohe and Gunzler in 10,000 × *g* supernatant [22]. For GPx enzymatic activity measurements, 1.0 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.5), 0.5 mM EDTA, 1.0 mM sodium azide, 0.24 units GSSG reductase, 1.0 mM GSH, 0.15 mM NADPH, 0.15 mM H_2O_2 and an appropriate amount of 10,000 × *g* supernatant.

The decrease in absorbance due to NADPH oxidation was measured at 340 nm on a double beam spectrophotometer.

2.3.2.9. Estimation of metallothionein (MT). The assay of the MT was performed according to the procedure given by Eaton and Toal [23]. For this assay, 10,000 × *g* supernatant was analyzed using radiolabeled cadmium (Cd) as a substrate. Briefly, 0.2 ml of carrier-free ^{109}Cd solution was incubated with the appropriate amount of sample for 10 min. This was followed by the addition of 0.1 ml of 2% bovine haemoglobin and subsequent boiling of the reaction tubes at 80 °C for 2 min. The tubes were centrifuged and 0.2 ml of the clear supernatant was transferred to disposable gamma counting tubes and the amount of activity was measured on a gamma counter. Blank samples without tissue homogenate were counted with each set of test reactions and the total amount of Cd binding MT was calculated.

2.3.3. Histopathological studies

For the histopathological observations at light microscopic level, fresh tissue pieces of liver were immersion fixed in 10% phosphate-buffered formalin. Following an overnight fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. Blocks were made and 5–7 μm thick sections were double stained with hematoxylin and eosin and observed under light microscope.

2.4. Statistical analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) and a multiple post hoc test (Student–Newman–Keuls) with 5% considered significant. The results are represented as mean ± S.D.

3. Results

All the results from various treatment groups have been compared with their normal controls. However, results from chlorpyrifos + zinc (G-4)-treated group have also been compared with the data of the chlorpyrifos-treated group (G-2).

3.1. GC–MS of chlorpyrifos

The results of the GC–MS analysis of chlorpyrifos are shown in Fig. 1. Based upon the prominent fragment patterns with high abundances (>30%) at mass/charge (*m*/*z*) ratios = 314, 285, 158, 197 and 97, respectively, and elucidation of the structure for chlorpyrifos with the available databases, it was determined that the purity of the compound is greater than 98%. These results suggested that chlorpyrifos compound is suitable for all animal model studies.

3.2. Body weights

The variations in the body weights of the animals subjected to different treatments are shown in Table 1.

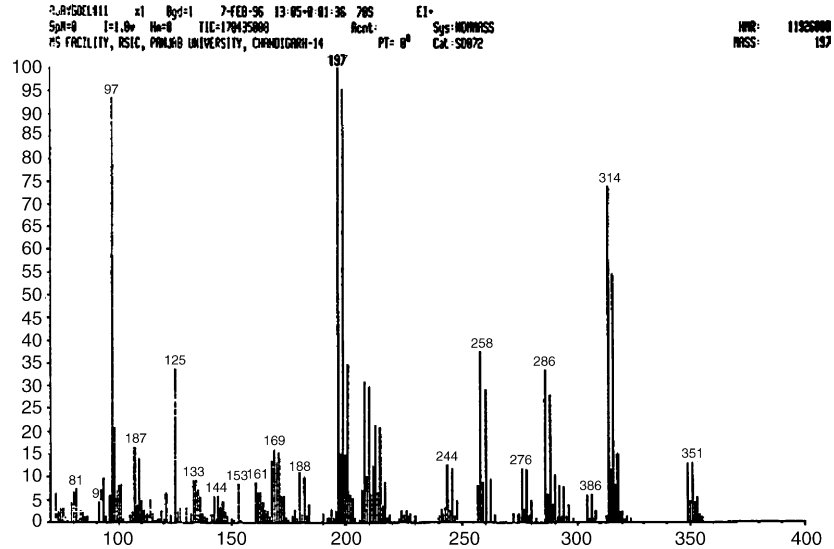

Fig. 1. A full-scan GC–mass spectrum of chlorpyrifos. The *x*-axis on the spectrum represents the mass/charge (*m*/*z*) ratio and the *y*-axis depicts the percent abundance for each fragment pattern. Based upon the prominent fragment patterns with high abundances, it was determined that the purity of the compound is greater than 98%.

Table 1
Alterations in the body weights of the animals following zinc treatment to chlorpyrifos-intoxicated rats

Groups	Body weight
G-1 (normal control)	245.00 ± 16.43 (11)
G-2 (chlorpyrifos)	221.25 ± 42.33 (9)
G-3 (zinc)	253.24 ± 33.40 (10)
G-4 (chlorpyrifos + zinc)	232.88 ± 32.70 (11)

Values are expressed as mean ± S.D. The values in parenthesis represent the number of animals used for each measurement.

During the course of present investigations, it was observed that the body weights of the untreated control animals and zinc-treated controls increased progressively throughout the study and recorded a net body weight gains of 67.8 and 69.79%, respectively. However, the net body weight gain of the animals intoxicated with chlorpyrifos was markedly less and was 36.41% only, as compared to the controls. Zinc treatment to the chlorpyrifos-intoxicated animals resulted in a net body weight gain of 48.8%.

3.3. Antioxidant defense system enzymes and lipid peroxidation

Chlorpyrifos treatment to control animals resulted in a significant increase ($p < 0.001$) in hepatic lipid peroxidation, as well as enzyme activities of SOD, G-PX and GR (Tables 2 and 3). In addition, chlorpyrifos treatment caused a statistically significant inhibition ($p < 0.001$) in the levels of GSH, catalase and GST. However, zinc treatment to chlorpyrifos-treated animals reversed the already raised levels of lipid peroxidation to within normal limits ($p < 0.05$–0.01). Moreover, zinc treatment to the chlorpyrifos-treated animals resulted in a significant elevation in the levels of GSH ($p < 0.05$), catalase ($p < 0.05$) and GST ($p < 0.01$) activities and a significant decrease in the levels of SOD ($p < 0.05$). However, activities of G-Px and GR did not alter significantly following zinc treatment to chlorpyrifos-intoxicated animals. No significant changes were observed in the control animals treated with zinc alone.

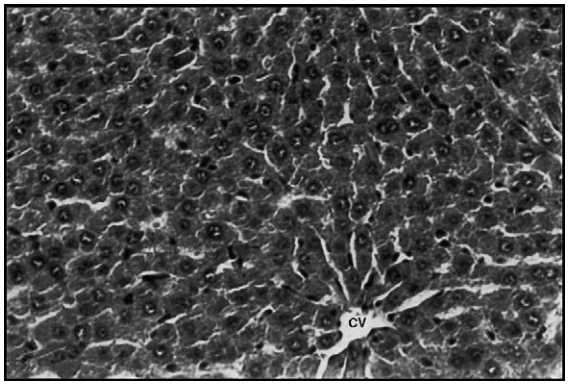

Fig. 2. A section of liver from a control animal showing a hepatic lobule with the uniform pattern of the polyhedral hepatocytes radiating from the central vein (CV) towards the periphery. Magnification: 400×.

3.4. Metallothionein

MT content of various treatment groups is presented in Table 4. A significant reduction ($p < 0.001$) in the concentration of this metal binding protein was observed following chlorpyrifos treatment. However, zinc treatment to normal controls, as well as chlorpyrifos-intoxicated animals responded by a significant elevation ($p < 0.001$) in the MT concentration. Moreover, the increase in the MT content was more pronounced in zinc-treated animals ($p < 0.001$) than the combined zinc- and chlorpyrifos-treated animals ($p < 0.00$), when compared to the normal controls.

3.5. Hepatic histoarchitecture

Histological findings of liver from various treatment groups are presented in Figs. 2–5. Normal control animals revealed clear cut hepatic lobules, separated by interlobular septa, traversed by portal veins (Fig. 2). Within the lobule, hexagonal array of hepatic plates

Table 2
Effect of zinc on the hepatic lipid peroxidation (LPO) and reduced glutathione (GSH) levels in rats subjected to zinc and chlorpyrifos treatment

Groups	LPO (nmol MDA formed min^{-1} mg^{-1} protein)	GSH (μmol GSH g^{-1} tissue)
G-1 (normal control)	0.119 ± 0.015 (8)	2.09 ± 0.05 (8)
G-2 (chlorpyrifos)	0.183 ± 0.031 (9)[c]	1.81 ± 0.04 (8)[c]
G-3 (zinc)	0.107 ± 0.038 (6)	1.98 ± 0.13 (7)
G-4 (chlorpyrifos + zinc)	0.125 ± 0.021 (7)[z]	2.09 ± 0.11 (7)[y]

Values are expressed as mean ± S.D. by Newman–Keuls test. The values in parenthesis represent the number of animals used for each analysis.
[c] $p < 0.001$ in comparison to G-1.
[y] $p < 0.01$ in comparison between G-2 and G-4.
[z] $p < 0.001$ in comparison between G-2 and G-4.

Table 3
Effect of zinc treatment on the hepatic antioxidant enzymes in rats subjected to chlorpyrifos intoxication

Groups	SOD (IU)	Catalase (μmol H_2O_2 decomposed min^{-1} mg^{-1} protein)	GSH-Px (μmol NADPH oxidized min^{-1} mg^{-1}protein)	GR (μmol NADPH oxidized min^{-1} mg^{-1}protein)	GST (μmol cojugate formed min^{-1} mg^{-1}protein)
G-1 (normal control)	8.08 ± 0.90 (7)	0.76 ± 0.16 (8)	0.317 ± 0.036 (7)	0.014 ± 0.0017 (9)	0.91 ± 0.18 (8)
G-2 (chlorpyrifos)	9.99 ± 0.74 (7)[c]	0.46 ± 0.12 (7)[c]	0.420 ± 0.031 (7)[c]	0.020 ± 0.0030 (7)[c]	0.62 ± 0.07 (7)[c]
G-3 (zinc)	8.19 ± 0.99 (8)	0.66 ± 0.08 (6)	0.331 ± 0.027 (8)	0.015 ± 0.0017 (7)	0.86 ± 0.08 (8)
G-4 (chlorpyrifos + zinc)	9.29 ± 0.91 (9)[b,x]	0.69 ± 0.24 (8)[x]	0.400 ± 0.070 (7)[b]	0.017 ± 0.0040 (8)[a]	0.78 ± 0.13 (7)[y]

Values are expressed as mean ± S.D. by Newman–Keuls test. The values in parenthesis represent the number of animals used for each analysis.
[a] $p < 0.05$ in comparison to G-1.
[b] $p < 0.01$ in comparison to G-1.
[c] $p < 0.001$ in comparison to G-1.
[x] $p < 0.05$.
[y] $p < 0.01$ in comparison between G-2 and G-4.

Table 4
Variations in the metallothionein (MT) content in liver following zinc treatment to chlorpyrifos-intoxicated rats.

Groups	Metallothionein (μ g^{-1} wet weight)
G-1 (normal control)	2.18 ± 0.38 (8)
G-2 (chlorpyrifos)	1.66 ± 0.23 (7)[b]
G-3 (zinc)	3.75 ± 0.55 (8)[c]
G-4 (chlorpyrifos + zinc)	3.29 ± 0.72 (7)[c,z]

Values are expressed as mean ± S.D. by Newman–Keuls test. The values in parenthesis represent the number of animals used for each analysis.
[b] $p < 0.01$ in comparison to G-1.
[c] $p < 0.001$ in comparison to G-1.
[z] $p < 0.001$ in comparison between G-2 and G-4.

radiating towards periphery from a central vein, were clearly visible. Between the laminae, hepatic sinusoids were quite clear. The hepatocytes were polyhedral. The nuclei were round and the size was roughly uniform, with the exception of a few binucleated cells. Similar observations were made for the control animals given zinc treatment.

Chlorpyrifos-intoxicated animals indicated marked alterations of hepatic pathology after 8 weeks of treatment. The hepatic cords were disrupted at most of the places (Fig. 3). A few hepatocytes were vacuolated and had lost the usual polyhedral shape. Vacuolization along with hepatocytes ballooning was more severe near the portal tracts with a resultant widening of the sinusoidal spaces and some degree of hepatic hypertrophy. There was a significant increase in the

Fig. 3. A liver section of a chlorpyrifos-treated animal demonstrating disrupted pattern of hepatic cords around the central vein (CV). Marked vacuolization and regions of necrosis (arrows) are evident in many parts of the section. Hepatocyte ballooning (circles) was a common occurrence, while a few binucleated cells were also seen. Sinusoidal spaces are widened (marked as "*"). Hypertrophy of the cytoplasmic structure is visible at certain places. Magnification: 400×.

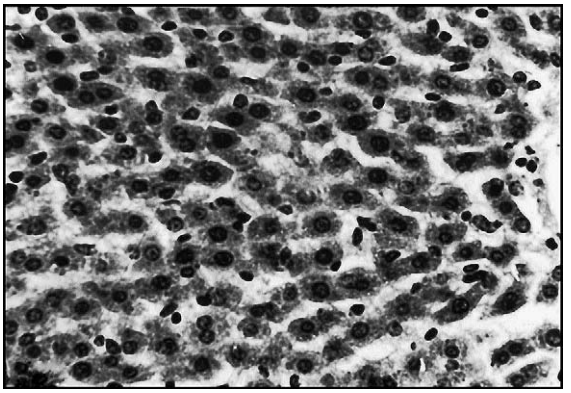

Fig. 4. A light micrograph from a zinc-treated animal depicting almost normal appearance in the arrangement of hepatocytes and the sinusoidal gaps. Magnification: 400×.

cells undergoing necrosis. Number of binucleated cells was also increased in these animals intoxicated with chlorpyrifos.

Zinc treatment alone did not result in any significant alteration in hepatic histoarchitecture as shown in Fig. 4. However, co-administration of zinc to chlorpyrifos-intoxicated rats resulted in marked improvement in the structure of the hepatocytes (Fig. 5), although some binucleated cells were still present. A few cells with disintegrating or dividing nucleus were also seen. Although, the lamellar pattern of hepatocytes was restored to almost normal, still the sinusoidal spacing was more widened in these animals, in comparison to the normal controls. Furthermore, the fine vacuolization observed was of very small proportion and was present around the hepatoportal tracts.

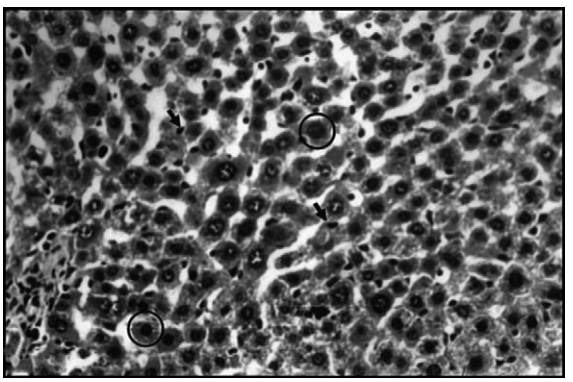

Fig. 5. Hepatic lobule from a zinc- and chlorpyrifos-treated animal showing some degree of necrosis (arrows) and moderate number of binucleated cells. A few ballooned cells (circles) were also present but were much lesser compared to chlorpyrifos-intoxicated rats. Magnification: 400×.

4. Discussion

The present study investigated the protective potential of zinc supplementation in animals subjected to chlorpyrifos intoxication. Here, we show that zinc treatment to chlorpyrifos-intoxicated animals normalized the otherwise raised levels of lipid peroxidation to within normal limits. Moreover, zinc treatment in these animals resulted in an elevation in the levels of GSH, catalase and GST activities, as well as a significant decrease in levels of SOD. Additionally, zinc supplementation in these animals resulted in marked improvement in hepatic histoarchitecture, emphasizing its protective potential.

The net body weight gain of the animals intoxicated with chlorpyrifos was markedly less as compared to the normal controls. These findings are in line with the previous work, where it was shown that there was a dose-dependent decrease in body weight gain in animals intoxicated with leptophos and isoprocarb [24,25]. In addition, we did not observe any appreciable change in the diet consumption of the rats following chlorpyrifos intoxication, suggesting that the poor body weight gain may be due to the overall increased degeneration of lipids and proteins as a result of the direct effects of the organophosphate. Zinc treatment to chlorpyrifos-treated animals showed a better body weight gain compared to chlorpyrifos-treated animals alone. Such protective effects of zinc have been reported previously and were attributed to the ability of zinc in reducing collagen accumulation in liver in these conditions [6,26,27].

In the present study, chlorpyrifos treatment to normal rats indicated a marked increase in the hepatic LPO. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and its occurrence in biological membranes causes impaired membrane function, structural integrity [28], decrease in membrane fluidity and inactivation of a several membrane bound enzymes. Although not entirely clear from the current data, it is plausible to speculate that chlorpyrifos treatment may result in peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids and ultimately result in cellular deterioration [29]. The normalization of LPO following zinc treatment is very likely due to its antiperoxidative properties, as has been shown previously [10,11]. Zinc has also been reported to interact with cell membranes to stabilize them against various damaging effects, including those due to oxidative injuries [30]. Similar observations have also been made earlier from our laboratory [13,14], where zinc supplementation improved the overall hepatic histoarchitecture in nickel-treated rats.

In our study, we observed a decrease in GSH levels in chlorpyrifos-intoxicated animals. Younes and Siegers [31] also observed a similar depletion of GSH and suggested that this may be responsible for enhanced LPO. GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including the GSH peroxidases [32]. Under oxidative stress, glutathione is consumed by the glutathione related enzymes to detoxify peroxides produced due to increased lipid peroxidation [33]. In this study, the observed increase in lipid peroxidation and a concomitant depletion in GSH activity, suggests that the increased peroxidation may be a consequence of depleted GSH stores. Treatment with zinc to chlorpyrifos-intoxicated animals showed normal levels of GSH, which may either be due to the effect of zinc in reducing net hepatic peroxidative activity of the hepatic cells [34].

The antioxidant enzymes SOD, G-Px and catalase limit the effects of oxidant molecules on tissues and are active in the defense against oxidative cell injury by means of their being free radical scavengers [35]. These enzymes work together to eliminate active oxygen species and small deviations in physiological concentrations may have a dramatic effect on the resistance of cellular lipids, proteins and DNA to oxidative damage [36,37]. The present study indicated a significant elevation in the activity of superoxide dismutase and glutathione peroxidase, but depressed levels of catalase in chlorpyrifos-intoxicated animals. These data suggest that chlorpyrifos treatment may result in increased formation of oxygen-free radicals, which could stimulate SOD activity to cope with this increased oxidative stress.

In the present study, we have observed low levels of catalase following 8 weeks of toxicity by chlorpyrifos, which could possibly be owed to the consumption of this enzyme in converting the H_2O_2 to H_2O. Another relevant consideration in H_2O_2 conversion by catalase and G-Px is that at lower concentrations, these hydrogen peroxides are preferentially metabolized by G-Px because of its lower K_m for H_2O_2. This is indicative of the rate of formation of free radicals [38]. In addition, we also observed significantly enhanced activities of glutathione reductase in chlorpyrifos-intoxicated animals, which is understandable in the light of depressed GSH levels. The increased activities of G-Px and glutathione reductase suggests that free radical scavenging processes in the cell are generally cooperative as catalase and G-Px combine to metabolize H_2O_2 produced by different substrates. Moreover, a statistically significant inhibition in the GST activity was noticed in the chlorpyrifos-treated animals, which usually is the case under oxidative stress conditions. This finding is in agreement with a other studies using chlorpyrifos and its oxygen analog, where the participation of GST in mediating the toxicity of these compounds was well-argued [39].

With regards to the animals, which were co-administered with zinc and chlorpyrifos, we observed normalization of lipid peroxidation and most antioxidant enzymes activities. These protective effects of zinc can be related to the antiperoxidative property of this metal ion. The observed normalization trend of GSH levels and GST activity following zinc treatment could possibly be because of either induced metallothionein content, or due to its indirect action in reducing the levels of oxygen reactive species [40]. Even though no definitive evidence is available for the protective effects of zinc on enzymes of oxidative stress especially with regard to SOD, catalase and G-Px, zinc deficiency has previously been documented to elevate the CuZn-SOD activity [41].

Significantly depressed levels of MT were observed in chlorpyrifos-intoxicated animals. Although MT synthesis is known to be induced in response to various pathological and physiological agents, the observed decrease in MT content could be ascribed to the zinc deficient state in these animals. Administration of zinc to the normal control as well as chlorpyrifos-intoxicated animals resulted in a marked elevation in the hepatic MT pool, which is suggestive of its property to induce metallothionein synthesis [42,43]. Metallothionein induction is mediated through transcriptional control of the genes coding for metallothionein in response to the various inducers, such as zinc [44].

With respect to the hepatic histoarchitecture of the chlorpyrifos-treated animals there was an increased vacuolization of hepatocytes and dilation of sinusoidal spaces in comparison to untreated normal controls. The number of binucleated cells was also increased in these animals. These observations indicated marked changes in the overall histoarchitecture of liver in response to chlorpyrifos, which could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of chlorpyrifos-treated animals are in corroboration with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. In support of the liver damage observed with chlorpyrifos toxicity, we have previously shown that chlorpyrifos intoxication alters serum and hepatic activities of liver maker enzymes including AST and ALT [5]. Co-administration of zinc to chlorpyrifos-treated animals resulted in normalizing the hepatic histoarchitecture appreciably. Similar hepatoprotective effects of zinc have also been observed by us earlier while evaluating

its potential in nickel and carbontetrachloride toxicity [5,13,26].

In view of these data, it can be concluded that zinc supplementation has hepatoprotective effects in chlorpyrifos-induced liver toxicity. The precise mechanism of the observed zinc mediated regulation of enzymatic activities and lipid peroxidation cannot be ascertained from this study and remains to be explored in future.

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