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# Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood

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**Abstract**

The present study determined the protective potential of zinc in attenuating the toxicity induced by chlorpyrifos in rat blood. Male Sparque Dawley (SD) rats received either oral chlorpyrifos (13.5 mg/kg body weight) treatment every alternate day, zinc alone (227 mg/l in drinking water) or combined chlorpyrifos plus zinc treatment for a total duration of 8 weeks. The effects of different treatments were studied on various parameters in rat blood including haemoglobin (Hb) levels, total leukocyte count (TLC), differential leukocyte count (DLC), zinc protoporphyrins (ZPP), serum trace elemental concentrations and Scanning Electron Microscopic (SEM) observation of the blood cells. Chlorpyrifos treatment to normal control animals resulted in a significant decrease in TLC and ZPP concentration after 4 and 8 weeks. Chlorpyrifos treated animals also showed significant neutrophilia and lymphopenia after 8 weeks of toxicity. In addition, a significant decrease in serum zinc and iron concentrations were observed following chlorpyrifos intoxication, however, these animals responded with increased serum copper levels following the toxic treatment with this organophosphate. SEM studies of the red blood cells from chlorpyrifos treated animals indicated marked alterations in the topographical morphology of the various cell types, with the prominent feature being common aniscocytosis of the erythrocytes. Oral zinc treatment to the chlorpyrifos treated animals significantly improved the total leukocyte, neutrophil and lymphocyte counts, as well as the otherwise reduced concentrations of ZPP and the levels of various serum trace elements. Protective effects of zinc were also evident in the electron microscopic observations where most blood cell types depicted reverted to a close to the normal appearance. Based upon these data, the present study is first of its kind and suggests that zinc treatment considerably attenuates chlorpyrifos induced toxicity induced in restoring the altered hematological indices and morphological changes.

## Introduction

Chlorpyrifos is a broad-spectrum organophosphate insecticide and is extensively being used to control agricultural pests, disease vectors and is preferred to chlorinated hydrocarbons for field applications because of its quick action, relatively shorter half-life and poor-accumulation in the food web (Kwong 2002). Chlorpyrifos manifests its mammalian toxicity through activation to its corresponding oxygen analog (chlorpyrifos-oxon), which in turn is responsible for the inhibition of the acetylcholinestrase (AChE) enzyme leading to neuropathy. Besides, the moderate to severe toxicity of chlorpyrifos in non-neuronal tissues in many mammalian species has also been attributed to the hydrolytic detoxification of the chlorpyrifos-oxon by a group of hepatic arylesterases (Costa

*et al*. 1990; Li *et al*. 1993). Previous reports from our laboratory have clearly indicated the adverse effects of chlorpyrifos intoxication on the profile of liver marker enzymes, antioxidant enzyme system and the hepatic levels of essential trace elements (Goel *et al*. 2000, 2005; Goel & Dhawan 2001). More specifically, in a recent study, Singh *et al*. (2004) reported a significant increase in lipid-peroxidation levels in rat erythrocytes following chlorpyrifos treatment. Although the primary toxicological target for chlorpyrifos is nervous system, it is very common to observe toxic manifestations of these compounds in other organs including hematological system. Curiously, besides the well-known effects of organophosphates on brain and some reports for their hepatic toxicity, there is limited evidence that these insecticides may have adverse effects on hematological profiles (Gibel & Lohs 1975; Mandal *et al*. 1986). Despite these sporadic reports, till date there is no clear information and understanding whether or not organophosphates in general, and chlorpyrifos in particular, mediates its toxic effects through alterations in the hematological indices in the laboratory animals.

Another challenging aspect of organophosphorus insecticide toxicity is lack of adequate preventative strategies that are safe and non-toxic for occupationally exposed human population. Various chemical compounds have been attempted for such preventive interventions, but without much success. More recently, much attention is being focused on the possible role of essential trace elements in providing the necessary preventive efficacy with least toxicity and side-effects (Xiu 1996; Kang & Zhou 2005; Zhou *et al*. 2005). In this context, zinc, a key constituent or cofactor of over 300 mammalian proteins, is intensively being studied for its protective efficacy in various models of animal toxicity (Joshi *et al*. 2004; Zhou *et al*. 2005). A number of studies have strongly suggested zinc to be a beneficial agent in mitigating the damage arising in the setting of increased oxidative stress (Cagen & Klaassen 1979; Cabre *et al*. 1999; Zhou *et al*. 2005). Data from our laboratory are in support of these observations and we have also demonstrated the protective effects of zinc in regulating the hepatic function in various models of hepatotoxicity (Dhawan & Goel 1994, 1995; Sidhu *et al*. 2004a, b).

Therefore, due to the paucity of information on the toxic manifestations of chlorpyrifos on hematological parameters, and the lack of information on the role of zinc in such conditions, it was of our interest in the present study to investigate whether zinc would ameliorate the damage inflicted on the blood cells of the rats intoxicated with chlorpyrifos. Here, we demonstrate that oral zinc treatment to the chlorpyrifos treated animals significantly improved altered hematological indices, serum trace elemental concentrations and morphological changes in blood cells. Collectively, these data suggest that zinc treatment can be potentially be considered as an intervention in human subjects with accidental exposures to acute doses of chlorpyrifos and related organophosphates.

## Materials and methods

### *Evaluation of chlorpyrifos purity*

Before the initiation of various treatments, the purity of chlorpyrifos procured from Montari Agro Industries, Bombay, India was evaluated using a VG70S-11-250J + Gas Chromatographic-Mass Spectrometer (GC-MS) at the Regional Sophisticated Centre, Panjab University, India.

### *Experimental design*

#### *Grouping of animals*

Male Sparque Dawley (SD) rats weighing $145 \pm 20$ 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 prior to 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. Animals in Group1 (G-1) served as normal controls and were fed only with normal diet and water *ad libitum*. 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 for a duration of 8 weeks. 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 of the animals. Group 4 animals were given a combined treatment with chlorpyrifos as well as zinc in a similar manner, as was given to Group 2 and Group 3 animals, respectively. All the treatments continued for a period of 8 weeks.

*Haematological observations*

*Blood collection*

Blood samples were drawn from various animals from all the treatment and control groups after 4 and 8 weeks treatment under light ether anaesthesia by puncturing the retero-orbital plexus of the animals with a fine sterilized glass capillary. The blood was collected in heparinized tubes for the estimation of haemoglobin (Hb), total leukocyte count (TLC), differential leukocyte count (DLC) and zinc protoporphyrins (ZPP).

*Haemoglobin (Hb)*

Haemoglobin content in the blood samples was assessed by oxyhaemoglobin method of Dacie and Lewis (Dacie and Lewis 1991). Briefly, 20 $\mu$l of fresh non-coagulated blood samples were diluted with 4.0 ml of 0.04% ammonia solution and were vortexed thoroughly before reading the absorbance of the colored complexes at 540 nm. A standard curve was plotted to calculate the Hb levels in unknown samples.

*Total leukocyte count (TLC)*

TLC in blood was carried out by the method of Dacie & Lewis (1991). Blood samples were diluted in Turk's fluid (2% acetic acid in distilled water with a pinch of crystal violet) in the ratio of 1:20 (v/v) and a drop of diluted blood was immediately poured on Neubaur's chamber and WBC's were counted.

*Differential leukocyte counts (DLC)*

DLC was also done by the method of Dacie & Lewis (1991). A drop of freshly drawn blood was taken on a clean microscopic glass slide, spreaded, airdried and finally methanol fixed for 10 min. The fixed blood films were than stained for 30 min in freshly prepared Giemsa stain and the percentage of different leukocytes were calculated.

*Zinc protoporphyrins (ZPP)*

ZPP, an index of iron deficiency anaemia were measured in unprocessed blood on a hematoflourimeter. ZPP in the erythrocytes fluoresces at 590 nm when the excited with suitable wavelength energy (422–430 nm). Oxyhaemoglobin, which strongly affects the resulting fluorescence is filtered and focused on the photo multiplier tube. Thus, the raw signal measured by the hematoflourimeter is a function of ZPP/Hb molar ratio and is independent of blood volume. Results were expressed as $\mu$moles ZPP $mol^{-1}$ heme.

*Elemental estimation*

*Preparation of serum*

The blood samples were allowed to clot at room temperature. The clotted blood samples were centrifuged at 1500 rpm for 10–15 min to separate the clot and the estimation of various elements was carried out in the this clear supernatant.

*Serum estimations of trace elements*

Determination of serum zinc, copper and iron were carried out by the method of Evenson and Anderson (Evenson & Anderson, Jr. 1975) using atomic absorption spectrophotometer. Briefly, 0.1 ml of serum was diluted to 1.0 ml with 10 mM nitric acid for each of the elements and the samples were analyzed on a Perkin Elmer 3100 Atomic Absorption Spectrophotometer. Standards for each of the trace elements were simultaneously run for each sample.

*Scanning Electron Microscopic study of blood cells*

Fresh blood samples were drawn from animals belonging to each of the treatment groups and a drop of blood was immediately immersion fixed in 2.5% glutaraldehyde made in 0.1 M phosphate buffer (pH 7.4). After 1 h of fixation, cells were centrifuged at 1000–1500 rpm and pellets were resuspended in triple distilled water. After 2–3 washings, the final pellet was suspended in triple distilled water. A drop of the sample was smeared on the metallic SEM stubs, which was loaded with a conductive silver tape on its top. The stubs were then coated with gold to a thickness of 100 Å using a sputter-ion coater, with a gold source, for 4–5 min and the specimens were finally observed

under Scanning Electron Microscope, JSM-6100, Jeol, Japan at Regional Sophisticated Instrumentation Centre (RSIC), Panjab University, Chandigarh, India.

*Statistical analysis*

The data were expressed as Mean ± S.D and the statistical significance of the data has been determined Students *t*-test.

## Results

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

*GC-MS of chlorpyrifos*

The purity of chlorpyrifos was evaluated using a VG 70S-11-250J + GC-MS. 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. It was confirmed that the purity of the compound was greater than 98% as previously described (Goel *et al.* 2005). These results suggested that chlorpyrifos compound is suitable for all animal model studies.

*Body weights*

The variations in the body weights of the animals subjected to different treatments are shown in Table 1. During the course of present investigations, it was observed that the body weights of the normal control animals (G-1) and zinc treated controls (G-3) increased progressively throughout the study, and recorded net body weight gains of 67.8% and 69.79% respectively, at the end of the study in proportion to their initial weights. However, the net body weight gain of the animals intoxicated with chlorpyrifos (G-2) was markedly less and was of the order of 36.41% only, as compared to that of normal animals. Additionally, even though zinc treatment to chlorpyrifos intoxicated animals (G-4) resulted in a marked net body weight gain (48.8%), but it was somewhat lesser (67.8% *versus* 48.8%) in comparison to the normal control animals, suggesting that zinc treatment was partially but not completely effective in mitigating chlorpyrifos toxicity.

*Table 1.* Alterations in the body weights of the animals following zinc treatment to chlorpyrifos intoxicated rats (Body weights are expressed in grams).

| Groups | Body weight |
|---|---|
| G-1 Normal control | 245.00 ± 16.43 |
| G-2 Chlorpyrifos | 221.25 ± 42.33 |
| G-3 Zinc | 253.24 ± 33.40 |
| G-4 Chlorpyrifos + Zinc | 232.88 ± 32.70 |

Values are expressed as mean ± S.D of a minimum of at least seven independent observations.

*Hematological parameters*

With regards to the effects of chlorpyrifos and zinc treatment, no change in hemoglobin levels was observed in any of the treatment groups during the course of this study. Chlorpyrifos treatment to normal control animals resulted in a statistically significant decrease in TLC at 4 weeks ($P < 0.05$), but was more profound at 8 weeks ($P < 0.01$), when compared to the control animals (Table 2). Zinc treatment to control animals did not show any significant change in TLC counts in any of the time intervals. However, zinc co-administration to chlorpyrifos treated animals raised the otherwise decreased TLC counts.

Table 3 demonstrates the DLC of the animals in various treatment groups. Chlorpyrifos treated animals showed a significant lymphopenia ($P < 0.05$) after 4 weeks, which deteriorated further at 8 weeks ($P < 0.001$). Further, these animals also showed significant neutrophilia after 8 weeks of intoxication with chlorpyrifos ($P < 0.01$). However, zinc treatment to the chlorpyrifos treated animals significantly improved the overall leukocyte and neutrophil count. Moreover, significant recoveries in neutrophil ($P < 0.054$) and lymphocyte ($P < 0.001$) counts were observed in these animals, when compared to chlorpyrifos treated animals. No changes were found in the total number of monocytes, eosinophils and basophils in any of the treatment groups.

*Table 2*. Effect of zinc on the Hemoglobin and Total leukocyte counts in the blood of rats subjected to chlorpyrifos treatment.

| Groups | Hemoglobin (g $dl^{-1}$) | | Total leukocyte count ($\times 10^3$ $mm^{-3}$) | |
|---|---|---|---|---|
| | 4 weeks | 8 weeks | 4 weeks | 8 weeks |
| G-1 Normal control | 13.18 ± 0.99 | 11.13 ± 0.97 | 5.25 ± 0.34 | 4.38 ± 0.30 |
| G-2 Chlorpyrifos | 13.08 ± 1.41 | 10.72 ± 1.36 | 4.61 ± 0.48[a] | 3.12 ± 0.25[b] |
| G-3 Zinc | 13.08 ± 1.68 | 11.37 ± 0.97 | 5.54 ± 0.37 | 4.23 ± 0.45 |
| G-4 Chlorpyrifos + Zinc | 12.20 ± 1.22 | 10.97 ± 0.99 | 5.38 ± 0.43 | 4.46 ± 0.25 |

Values are expressed as mean ± S.D. A minimum of six animals were used for each independent analysis.[a] $P < 0.01$ and [b] $P < 0.001$ in comparison to G-1.

*Table 3*. Alterations in the differential leukocyte counts following zinc treatment to chlorpyrifos intoxicated rats (DLC counts are expressed as percent counts).

| | G-1 Control | G-2 Chlorpyrifos | G-3 Zinc | G-4 Zinc + Chlorpyrifos |
|---|---|---|---|---|
| 4 weeks | | | | |
| Neutrophils | 35.78 ± 4.73 | 39.28 ± 5.84 | 35.90 ± 5.16 | 38.42 ± 4.88 |
| Lymphocytes | 52.37 ± 6.11 | 45.73 ± 2.52[a] | 51.19 ± 8.71 | 48.13 ± 2.97 |
| Monocytes | 7.01 ± 1.32 | 7.52 ± 1.39 | 7.58 ± 1.73 | 8.20 ± 1.37 |
| Eosinophils | 3.33 ± 0.71 | 3.79 ± 0.88 | 3.49 ± 0.27 | 3.82 ± 0.19 |
| Basophils | 1.51 ± 0.09 | 1.68 ± 0.17 | 1.84 ± 0.19 | 1.43 ± 0.08 |
| 8 weeks | | | | |
| Neutrophils | 35.02 ± 6.09 | 42.61 ± 4.73[b] | 35.19 ± 7.14 | 37.57 ± 4.19[#] |
| Lymphocytes | 53.18 ± 4.84 | 44.86 ± 2.94[c] | 52.63 ± 4.19 | 50.74 ± 2.75[##] |
| Monocytes | 7.12 ± 1.19 | 7.63 ± 0.59 | 7.78 ± 1.13 | 7.33 ± 1.13 |
| Eosinophils | 3.27 ± 0.42 | 3.58 ± 0.19 | 3.17 ± 0.22 | 3.02 ± 0.12 |
| Basophils | 1.41 ± 0.14 | 1.32 ± 0.09 | 1.23 ± 0.10 | 1.34 ± 0.06 |

Values are expressed as mean ± S.D.; An average of 6–8 animals were used for each independent analysis. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ in comparison to G-1. [#] $P < 0.05$, [##] $P < 0.001$ in comparison to G-2.

*Table 4*. Effect of zinc on the Zinc Protoporphyrins in the blood of rats subjected to chlorpyrifos treatment (ZPP levels are expressed as $\mu$mol ZPP $mol^{-1}$ heme).

| Groups | Zinc protoporphyrins | |
|---|---|---|
| | 4 weeks | 8 weeks |
| G-1 Normal control | 119.33 ± 12.3 | 132.27 ± 14.11 |
| G-2 Chlorpyrifos | 94.29 ± 10.2[b] | 102.19 ± 17.19[a] |
| G-3 Zinc | 108.75 ± 13.3 | 141.06 ± 9.45 |
| G-4 Chlorpyrifos + Zinc | 117.50 ± 13.9[#] | 138.76 ± 12.11[##] |

Values are expressed as mean ± S.D; A minimum of seven animals were used for each independent analysis. [a] $P < 0.01$ and [b] $P < 0.001$ in comparison to G-1. [#] $P < 0.01$, [##] $P < 0.001$ in comparison to G-2.

Blood levels of ZPP are illustrated in Table 4. Animals which received chlorpyrifos treatment resulted with significantly depressed ZPP concentrations after 4 weeks ($P < 0.001$; 20.9%) and 8 weeks ($P < 0.01$; 22.7%). Simultaneous zinc treatment to chlorpyrifos treated animals resulted in normalization of these decreased ZPP levels.

### *Serum elemental estimations*

Serum zinc and iron levels were observed to be significant lowered after 4 and 8 weeks of chlorpyrifos treatment to normal animals (Tables 5 and 6), whereas the levels of copper were significantly increased in these animals (Table 7). Nonetheless, a significant improvement was observed in the iron and copper levels following zinc treatment to chlorpyrifos treated animals. In addition, zinc levels were also found to be raised in the combined treatment group but the increase was statistically not significant.

*Table 5*. Variations in serum Zinc levels following zinc treatment to chlorpyrifos intoxicated rats ($\mu$g ml$^{-1}$ of serum).

| Groups | 4 weeks | 8 weeks |
|---|---|---|
| G-1 Normal control | $4.73 \pm 0.43$ | $4.88 \pm 0.72$ |
| G-2 Chlorpyrifos | $3.68 \pm 0.59^{c}$ | $3.91 \pm 0.63^{b}$ |
| G-3 Zinc | $4.67 \pm 0.88$ | $4.94 \pm 0.79$ |
| G-4 Chlorpyrifos + Zinc | $3.87 \pm 0.69^{a}$ | $4.29 \pm 0.47$ |

Values are expressed as mean ± S.D. A minimum of seven animals were used for each independent analysis. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ in comparison to G-1.

*Table 6*. Variations in serum copper levels following zinc treatment to chlorpyrifos intoxicated rats ($\mu$g ml$^{-1}$ of serum).

| Groups | 4 weeks | 8 weeks |
|---|---|---|
| G-1 Normal control | $1.59 \pm 0.16$ | $1.54 \pm 0.18$ |
| G-2 Chlorpyrifos | $1.88 \pm 0.26^{a}$ | $1.90 \pm 0.23^{b}$ |
| G-3 Zinc | $1.70 \pm 0.23$ | $1.52 \pm 0.24$ |
| G-4 Chlorpyrifos + Zinc | $1.52 \pm 0.17^{\#}$ | $1.51 \pm 0.22^{\#}$ |

Values are expressed as mean ± S.D. A minimum of seven animals were used for each independent analysis. $^{a}P < 0.05$ and $^{b}P < 0.01$ in comparison to G-1. $^{\#}P < 0.01$ in comparison to G-2.

*Table 7*. Variations in serum iron levels following zinc treatment to chlorpyrifos intoxicated rats ($\mu$g ml$^{-1}$ of serum).

| Groups | 4 weeks | 8 weeks |
|---|---|---|
| G-1 Normal control | $5.86 \pm 1.02$ | $5.47 \pm 1.17$ |
| G-2 Chlorpyrifos | $4.11 \pm 0.83^{b}$ | $3.89 \pm 0.73^{a}$ |
| G-3 Zinc | $5.56 \pm 1.01$ | $5.27 \pm 1.19$ |
| G-4 Chlorpyrifos + Zinc | $4.81 \pm 0.99$ | $4.92 \pm 1.10$ |

Values are expressed as mean ± S.D. A minimum of eight animals were used for each independent analysis.$^{a}P < 0.01$ and $^{b}P < 0.001$ in comparison to G-1.

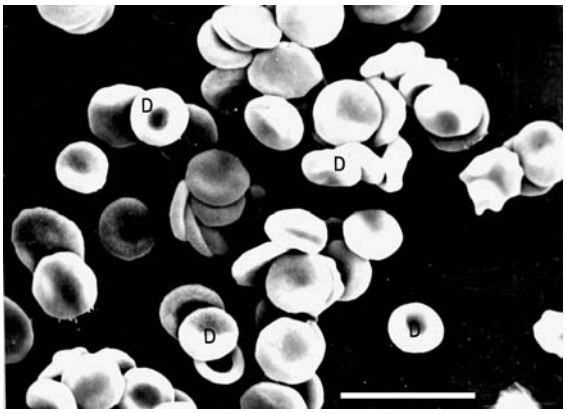


*Figure 1*. A Scanning Electron Micrograph from a control rat revealing typical biconcave discocytes (D). Bar scale = 10 $\mu$m.

### *Scanning Electron Microscopic observations of red blood cells*

Morphological studies of the blood cells were performed using Scanning Electron Microscope and these results are depicted in Figures 1–6. Normal control animals (G-1) showed typical appearance for erythrocytes where most of the cells were perfect discocytes and a few cup shaped stomatocytes (Figure 1). However, animals intoxicated with chlorpyrifos showed marked alterations in the morphological appearance of the blood cells, with almost no normal discocytes. Most of the cells changed either to cup shaped stomatocytes or were filled and changed to rounded spherocytes (Figures 2a and b). Increased aniscocytosis was a prominent feature, whereby variation in shapes and size of the cell was noticed. Certain irregularly crenated and contracted cells with numerous projections (echinocytes) were also visible. Furthermore, many cells transformed to acanthocytes, which had few irregularly spaced spicules that were bent back at their tips.

Protective effects of zinc were also evident in the combined zinc and chlorpyrifos treated group, whereby the drastic alterations in the shape of the blood cells were reverted significantly close to the normal appearance of the cells (Figures 4a and b). Despite all these protective effects of zinc, moderate population of spherocytes, acanthocytes and echinocytes were still persistent. Zinc treated control animals had almost normal discocytes. No morphological changes in blood cells were observed in the animals treated with zinc alone (Figure 3).

## Discussion

The present study investigated the protective effects of zinc supplementation in animals subjected to chlorpyrifos intoxication. Here, we demonstrate that chlorpyrifos treatment to animals for 8 weeks resulted in significant abrogation of the hematological indices as well as the morphological appearance of the blood cells as observed by electron microscopic studies. However, simultaneous

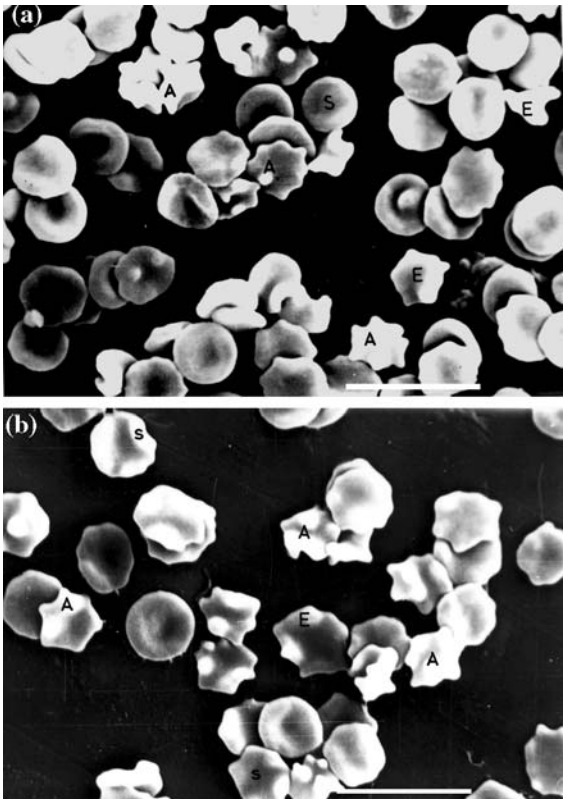


*Figure 2*. (a) A SEM image from blood cells from a chlorpyrifos treated animal showing numerous acanthocytes (A) with small spicules, a few contracted echinocytes (E) and a small number of round filled spherocytes (S). Bar scale = 10 $\mu$m. (b) Morphological view of the erythrocytes from chlorpyrifos intoxicated animals showing increased population of blebbed acanthocytes (A) and a few cup shaped stomatocytes (s). Bar scale = 10 $\mu$m.

co-administration of zinc to chlorpyrifos treated animals normalized the otherwise altered levels of blood cell counts, the ZPP levels, and the serum trace elemental concentrations. Morphological studies of blood cells on animals in this group also showed partial protective effects of zinc in conditions of chlorpyrifos induced toxicity.

The observed body weight gain of the animals intoxicated with chlorpyrifos was markedly less as compared to the normal controls which is in line with few previous studies, wherein, a similar dose dependent decrease in body weight was observed using related organophosphorus insecticides (Abou-Donia 1981; Rahman *et al*. 1990). However, in our study, we did not observe any appreciable change in the diet consumption of the rats following toxic treatment with chlorpyrifos, and

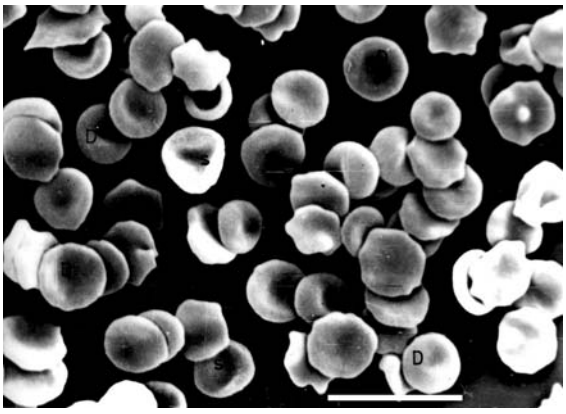


*Figure 3*. Erythrocytes from a zinc treated animal showing more number of discocytes (D) along with a few cup shaped stomatocytes (s). Bar scale = 10 $\mu$m.

thus it is anticipated that this effect could possibly be due to the overall increased detoxification of lipids and proteins as a direct result of chlorpyrifos toxicity or due to increased peroxidative damage (Rajini *et al*. 1987; Rahman *et al*. 1990). However, zinc treatment to chlorpyrifos intoxicated animals resulted in significant net body weight gain. Similar protective effects of zinc in improving the body weight gain have also been emphasized in an earlier study, whereby the protective effect of zinc was attributed to its ability to reduce collagen accumulation in liver in the wake of augmented toxic stress (Dhawan & Goel 1994).

During the present study, no significant decrease in haemoglobin content was observed after 8 weeks of chlorpyrifos treatment as compared to normal animals. Although, there is no information available on the effects of chlorpyrifos intoxication on the hemoglobin content, but an increase in the haemoglobin content was reported with other organophosphorus and organochlorine insecticides (Rahman *et al*. 1990; Rajini *et al*. 1987; Janardhan and Sisodia 1990).

As regards to the total and differential leukocyte counts, a significant decrease in the TLC was observed in chlorpyrifos intoxicated animals after 4 and 8 weeks. Likewise, decreased lymphocyte counts were also seen in these animals. On the contrary, neutrophil counts were found to be elevated significantly after 8 weeks. Neutrophils are the first line of defense against infectious agents, tissue injury, parasites and inflammatory or foreign materials (Kobayashi *et al*. 2003) and exert their activity by eliminating foreign material by

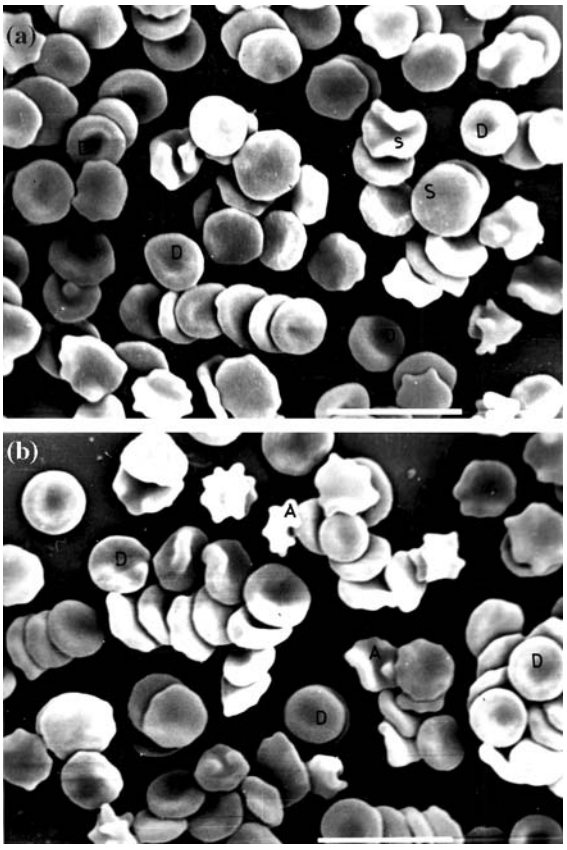


*Figure 4*. (a) Blood cells from the combined chlorpyrifos and zinc treated rats showing mostly discocytes (D), moderate number of round filled spherocytes (S) and cup shaped stomatocytes (s). Bar scale = 10 $\mu$m. (b) Morphological view of the blood cells from the combined chlorpyrifos and zinc treated animal showing few blebbed acanthocytes (A) and a few discocytes (D). Bar scale = 10 $\mu$m.

phagocytosis. So, the observed decrease in leukocyte counts following intoxication with chlorpyrifos could be attributed either to the slower rate of production of leukocytes or due to their inhibited release into the blood circulation. Although there is no report on the effects of chlorpyrifos on the hematological indices, a similar decrease in leukocyte counts was observed in rodents which were intoxicated with another organophosphate, monochrotophos for a longer term study (Janardhan & Sisodia 1990). A significant decrease in the total bone marrow cell count was indicated to be a plausible rationale for the observed depression in total and differential counts of the rats exposed to chronic doses of primiphos-methyl (Rajini *et al.* 1987). Interestingly, zinc treatment to chlorpyrifos intoxicated animals preserved the levels of total as well as differential leukocytes to within the normal limits, throughout the study. This property of zinc could be attributed to the fact that leukocytes contain high amounts of zinc and the zinc deficient conditions may have triggered a compensatory signal in these animals (Prasad 1983).

Chlorpyrifos treatment to normal control animals resulted in a significant decrease in zinc protoporphyrins levels that are the precursors of hemoglobin. The observed decrease in the ZPP levels are understandable in the light of the fact that zinc is required as a cofactor in the synthesis of ZPP (Walden *et al.* 1984). Moreover, zinc supplementation to chlorpyrifos treated animals normalized the ZPP levels, probably because of the increased availability of zinc, which is required for the synthesis of this heme moiety.

In this study, significantly depressed serum zinc levels were observed in chlorpyrifos treated animals. Abnormalities in the zinc metabolism leading to its deficiency are generally attributed to various factors like, malabsorption, malnutrition, decreased intestinal zinc binding factors or the increased excretion of the zinc via the gastrointestinal tract or excretion through urine are of common occurrence in chronic liver disorders (McClain & Su 1983). It has also been cited, that a direct correlation exists between zinc and copper, and when zinc concentrations are low, it results in increased copper levels (McCall *et al.* 1971), thus indicating an antagonistic nature for these two metal ions. Zinc acts by displacing copper from transport proteins and stimulates the synthesis of metallothionein, as a consequence of which, copper binds preferentially to the mucosal metallothionein and is associated with a reduced rate of transfer across the membrane into the plasma (McCall *et al.* 1971). These suggestions support our findings for the increased copper levels in zinc deficient animals following chlorpyrifos intoxication. Furthermore, inhibited levels of serum iron may be due to the fact that more iron is being transported into the liver causing its deficiency into the blood pool.

Scanning Electron Microscopic studies revealed drastic alterations in the red cell morphology in the blood cells of the animals following chlorpyrifos treatment. The prominent features were the transformation of the normal discocytic appearance of the RBCs to many different forms including echinocytes, spherocytes, stomatocytes and acanthocytes. Due to lack of any related

studies on this aspect, some of the plausible explanations for such findings could be attributed to the general causes such as abnormal erythropoiesis, inadequate haemoglobin formation, effects on the erythrocyte membrane lipid bilayer, accelerated erythrocyte aging, decreased water permeability across erythrocyte membranes, decreased erythrocyte thermostability, deformability and the rate of oxygen release by erythrocytes or increased erythropoiesis to compensate for the anaemia (Tkeshelashvili *et al*. 1989). Modifications in the shape of the RBCs' has been attributed to the changes in the membrane lipid composition, causing deformations in the blood cell shape in response to various adverse treatments (Bessis 1972). Co-administration of zinc to chlorpyrifos intoxicated animals significantly improved the morphology of the red blood cells. The protective effects of zinc are most likely due to its antioxidant potential, whereby, it regulates the membrane lipid composition and maintains the integrity of the membranes.

It is worth mentioning that even though the zinc concentrations used in this study were somewhat higher than the daily recommended allowance, still, no toxic effects were observed of zinc by itself in animals treated with zinc alone. Additionally, the repeated treatment of animals with chlorpyrifos may suggest that the observed toxic effects of chlorpyrifos may be better representative of an 'acute' rather than 'chronic' exposure to this organophosphate. Accordingly, it would seem that in the best scenario for zinc protection, it may be rationale to consider this as an agent of choice for intervention of human subjects with acute accidental exposures to chlorpyrifos, and probably other related organophosphates.

Hence, the current study is a first demonstration wherein a relevant correlation has been observed between the administered chlorpyrifos dose and the blood toxicity. In view of these data, it can be concluded that zinc supplementation has protective effects in chlorpyrifos induced blood toxicity. Although these effects of zinc are very promising, but the precise mechanisms of its protective action cannot be ascertained from this study and remains to be explored further.

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