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# Ameliorating and Hypoglycemic Effects of Zinc Against Acute Hepatotoxic Effect of Chlorpyrifos

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**Abstract:** The protective effects of zinc on liver injury induced by chlorpyrifos (CPF) were investigated in thirty male Wistar rats. Group 1 control group administered corn oil (vehicle of CPF), group 2 were orally administered CPF at a dose of 31.5 mg/kg once daily for 3 consecutive days. Group 3 received the same dose of chlorpyrifos orally concomitantly with zinc (Zn, 227 mg $l^{-1}$) in drinking water for 3 consecutive days then continue supplementation of zinc only till tenth day. Administration of CPF resulted in a significant increase in serum glucose level, similarly, a significant increase in the levels of various serum hepatic marker enzymes [aminotransferases (AST and ALT) and alkaline phosphatase] and significant increase in the levels of hepatic tissue malondialdehyde (MDA) while induced significant decrease in the activities of superoxide dismutase (SOD) compared with the control group. In contrast, co-administration and post-treatment with Zn to CPF-treated animals restored most of these biochemical parameters to within normal levels. From the obtained data in this study, it can deduce that CPF-induced hyperglycemia, lipid peroxidation, oxidative stress in male rats and conjunction supplementation and treatment with Zn has resulted in pronounced ameliorating effect especially at the end of the experiment emphasizing its antioxidant role.

**Key words:** Hypoglycemic · Chlorpyrifos · Zinc · Hepatotoxic Effect · Hepatic Marker Enzymes

## INTRODUCTION

Zinc (Zn) is an essential trace element, relatively nontoxic and integral to several key functions in human metabolism [1, 2]. It is a critical component of biomembranes and is essential for proper membrane structure and function and the activity of numerous enzymes [3], Zinc is essential for cell proliferation and differentiation. It can prevent free radical formation, protect biological structures from damage and correct the immune functions [4]**.** Also, it plays an important role in regulation of cellular glutathione that is vital to cellular antioxidant defense [5]. Previous studies illustrated the efficacy of zinc in regulating the liver functions in various animal models of increased oxidative stress [6-9].

Pesticide use in public health protection and agricultural programs is pervasive and growing and serious adverse health effects on animal populations and on humans are widespread and common.

Chlorpyrifos[O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide and nematicide [10]. It is utilized extensively in agriculture and for residential pest control throughout the world [11-13].

Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation [14]. After CPF absorption, It has been demonstrated that its activation to the corresponding chlorpyrifos-oxon, is achieved through oxidative desulfuration by cytochrome P450-dependent enzymes [15], this oxygen analog in turn is responsible for its mammalian toxicity through AChE inhibition [16, 17]. Elimination of CPF occurs mainly through the kidneys [18, 19]. Liver is a major site for metabolism of exogenous chemicals (pesticides, drugs, metals), resulting in the formation of metabolites which may be more or less toxic than the parent compound. It is also, apart from the gastrointestinal tract, the first major

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organ to be exposed to ingested toxins due to its portal blood supply and toxins may be, at least partially, removed from the circulation during the first pass, providing protection to other organs while increasing the likelihood of hepatic injury [20, 21]. The metabolic bioactivation and detoxification of CPF occur primarily in the liver by cytochrome P450 enzymes (CYP) [22]. CPF induces a number of adverse effects, including hepatic dysfunction, hematological changes, immuno-, embryo-, geno-, terato- and neuro-toxicity and neurobehavioral changes [23-27]. It has been shown that repeated doses of chlorpyrifos were able to cause significant hepatic atrophy [28]. The hepatotoxic actions of chlorpyrifos, also appeared through adverse effect on the profile of liver marker enzymes, antioxidant enzymes and essential trace element in intoxicated rats [29].

Increased oxidative stress in the body at CPF treatment was evidenced by enhanced levels of thiobarbituric acid reactive substances (TBARS), accompanied by a concomitant decrease in the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in liver, kidney and spleen [30]. Available reports indicate that organophosphates insecticides including CPF alter the enzyme activities associated with antioxidant defense mechanisms [31, 32]. Therefore, the present study was conducted to evaluate the protective and treatment effects of zinc with regard to its anti-oxidative potential to ameliorate the oxidative stress and restore the altered biochemical enzyme activities, lipid peroxidation induced by acute intoxication of CPF in experimental male rats.

## MATERIALS AND METHODS

**Animals:** Thirty male Wistar rats (weighing approximately 150-180g) were used in this study. They were obtained from Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The animals were housed in plastic cages, fed clean tap water and balanced ration *ad libitum*, exposed to a 12 h light/dark cycle. The animals were quarantined for 10 days before beginning the experiments.

### Chemicals

**Chlorpyrifos Insecticide:** In Dursban H 48% EC was manufactured by Dow Agroscience in England (imported by Agreen Serve, Egypt).

**$ZnSO_4.7H_2O$:** Of mass molarity 287.53g/ml was obtained from Sigma–Aldrich Company, Germany.

**Enzyme Kits:** Kits for determination of serum glucose, GOT (AST) and GPT (ALT) were obtained from Diamond Diagnostic Company, Holiopolis, Cairo, Egypt. ALP (alkaline phosphatase) was purchased from Egyptian Company for Biotechnology (Spectrum).

**Chemicals Used in Determination of Malondialdehyde (MDA) and Super Oxide Dismutase (SOD):** Thiobarbituric acid (TBA), phosphoric acid, N- butanol and Standard Malondialdehyde (1,1,3,3- tetraethoxy popane) for MDA and Hepes 150 m mol**,** $Na_2$ EDTA 0.1 Mm, $Na_2Co_3$, Methionine, NBT (nitroblue tetrazolium) and Riboflavin for SOD. All were purchased from mid Egypt Company (Dokki, Cairo, Egypt).

**Experimental Design:** The animals were divided into three groups 10 animals in each, group 1 (G1) control group, received an equivalent volume of corn oil (vehicle) orally by stomach tube once daily for three consecutive days, group 2 (G2) treated orally once daily with 25% of oral $LD_{50}$ (31.5 mg/kg) of chlorpyrifos (CPF) dissolved in corn oil for 3 consecutive days. $LD_{50}$ was determined by Bebe and Panemanglore [30], group 3 (G3) received the same dose of chlorpyrifos orally once daily concomitantly with zinc in zinc sulphate in drinking water at a concentration of 227 mg $l^{-1}$ [29, 33] for three consecutive days. From the fourth to the tenth day, the animals in group 1 were kept as a control and left without any treatment; also the animals in group 2 previously treated with chlorpyrifos were left without treatment. However the animals in group 3 were treated with zinc only in drinking water at a concentration of 227 mg $l^{-1}$ from the fourth to the tenth day. Five animals from each group were anaesthetized with diethyl ether and sacrificed for collection of blood and tissues samples after the third and tenth day by 24 hours.

**Biochemical assay:**

**Collection of Blood Samples:** Blood samples were withdrawn from the animals under diethyl ether anaesthesia by puncturing the retro-orbital venous plexus (inner canthus of the eye) with a fine sterilized glass capillary tube. Blood was collected in non-heparinized centrifuge tubes and left for 20 min at room temperature, then centrifuged at 3000 rpm. for 10 min to separate the sera. The clean serum was aspirated by means of automatic pipette and finally stored at - 20 in clean, dry and labeled Epindorff tubes for use in biochemical investigations (serum glucose and AST, ALT and ALP as indicators of liver dysfunction).

**Preparation of Tissue Samples:** After soon evisceration, the liver of each rat was removed, washed in saline; part of liver was homogenized and stored at - 20°C for further determination of antioxidant enzymes activities and MDA level. Liver homogenate was prepared according to Combs *et al*. [34].

**Determination of Serum Glucose Level:** Serum glucose was estimated colorimetrically according to Young [35].

**Determination of Serum Hepatic Marker Enzymes Levels:** Serum AST and ALT enzymes were estimated colorimetrically according to Tietz [36]. Serum ALP enzyme activity was estimated colorimetrically according to Belfield and Goldberg [37].

**Hepatic Lipid Peroxidation and Antioxidant Enzyme Measurement of Liver Malondialdehyde (MDA Concentration):** Liver microsomal lipid peroxidation product such as malondialdehyde (MDA) was determined according to Yashkochi and Masters [38]. Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) in an acid medium giving a colored TBA-complex that could be measured colorimetrically at 520-535nm against blank and MDA values were expressed as n moles MDA/mg protein.

**Measurement of Liver Superoxide Dismutase Activity:** Superoxide dismutase (SOD) activity was estimated according to [39]. The optical absorbance was measured at wave length 560 nm against blank reagent.

Superoxide dismutase (SOD) = Reading (absorbance) of (SOD)/ mg protein.

**Protein Determination:** The total protein concentration of supernatant was determined according to Lowry *et al*. [40].

**Statistics:** The values were expressed as means ± standard error (SE). One way ANOVA was used to compare between the values of treated groups (G2 and G3) and that of the control (G1) and values of CPF + Zn (G3)-treated group have also been compared with the data of the CPF treated group (G2). All statistical analyses were performed using SPSS (Statistical package for Social Sciences 10.0 for windows) [41].

## RESULTS

The intoxicated rats with CPF in a dose level of 31.5 mg/kg during the experiment (G2) showed no mortality, however some clinical signs appeared on them as lacrimation, simple tremors and rapid respiratory rate. These signs were recorded only within hours of treatment and then disappear and the animals return to rest state.

**Glucose Level:** Serum glucose level was found to be significantly increased ($p < 0.005$) in chlorpyrifos-treated animals (G2) in comparison to untreated normal controls (G1) as shown in Table 1. However, zinc treatment to chlorpyrifos-treated animals reversed the increased glucose levels to within normal limits (G3).

**Liver Function**

**Serum ALT:** Serum ALT level was found to be significantly increased ($p < 0.005$) in chlorpyrifos-treated animals in comparison to untreated normal controls as shown in Table 2. Supplementation of zinc to CPF-treated groups normalized the levels of ALT.

**Serum AST:** Serum AST level was found to be significantly increased ($p < 0.005$) in chlorpyrifos-treated animals in comparison to untreated normal controls as shown in Table 3. While supplementation of zinc to CPF-treated groups significantly decreased AST level in comparison to chlorpyrifos-treated animals.

Table 1: Effect of zinc on the serum glucose concentration (mg/dl) in rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 61.69±5.64 | 99.74±10.39[a] | 79.93±3.36 |
| 10 days | 4.97±95.77 | 122.8±13.54[a] | 68.75±0.89[bc] |

- Mean value ± standard error.
- a significant in comparison G2 to G-1 $P < 0.05$.
- b significant in comparison between G-3 and G-1 $P < 0.05$.
- c significant in comparison between G-3 and G-2 $P < 0.05$.

Table 2: Effect of zinc on the serum ALT concentration (U/I) in rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 66.20±3.17 | 83.80±3.97[a] | 66.40±0.51[c] |
| 10 days | 67.40±0.98 | 84±0.84[a] | 78.40±2.52[bc] |

- Mean value ± standard error.
- a significant in comparison G2 to G-1 $P < 0.05$.
- b significant in comparison between G-3 and G-1 $P < 0.05$.
- c significant in comparison between G-3 and G-2 $P < 0.05$.

Table 3: Effect of zinc on the serum AST concentration (U/I)) in rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 71.20±3.58 | 83.25±2.20[a] | 66.53±1.80[c] |
| 10 days | 57.75±1.62 | 64.40±1.33[a] | 55.40±2.04[c] |

- Mean value ± standard error.
- a $P < 0.05$ in comparison to G-1.
- c $P < 0.05$ in comparison between G-2 and G-3.

Table 4: Effect of zinc on the serum alkaline phosphatase concentration (IU/L) in rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 155.70±8.36 | 137.8±18.22 | 145.9±14.39 |
| 10 days | 163.8±8.24 | 284.9 ±35.60[a] | 195.7±26.33[c] |

- Mean value ± standard error.
- a $P < 0.05$ in comparison to G-1.
- c $P < 0.05$ in comparison between G-2 and G-3.

Table 5: Effect of zinc on the malondialdehyde (MDA) concentration (nm/mg tissue protein) in liver of rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 0.73±0.05 | 1.16±0.06[a] | 1.07±0.07[b] |
| 10 days | 0.62±0.10 | 1.94±0.18 [a] | 0.99±0.03[bc] |

- Mean value ± standard error.
- a $P < 0.05$ in comparison to G-1.
- b significant in comparison between G-3 and G-1 $P < 0.05$.
- c significant in comparison between G-3 and G-2 $P < 0.05$.

Table 6: Effect of zinc on the superoxide dismutase activity (IU/mg tissue protein) in liver of rats subjected to chlorpyrifos treatment

| | Groups | | |
|---|---|---|---|
| Time (days) | G1 (control) | G2 (Chlorpyrifos) | G3 (Chlorpyrifos + zinc) |
| 3 days | 0.14±0.02 | 0.10±0.01[a] | 0.13±0.01 |
| 10 days | 0.14±0.01 | 0.10±0.01[a] | 0.21 ± 0.02[bc] |

- Mean value ± standard error.
- a $P < 0.05$ in comparison to G-1.
- b significant in comparison between G-3 and G-1 $P < 0.05$.
- c significant in comparison between G-3 and G-2 $P < 0.05$.

**Serum Alkaline Phosphatase:** Serum Alkaline phosphatase level was found to be significantly increased ($p < 0.005$) in chlorpyrifos-treated animals in comparison to untreated normal controls as shown in Table 4. Supplementation of zinc to CPF-treated groups normalized the levels of alkaline phosphatase.

**Antioxidant Enzymes and Lipid Peroxidation**

**MDA Levels:** The level of MDA was significantly increased in chlorpyrifos-treated animals in comparison to untreated normal controls, as shown in Table 5. While supplementation of zinc to CPF-treated groups significantly decreased MDA level in comparison to chlorpyrifos-treated animals.

**SOD Activity:** A significant decrease in SOD activity was observed in chlorpyrifos-treated animals in comparison to untreated normal controls, as shown in Table 6. While supplementation of zinc to CPF-treated groups significantly increased SOD activity in comparison to chlorpyrifos-treated animals.

## DISCUSSION

Zinc is a component of over 300 enzymes and regulatory proteins [42], as well as, zinc (Zn) is an essential trace element, relatively nontoxic and integral to several key functions in human metabolism [1, 2].

Chlorpyrifos (CPF) is a broad spectrum OP widely used for a variety of agricultural and public health applications [43].

In the current study, we reported the hyperglycemic effects of CPF in rat and hypoglycemic effect of zinc to chlorpyrifos-treated animals.

This result is in agree with that reported by Acker and Nogueira [44] who reported that single acute administration of CPF in rats induced hyperglycemic and hyperlipidemic effects.

The mechanisms involved in the hyperglycemia induced by Ops are stimulation of hepatic gluconeogenesis and glycogenolysis [45]. Another proposed mechanism of OPs-induced hyperglycemia is the activation of the hypothalamus–pituitary–adrenal (HPA) axis. The activation of HPA axis by OPs causes secretion of glucocorticoids from adrenal cortex that in turn increases blood glucose by induction of gluconeogenesis pathway [46].

Zinc plays an important role in insulin action and carbohydrate and protein metabolism [47]. The molecular mechanism responsible for the insulin-like effects of Zn compounds involves the activation of several key components of the insulin signaling pathways enhancing glucose uptake [48]. Zinc have common "insulin-mimetic"activities. Zn treatment has been found to improve carbohydrate and lipid metabolism in rodent models of diabetes. In isolated cells, it enhances glucose transport, glycogen and lipid synthesis and inhibits gluconeogenesis and lipolysis [49].

In the present study, chlorpyrifos treatment to normal rats indicated a marked increase in the liver marker enzymes including AST, ALT and Alkaline phosphatase

while Co-administration of zinc to chlorpyrifos-treated animals resulted in normalizing the hepatic marker enzymes.

This result is an indicator of liver injury, when the liver cell membrane is damaged; varieties of enzymes normally located on the cytosol (cellular enzymes) are released into the blood stream [50]. Also, the elevation in alkaline phosphatase level suggests an increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity. Kalender *et al*. and Etim *et al*. [51, 52] reported that increase of alkaline phosphatase level after Diazinon and Lindane induced hepatotoxicity.

Goel *et al*. [29] concluded that zinc supplementation has hepatoprotective effects in chlorpyrifos-induced liver toxicity.

Also, the profile of liver marker enzymes and essential trace element were found to be adversely affected in rats subjected to chlorpyrifos treatment [7, 8].

MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (LPO) [53, 54].

CPF induced oxidative stress leading to the generation of free radicals which play an important role in lipid peroxidation, DNA damage and protein oxidation [55]. The oxidative stress damage may \alter cell function through changes in intracellular calcium or intracellular pH and eventually can lead to cell death [56]. Therefore, it could be suggested that the oxidative stress induced by CPF may mediate the disturbance in hepatic function which is reflected by the recorded increase in ALT and AST as a result of cell injury.

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 levels of lipid peroxidation to within normal limits. These protective effects of zinc can be related to the antiperoxidative property of this metal ion.

Mansour and Mossa [57] reported that administration of chlorpyrifos resulted in a significant increase in lipid peroxidation (LPO) level and revealed the pronounced ameliorating effect of zinc in chlorpyrifos- intoxicated rats.

The antioxidant enzymes SOD, GST and CAT 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 scavenger and conversion of superoxide radical to hydrogen peroxide. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS [58, 59].

The present study indicated a significant decrease in the activity of superoxide dismutase in chlorpyrifos-intoxicated animals. Supplementation of zinc to chlorpyrifos-treated groups of rats normalized the levels of SOD. The decrease in the activity of superoxide dismutase in chlorpyrifos-intoxicated animals may be owed to the consumption of this enzyme in converting superoxide ($O_2^{\bullet}$) to hydrogen peroxide ($H_2O_2$), a more stable ROS. $H_2O_2$ is then converted to $H_2O$ by GST and CAT enzymes [58, 59]. Super oxide dismutase (SOD) protects tissues from oxidative stress and damage by catalyzing the conversion of ($O_2^{\bullet}$) to $H_2O$. SOD contains both copper and zinc, zinc is known to induce the production of metallothionein, which is very rich in cysteine and is an excellent scavenger of OH [60].

This result is in agree with that reported by Mansour and Mossa [57] who reported that administration of chlorpyrifos resulted in a significant decrease in SOD level and supplementation of zinc to chlorpyrifos-treated groups of rats normalized the levels of SOD.

On the other hand, Goel *et al*. [29] indicated a significant elevation in the activity of superoxide dismutase in chlorpyrifos-intoxicated animals. These data suggest that chlorpyrifos treatment may result in increased formation of oxygen-free radicals, which could stimulate to SOD activity to cope with this increased oxidative stress.

At viewing of these data, it can be concluded that zinc supplementation has hepatoprotective and treatment effects in chlorpyrifos-induced liver toxicity via its antioxidant powerful mechanism and can reverse the hyperglycemic effect induced by CPF in male albino rats.

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