**Hepato-Renal Toxicity Induced by Chlorpyrifos, Diazinon and their Mixture to Male Rats with Special Concern to the Effect of Zinc Supplementation**

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**Abstract**

**Background:** In real life, exposure to pesticide mixtures is unavoidable especially from consumption of vegetables and fruits sprayed with a cocktail of pesticides during their growing seasons in the fields. Therefore, the issue of mixture toxicity occupies the major concern of the scientific and regulatory communities. This work was undertaking to assess some toxicological effects following exposure of male rats to chlorpyrifos (CPF), diazinon (DIZ) and their combination (CPF+DIZ), and to evaluate the ameliorative effect of co-administration of zinc.

**Methods:** Sixty-four adult male Wistar rats were divided into equal eight groups. Three groups were designated for (CPF), (DIZ) and the mixture (CPF+DIZ) treatments. Other three groups were designated for zinc in conjunction with the pesticides. Two groups; one received water only (control), and the other represented positive zinc treatment. A total number of 10 liver and kidney function biomarkers (e.g., AST, ALT, ALP, LDH, Albumin, ChE, Total Protein, Total Lipids, Creatinine and Uric Acid), as well as histopathological changes in liver, kidneys and testes were studied.

**Results:** Generally, CPF and DIZ individually and the mixture CPF+DIZ induced significant (P≤ 0.05) alterations in the measured parameters. Zinc supplementation in conjunction with the tested pesticides achieved considerable ameliorative effect expressed in terms of amelioration index (AI). In the majority of estimations, such index was closely amounted to (1.0) indicating high alleviation of the toxic effects due to zinc supplementation.

**Conclusion:** It was concluded that co-administration of zinc acted as a powerful antioxidant against liver and kidney dysfunction induced by CPF, DIZ and their combination. This may be useful especially in individuals who are occupationally exposed daily to low doses of such insecticides.

**Keywords:** Chloropyrifos; Diazinon; Pesticide mixtures; Hepatorenal toxicity; Histopathology; Zinc supplementation; Amelioration index

**Introduction**

The organophosphorus (OP) compounds are among the major insecticide classes used globally. As quantity, they amounted to 44% of the total insecticides used in Egypt during the 90’s [1]. The OP pesticides are mainly anticholinesterase compounds, and thus affect severely on the central or peripheral nervous system. Therefore, exposure of agricultural workers to these pesticides may represent a risk issue to their health. Recent studies identified reactive oxygen species (ROS) as a cause of toxic effects exerted by these pesticides [2]. OP compounds are highly toxic to insects and mammals, however they are rapidly metabolized. The annual accidental poisonings and death of humans by using these pesticides, especially in developing countries, is well documented [3].

Pesticide residues in food have direct impacts on human health and international trade [4]. Monitoring programs concerned with pesticide residues in food have shown that a given food item, such as an agricultural fruit, might be contaminated with a large number of pesticides and their residues; i.e. “multi residue contamination”. For instance, different varieties of OP pesticides, in addition to carbamates (CM) and organochlorines (OC), were found as residues in Egyptian fruits and Vegetables [5-8].

Both chlorpyrifos (CPF), [O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate], and diazinon (DIZ), [phosphoric acid, O,O-diethyl O (2-isopropyl-6-methyl-4-pyridinyl) phosphorothioate], are anticholinesterase insecticides with contact, stomach, and respiratory action. They are used widely to combat insect pests in different crops. Therefore, their residues may be found in the same food commodities such as vegetables and fruits. Monitoring studies conducted in different countries revealed presence of residues of both insecticides among other ones in different varieties of food commodities. For examples, vegetables (e.g., spinach, lettuce, cabbage, tomato and onion) and soil samples in Borno State, Nigeria were found to contain high concentrations of diclorvos, diazinon, chlorpyrifos and fenithrothion [9]. One hundred thirty two samples representing herbs, vegetables and fruits were randomly collected from the local markets at Cairo, Egypt. Residues of 17 pesticides, including CPF and DIZ, were detected in the analyzed samples [10]. A total of 12 OP insecticides including CPF and DIZ and their metabolites, dialkylphosphates (DAPs), were identified in some vegetables and fruits prior to entering the channels of trade by California producers and shippers [11]. Even in samples of honey bees (Apis mellifera), honey and pollen, residues of 14 OP insecticides were detected; pollen contained the greatest concentrations of OP pesticides. (e.g., profenofos, chlorpyrifos, malation and diazinon) [12]. Indeed, the multi-pesticide residues in food raised the concern about exposure to multi toxicansts and explored the fact that exposure to more than one toxic compound (e.g. pesticides) is common in real life and such exposure may occur in air, water and food. As a result, the multi-tissue and multi-organ health hazard of chemical mixtures is a challenging toxicological problem, and a subject of major current concern to both the scientific and regulatory communities [13].

Biologically active substances (e.g., pesticides) enhance the formation of reactive oxygen species (ROS). These ROS are responsible of inducing oxidative stress in the tissues and chronic permanent damage in animal organs and their functions [3,14]. This raised the interest of scientists to search for antioxidants which might alleviate oxidative stress caused by pesticides. Several substances including naturally available plant oils, vitamins, and essential mineral elements were used to alleviate toxic hazards of OP pesticides-induced oxidative stress in experimental animals. For examples, against chlorpyrifos, these substances were tested: fenel (Foeniculum vulgare Mill.) essential oil [15]; wheat germ oil and grape seed oil [16]; Vitamin E (α-tocopherol) [17]; and zinc [18,19]. White grape seed oil [20]; Vitamin E (α-tocopherol) [21,22] and a combination of vitamin C and E were used against diazinon-induced oxidative stress in rats [23].

The role of certain essential metals in modulating the effects of different toxicants is an area of recent interest. Recent studies have...
shown that zinc can protect against oxidative damage caused by several toxicants and thus may have antioxidant properties [24].

The present investigation was carried out to assess some toxicological effects in male rats following exposure to CPF, DIZ and their combination, and to evaluate the ameliorative effect of co-administration of zinc. Some biomarkers of liver and kidney functions, as well as histopathological changes in liver, kidneys and testes were studied.

**Materials and Methods**

**Chemicals**

Chlorpyrifos was obtained from the National Company for Agrochemicals and Investment (Agrochem), Alexandria, Egypt as Pestan® (48% EC). Diazinon was procured from the Egyptian Mud Engineering and Chemicals Company (EMEC), Alexandria, Egypt as Kanzinon® (60% EC). Zinc Chloride (ZnCl₂) powder (M.W. 136.29), a product of Oxford Laboratory Reagent, UK was purchased from the local market.

**Reagents (diagnostic kits)**

Diagnostic kits used in the present study were obtained from Biodiagnostic Co., Dokki, Giza, Egypt. These were liver biomarkers (e., g., Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Albumin, Total protein, Total lipids and Cholinesterase (ChE)) and kidney biomarkers (e., g., Uric acid and Creatinine).

**Animals**

Healthy male albino rats of the Wistar strain (Rattus norvegicus), 60 days of age and with average weight of 110 ± 20 g, were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (23 ± 2°C) on standard pellet diet and had free access to water in daily dark/light cycle of 12/12 hrs. Rats were acclimatized for 1 week prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, College of Agriculture in Damanhour, Egypt, and according to the guidance for care and use of laboratory animals [25].

**Determination of oral LD₅₀ for the tested insecticides**

Preliminary tests were carried out to determine the median lethal dose (LD₅₀) for commercial formulations of chlorpyrifos and diazinon on male rats. For each insecticide, three doses were prepared in water based on active ingredient, a.i., contents (e.g., 33.75, 135 & 189 mg/kg b.w. for chlorpyrifos; and 300, 350 & 400 mg/kg b.w. for diazinon).

Four rats were used for each tested dose, in addition to four rats given water only and served as control group. Dosing was performed by gavages with 0.5 ml solutions. The 24h-LD₅₀ values were estimated according to Finney [26]. Based on the obtained LD₅₀ values, the equivalent to the 1/10 was used in the present study.

**Dosing and treatments**

A total of 64 male rats were divided into eight groups (Gs), each contained eight animals. G1: (Cont.) received water free of any pesticide and served as control. G2 (Zn): administered ZnCl₂ in drinking water at a concentration of 227 mg/L (as Zn) according to Goel et al. [27]. G3: (CPF) and G4: (DIZ) were orally administered 8.7 and 40.84 mg/kg b.w. for chlorpyrifos and diazinon, respectively. G5: (CPF+DIZ) was given both insecticides at their respective doses singly. Groups 6, 7 and 8 were respectively administered CPF, DIZ and CPF+DIZ by oral gavages in addition to Zn in drinking water. The insecticides were given every 48h intervals; zinc solution was introduced in sufficient amounts a day after another, while water in G1 was permitted ad libitum. The experimental duration was extended up to 42 days, and the doses of insecticides were adjusted weekly according to changes in body weights of the tested animals.

**Blood and organs collection**

Body weight changes of male rats were recorded weekly during the experimental period (6 weeks). At the end of this period, blood samples were withdrawn from the animals under ether anesthesia by puncturing the retro-orbital venous plexus with a fine sterilized glass capillary. Blood was collected into non-heparinized glass tubes to separate serum. The tubes were left for 20 min at room temperature, then centrifuged at 3000 rpm (600g) for 10 minutes using BOECO centrifuge model C-28, Germany, and kept in a deep freezer (-20°C) until analyzed within one week maximum. Finally, the rats were sacrificed by decapitation. Liver, kidneys, and testes were quickly removed weighed individually and reserved in 10% formalin saline for histological examinations.

**Biochemical estimations**

Enzymatic analyses were measured on Jenway 6305 UV/VIS Spectrophotometer at the specified wavelengths. Liver and kidney biomarkers were measured in sera and the analyses were carried out in accordance to the pamphlet instructions given by the manufacturers, and as briefly described below.

**Liver function parameters**

Aspartate aminotransferase; AST (EC.2.6.1.1) activity was determined according to the method described by Reitman and Frankel [28]. This method depends on photometric determination of the oxaloacetate haemolysis concentration formed with 2, 4-dinitrophenylhydrazine at 546nm, expressing the enzyme’s activity in terms of U/L.

Alanine aminotransferase; ALT (EC.2.6.1.2) activity was determined according to Reitman and Frankel [28] by measuring the concentration of pyruvate haemolysis formed with 2, 4-dinitrophenylhydrazine at 546 nm. The enzyme’s activity is given as U/L.

Alkaline phosphatase; ALP (EC 3.1.1.31) was determined by the method of Rosalki and Foo [29]. The enzyme ALP splits the substrate p-nitrophenylphosphate into phosphate and the yellow colored indicator p-nitrophenol which could be measured at 405 nm. The enzyme’s activity is given as U/L.

The lactate dehydrogenase (LDH) catalyzes the reaction between pyruvic acid and NADH giving lactic acid and NAD⁺. Oxidation of NADH is proportional to the activity (U/L) of the LDH enzyme in the sample [30]. The measurements were carried out at 340 nm.

Albumin (Alb) determination was carried out according to the method reported by Doumas et al. [31], which based on formation of an albumin/bromocresol - green complex at pH 4.2 and photometric measurement of the absorbance at 630 nm (620 - 640 nm); expressing albumin concentration in g/dl.

Total lipids (TL) were determined according to Knight et al. [32]. Lipids are hydrolyzed by sulphuric acid, then treated with phosphor- vanillin mixture to produce sulpho-phosphovanillin complex of rose coloration which was measured photometrically at 520-540 nm. Concentration of total lipids was given in g/dl.

Total protein was determined according to the method described by Armstrong and Henry [33]. The method depends upon formation of a colored complex between protein and cupric ions in an alkaline medium, which can be measured at 546 nm. Total protein concentration was expressed in terms of g/dl.

Cholinesterase; ChE (EC 3.1.1.8) catalyzes the hydrolysis of the...
neurotransmitter acetylcholine into choline base and acetic acid. The
determination of ChE activity was carried out according to the method
reported by Knedel and Böttger [34]. The absorbance of the sample
against reagent blank was read at 405 nm; expressing the enzyme’s
activity in terms of U/L.

Kidney function parameters

Creatinine (Cr) was determined according to the method of
Mitchell [35] which depends on color complex formed by the reaction
between creatinine in an alkaline solution and picrate yielding a color
measurable at 546 nm. Creatinine concentration was given in µg/dl.

Uric acid (UA) determination was carried out according to the
method reported by Morgenstern et al. [36]. Quinoneminine yielded due
to transformation of uric acid by the action of uricase is proportional
to concentration of uric acid in the sample. The measurements were
carried out at 546 nm and uric acid concentration was given in µg/dl

Histopathological studies

Autopsy samples were taken from the liver, kidneys and testes
from rats of different groups and fixed in 10% formalin saline for 24 h.
Washing was done in tap water and then dehydrated in ascending
grades of alcohol. Specimens were cleared in xylene and embedded in
paraffin bees at 56 degree in hot air oven for 24 h. Paraffin blocks were
prepared for sectioning at 4 microns thickness by slide microtome. The
obtained tissue sections were collected on glass slides, deparaffinized
and stained by hematoxylin and eosin (H&E) stain. Two slides were
prepared for each animal; each slide contained two sections for each
organ. Ten field areas for each section were selected and examined for
histopathological changes under light microscope [37] at 400x
magnification. The histopathology was carried out in the Pathology
Department, Faculty of Veterinary Medicine, Cairo University, Giza,
Egypt. Tissue injury in the examined organs was scored in terms of
degree of cell damage, according to Brunt et al. [38].

Statistical analysis and data presentation

All obtained data were statistically analyzed using Statistical
analitics/stat.html). Data were analyzed as factorial arrangement of
kind of emulsifying and storage period in complete randomized design
with three replicates. Comparisons among the means of different
treatments were achieved by using the least significant difference
procedure (LSD) at P = 0.05 and 0.01 levels.

Data of biochemical measurements were further subjected to
estimation of percent of changes caused by exposure to the tested
pesticides, and the improvement achieved by co-administration of zinc
in terms of amelioration index (AI); according to Mansour and Gamet-
pesticides, and the improvement achieved by co-administration of zinc

Table 1: Effect of chlorpyrifos, diazinon and their mixture, with and without zinc, on body and organs weights of male rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Absolute organs weights (g)</th>
<th>Relative organs weights (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Kidneys</td>
</tr>
<tr>
<td>Control</td>
<td>211.71 ± 4.11</td>
<td>6.38 ± 0.03</td>
<td>2.17 ± 0.06</td>
</tr>
<tr>
<td>Zn</td>
<td>222.28 ± 2.56</td>
<td>6.00 ± 0.03</td>
<td>2.01 ± 0.03</td>
</tr>
<tr>
<td>CPF</td>
<td>176.00 ± 2.58**</td>
<td>5.80 ± 0.37**</td>
<td>1.38 ± 0.05**</td>
</tr>
<tr>
<td>DIZ</td>
<td>167.71 ± 3.63**</td>
<td>4.99 ± 0.39**</td>
<td>1.25 ± 0.04**</td>
</tr>
<tr>
<td>CPF + DIZ</td>
<td>175.14 ± 3.62**</td>
<td>5.58 ± 0.01**</td>
<td>1.44 ± 0.01**</td>
</tr>
<tr>
<td>CPF + Zn</td>
<td>197.00 ± 3.41</td>
<td>5.89 ± 0.03</td>
<td>1.85 ± 0.01</td>
</tr>
<tr>
<td>DIZ + Zn</td>
<td>199.28 ± 5.12</td>
<td>5.39 ± 0.37</td>
<td>1.53 ± 0.01</td>
</tr>
<tr>
<td>CPF + DIZ + Zn</td>
<td>210.71 ± 2.98</td>
<td>5.94 ± 0.02</td>
<td>1.69 ± 0.02</td>
</tr>
</tbody>
</table>

- CPF = Chlorpyrifos, DIZ = Diazinon. - Each value is a mean ± SD; n = 8.
- Statistical difference from the control: *: significant at P > 0.05; **: highly significant at P < 0.01; values of P ≥ 0.05 are not significantly
different than control value.
- Relative organs weights (%) = (Weight of organ / Body weight) x 100.

Citation: Mansour SAK, Abbassy MAL, Shaldam HA. Hepato-Renal Toxicity Induced by Chlorpyrifos, Diazinon and their Mixture to Male Rats with Special Concern to the Effect of Zinc Supplementation. J Toxicol Pharmacol 2017; 1:015.

**Acute oral toxicity of the tested insecticides**

The estimated oral LD₅₀ for chlorpyrifos and diazinon against the
used male rats indicated that chlorpyrifos (CPF) was more toxic
(87.7 mg/kg b.w.) than diazinon (DIZ) (408.5 mg/kg b.w.). These
values were used to calculate the doses used in the present study (1/10
LD₅₀; i.e., 8.77 and 40.85 mg/kg b.w from chlorpyrifos and diazinon,
respectively).

Effect on body and organs weights

Body and organs weights of rats dosed with CPF, DIZ, CPF+DIZ, with
and without Zn administration, are presented in Table 1. Control
rats showed a mean body weight of 211.7 g which was significantly
higher (P ≤ 0.01) than those recorded for CPF (176.0 g), DIZ (167.7 g)
and the mixture, CPF+DIZ (175.1 g). The body weights in the
treatments of Zn alone or in combination with the tested pesticides
showed values of no statistical difference than those of control value.

The absolute weights of liver, kidneys and testes from control rats
recorded 6.38, 2.17 and 2.94 g, respectively. Except Zn, the values of
the tested treatments were generally lower than those of the control
ones with different degrees of significances (P ≤ 0.05 & P ≤ 0.01).
Generally, co-administration of Zn minimized the observed differences
to some extent (Table 1).

The relative liver weight in control group accounted to 3.01%,
CPF and CPF+DIZ caused high elevation in the relative liver weights
(e.g., 3.3% and 3.19%, respectively), while DIZ caused the opposite
(2.98%). In control treatment, the relative weight of kidneys equaled
1.02%, the values estimated for CPF, DIZ and CPF+DIZ (0.78, 0.75 and
0.82%, respectively) were significantly lower at P ≤ 0.01. Generally,
co-administration of Zn minimized the observed differences to some
extent (Table 1).

The relative weight of testes in control rats was found 1.39%; a value
which was insignificantly differed than the values estimated for
Zn, CPF+DIZ and CPF+DIZ+Zn. However, the testes in DIZ and
DIZ+Zn recorded 0.73% and 0.78%, respectively; achieving high
significant differences (P ≤ 0.01). Generally, co-administration of Zn
minimized the observed differences to some extent (Table 1).

Effect on liver biochemical parameters

Figures 1-3 show the effect of the tested doses, with and without
zinc, on a number of liver biochemical parameters measured in
the rat sera. Control rats and those administered Zn showed similar
serum AST activity (34.41 and 34.27 U/L, respectively) (Figure 1a).
Compared with control value, the rats treated with CPF, DIZ and their
mixture (CPF+DIZ) showed high significant (P ≤ 0.01) elevation in
AST activity; accounting to 44.08, 43.42 and 53.17 U/L, respectively. Supplementation of Zn with these pesticides limited the enzyme elevation to some extent, but still significantly ($P \leq 0.05$) deferred than the control value; where the serum activity was found 36.05, 36.27 and 36.24 U/L, respectively for CPF+Zn, DIZ+Zn and CPF+DIZ+Zn.

The serum ALT activity (Figure 1b) showed values nearly similar to those of serum AST activity. Also, there was a similar trend regarding to high significant ($P \leq 0.01$) increase of ALT activity in CPF, DIZ and CPF+DIZ treatments, and only significant increase ($P \leq 0.05$) in the treatments given Zn with CPF or DIZ. Supplementation of Zn with the mixture CPF+DIZ didn’t effectively limit high elevation of ALT.

The serum ALP activity in control rats recorded 55.66 U/L, a value which was insignificantly differed than the value recorded for Zn treatment (Figure 1c). CPF, DIZ and their mixture induced high significant ($P \leq 0.01$) increase accounting to 9.18, 9.29 and 11.44 g/dl, respectively. Co-administration of Zn with CPF or DIZ achieved values of 6.25 and 6.54 g/dl, respectively which were insignificantly differed than control values ($P \leq 0.05$). However, the CPF+DIZ+Zn treatment recorded protein concentration accounting to 7.24 g/dl; a value which was significantly higher than control value at ($P \leq 0.05$).

The activity of serum cholinesterase (ChE) enzyme in control rats recorded 2178.4 U/L; a value which was insignificantly different than those estimated for Zn treatment (2126.82 U/L). The decline in ChE activity in the obtained LDH activity for the control rat treatment (157.74 U/L), the tested toxicants, either with or without Zn, caused high elevation ($P \leq 0.01$) in the activity of this enzyme (Figure 2a). However, such elevation was much lower in the treatments which included Zn administration. For example, the LDH activity in CPF+Zn, DIZ+Zn and CPF+DIZ+Zn treatments was 261.34, 265.34 and 271.34 U/L, respectively, which were significantly higher than control value ($P \leq 0.05$). Therefore, the concentration of total lipids recorded 6.27 g/dl in control rats; all the tested substances induced elevation in the concentration levels of total lipids (Figure 2c). The elevation was significant ($P \leq 0.05$) in the treatments of CPF+Zn, DIZ+Zn and CPF+DIZ+Zn, but was highly significant ($P \leq 0.01$) in the rest of treatments (except that of Zn alone which had insignificant difference compared with control).

The total protein concentrations recorded 6.67 and 6.39 g/dl in the sera of control and Zn treatments, respectively (Figure 3a). CPF, DIZ and their mixture achieved significant ($P \leq 0.01$) increase, but the use of Zn with DIZ and the mixture (CPF+DIZ) achieved significant ($P \leq 0.05$) increase in the ALP activity compared with the control value. There was no significant difference between the ALP activity of CPF+Zn and that of the control treatment.

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Effect on kidney biochemical parameters

Compared to control and Zn treatments of 0.68 µg/dl serum creatinine concentration, all the insecticidal treatments induced high increase ($P \leq 0.01$) in creatinine levels. The highest concentration value was achieved by the treatment of CPF+DIZ (1.00 µg/dl), while the lowest (0.83 µg/dl) was entitled to CPF+Zn treatment (Fig. 4a).

Uric acid concentration in serum of the untreated (control) rats and those received Zn only were accounted to 5.73 and 5.44µg/dl, respectively (Figure 4b). In comparison, the treatments of CPF, DIZ and CPF+DIZ caused high significant increase ($P \leq 0.01$) accounting to 7.73, 7.87 and 7.64µg/dl, respectively. Co-administration of Zn with these insecticides limited such elevation to some extent ($P \leq 0.05$).

Quantitative estimation of biochemical alterations with and without zinc supplementation

In this context, Tables 2 demonstrates the results of biochemical alterations due to exposure of the experimental rats to the tested insecticides, with and without zinc supplementation. As ameliorative index (AI) approaches “1”, the improvement reaches high degree of normalization to the control value.

Percent of changes in aminotransferases (AST, ALT), ALP and LDH due to CPF exposure were 28.1, 24.2, 34.1 and 63.3%, respectively (Table 2). However, Zn supplementation normalized activities of these enzymes; giving rise to amelioration indices (AI) nearly equaled 1.00 (e.g., 1.05, 1.09, 0.97 and 1.03, respectively). Estimated percent of changes in albumin, t-lipid, t-protein, ChE, creatinine and uric acid were found (-6.3%), (47.7%), (37.6%), (-30.7%), (41.2%) and (34.9%), respectively following exposure to CPF. With Zn supplementation, the lowest AI (0.76) was entitled to ChE, while ranged between 0.94 and 1.2 for the other parameters. In the treatments of DIZ alone, the highest change was accounted to LDH (65.7%), and Zn caused modulation of LDH activity to the normal value (i.e., AI = 1.03).

Treatments of the mixture (CPF+DIZ) caused changes in the levels of the measured biochemical parameters. The changes were very higher than those occurred in the single treatments. For example, the changes reached 72.7% for LDH, 71.5% for t-protein, 60.6% for t-lipids and -48.5% for ChE. However, administration of Zn with this mixture reflected in noticeable improvement in terms of AI values accounted to 1.07, 1.08, 1.08 and 0.84, respectively (Table 2).

Histological effects on rat organs

Histological examination of liver, kidney and testis of male rats treated with CPF, DIZ and their mixture are shown in Figures 5-7. The prepared sections were stained by H & E and examined microscopically at 400 x magnification power. Tissue injury in the examined organs is scored in terms of degree of cell damage as presented in Table 3.

Liver: Sections from control or Zn treatments showed normal histological structure of hepatic lobule (Slide 5A). Rats treated with CPF showed kupffer cells activation (arrow a) and presence of leucocytes in the hepatic sinusoids (arrow b) (Slide 5B), while those treated with DIZ showed multiple focal hepatic necroses associated with leucocytic cells infiltration (arrows a) (Slide 5C). The mixture CPF+DIZ (Slide 5D) showed kupffer cells activation (arrow a), focal hepatic necrosis associated with leucocytic cells infiltration (arrow b) and necrosis of sporadic hepatocytes (arrow c). Hepatic injury was accounted to mild, moderate and severe, respectively in DIZ, CPF and CPF+DIZ (Table 3).

Kidneys: Sections from control or Zn treatments showed normal histological structure of renal parenchyma (Slide 6A). Rats treated with activity in CPF+DIZ+Zn treatment was significantly ($P \leq 0.05$) lower than control. All the other tested treatments recorded noticeable decline ($P \leq 0.01$) in the activity of this enzyme (Fig. 3b).
**Table 2:** Percent of change in some biochemical parameters, related to liver and kidney functions, in male rats induced by chlorpyrifos (CPF), diazinon (DIZ) and the mixture (CPF+DIZ), and the ameliorative effect of zinc supplementation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biochemical Parameters</th>
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<td>9.18</td>
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<td>38.64</td>
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<td>Ameliorative Index**</td>
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<tr>
<td>% of Change**</td>
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<tr>
<td>Ameliorative Index**</td>
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<td>1.30</td>
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</table>

- Data refer to Figs. 1- 4 and each value is a mean of 8 values.

* % of change in the biochemical parameter in question (**effect of pesticide**) = [(b – a) / a] x 100 ;

"Ameliorative Index (AI) (**effect of zinc co-administration**) = c / a

The organs from control animals (**Slide A**) showed normal histological structure of hepatic lobule. (H & E, 400x). Rats treated with CPF showed kupffer cells activation (arrow a) and presence of leucocytes in the hepatic sinusooids (arrow b) (**Slide B**), while those treated with DIZ showed multiple focal hepatic necroses associated with leucocytic cells infiltration (arrows a) (**Slide C**). The mixture CPF+DIZ (**Slide D**) showed kupffer cells activation (arrow a), focal hepatic necrosis associated with leucocytes cells infiltration (arrow b) and necrosis of sporadic hepatocytes (arrow c) (H & E, 400x).

**N.B.:** Compared with the above mentioned histological changes induced by the tested pesticides, co-administration of Zn retained the histopathological alterations to the normal picture.

**Figure 5:** Histological examination of Liver of male rats treated with chlorpyrifos (CPF), diazinon (DIZ) and their mixture compared with normal (control) rats.

CPF showed vacuolations of endothelial lining glomerular tufts (arrow a) and vacuolations of epithelial lining renal tubules (arrow b) (**Slide 6B**), while those treated with DIZ showed eosinophilic protein cast in the lumen of renal tubules (arrow a) (**Slide 6C**). The mixture CPF+DIZ (**Slide 6D**) showed vacuolations of endothelial lining glomerular tuft (arrow a) and epithelial lining renal tubules (arrow b) as well as atrophy of some glomerular tuft (arrow c). Renal injury was accounted to mild, moderate and severe, respectively in DIZ, CPF and CPF+DIZ (**Table 3**). The organs from control animals (**Slide A**) showed normal histological structure of renal parenchyma. Rats treated with CPF showed vacuolations of endothelial lining glomerular tufts (arrow a) and vacuolations of epithelial lining renal tubules (arrow b) (**Slide B**), while those treated with DIZ showed eosinophilic protein cast in the lumen of renal tubules (arrow a) (**Slide C**). The mixture CPF+DIZ (**Slide D**) showed vacuolations of endothelial lining glomerular tuft (arrow a) and epithelial lining renal tubules (arrow b) as well as atrophy of some glomerular tuft (arrow c) (H & E, 400x).

**N.B.:** Compared with the above mentioned histological changes induced by the tested pesticides, co-administration of Zn retained the histopathological alterations to the normal picture.

**Figure 6:** Histological examination of kidney of male rats treated with chlorpyrifos (CPF), diazinon (DIZ) and their mixture compared with normal (control) rats.

**Tests:** Sections from control or Zn treatments showed normal histological structure and normal seminiferous tubule (**Slide 7A**). Rats treated with CPF showed spermatogonial cell debris in the lumen of seminiferous tubule (arrow a) (**Slide 7B**), while those treated with DIZ showed necrosis and sloughing of germ cells lining sommiferous tubule (arrow a) (**Slide 7C**). The mixture CPF+DIZ (**Slide 7D**) showed necrosis and absence of spermatogonial cells lining sommiferous tubules (arrow a) and intra luminal spermatid giant cell (arrow b) (H & E, 400x). Tests injury was accounted
Table 3: Histopathological changes in the liver, kidney and testis of experimental rats, based on scoring severity of injury† in the examined organs.

<table>
<thead>
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<th>Treatment</th>
<th>Hepatic injury</th>
<th>Renal injury</th>
<th>Testis injury</th>
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<tbody>
<tr>
<td></td>
<td>Score (average)</td>
<td>Severity</td>
<td>Score (average)</td>
</tr>
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<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Zinc</td>
<td>0</td>
<td>nil</td>
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</tr>
<tr>
<td>CPF</td>
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<td>moderate</td>
<td>2</td>
</tr>
<tr>
<td>DIZ</td>
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<td>mild</td>
<td>1</td>
</tr>
<tr>
<td>CPF + DIZ</td>
<td>3</td>
<td>severe</td>
<td>3</td>
</tr>
</tbody>
</table>

† Tissue injury in the examined organs scored in terms of degree of cell damage, according to Brunt et al. as follows:
(0) = no change;
(1) = mild change (e.g., <25% of cells showing damage);
(2) = moderate change (e.g., 25–50% cell damage);
(3) = severe change (e.g., >50% cell damage).

The organs from control animals (Slide A) showed normal seminiferous tubule (Slide A). Rats treated with CPF showed spermatogetic cell debris in the lumen of seminiferous tubule (arrow a) (Slide B), while those treated with DIZ showed necrosis and sloughing of germ cells lining seminiferous tubule (arrow a) (Slide C). The mixture CPF+DIZ (Slide D) showed necrosis and absence of spermatogetic cells lining seminiferous tubules (arrow a) and intra luminal spermatic giant cell (arrow b) (H & E, 400x).

N.B.: Compared with the above mentioned histological changes induced by the tested pesticides, co-administration of Zn retained the histopathological alterations to the normal picture.

Figure 7: Histological examination of testis of male rats treated with chlorpyrifos (CPF), diazinon (DIZ) and their mixture compared with normal (control) rats.

Compared with the above mentioned histological changes induced by the tested pesticides, co-administration of Zn retained the histopathological alterations to the normal picture nearly.

Discussion

The oral LD₅₀ for technical grade chlorpyrifos and diazinon in rats are 95 - 270 mg/kg ([extoxnet.orst.edu/pips/chlorpyr.htm](http://extoxnet.orst.edu/pips/chlorpyr.htm)) and 300 – 400 mg/kg ([extoxnet.orst.edu/pips/diazinon.htm](http://extoxnet.orst.edu/pips/diazinon.htm)), respectively. The estimated oral LD₅₀ for the used commercial products of CPF and DIZ to male rats were found 87.71 mg/kg (72.98 – 103.83) and 408.49 mg/kg (400.11 – 416.12), respectively. Such differences may refer to the nature of the pesticide formulation and other factors related to test conditions. On the other side, the experimentally obtained LD₅₀ values indicate that the acute toxicity of CPF is about five times that of DIZ.

In toxicological studies, it has been reported that body and organs weights are considered important criteria for evaluating organ toxicity. The increase or decrease in body weight is a sign of toxic effects [39]. Generally, CPF, DIZ and their mixture caused decrease of body and organs (e.g., liver, kidneys and testes) weights compared with control rats (Table 1). However, co-administration of Zn normalized body weights and limited decline of organs weights to some extent. It was reported that reduction in body weight in experimental animals exposed to OP intoxication may refer to increased degradation of lipids and proteins [27]. On the other hand, hepatic and renal toxicity may cause reduction of liver and kidney size due to either acute or chronic hepatic or renal injury resulting in cell losses [27].

In agreement with our findings, Joshi et al. [40] reported significant decrease of testes weights in chlorpyrifos and diazinon treated rats compared with the control group. Such decrease may refer to reduced tubular size as a result of impairment at testicular, pituitary, or hypothalamic level [41]. Similar results were reported by El-Kashoury [42] in rats treated with the OP insecticide, profenofos, and in rats treated with the OC pesticide, endosulfan [43].

High elevation of the liver enzymes (e.g., AST, ALT, ALP, LDH) are in agreement with findings of many researchers on experimental animals exposed to several OP insecticides including CPF and DIZ [18,19,44]. Their elevation reflect the increase of plasma membrane permeability resulting from the damage of hepatocytes [45]. Moreover, altered liver enzyme activities have been previously reported among pesticide workers exposed to an OP pesticide alone or in combination with other pesticides [46]. It was previously reported by Salih [44] that cellular damage caused by toxic substances exhibited good correlation with the enzyme leakage and was frequently accompanied with increasing permeability of cell membranes.

In general, the present study showed that exposure of male albino rats to CPF, DIZ and their combination have increased levels of total lipids (Figure 2c) and total protein (Figure 3a), and decreased albumin (Figure 2b) content. Our findings are supported by the results of previous investigators who reported that the OP insecticides generally cause an increase in level of total lipids and total protein and decrease in albumin level according to liver function disorders after all treatments [47,48].

Cholinesterase (ChE), also known as pseudocholinesterase, is synthesized mainly in hepatocytes and secreted into the blood stream [49]. ChE activity is declined in liver dysfunction due to reduced synthesis, in contrast to other serum enzymes attributed to the clinical assessment of liver function whose activities increase as a result of increased release from their cellular sources following damage of cell membrane [50]. In the present study, results in Fig. 3b show the effect of CPF, DIZ and their mixture on the activity of ChE in serum of male albino rats after 6 weeks of treatment. These findings are in agreement with those reported by previous investigators on chlorpyrifos in rat experiments [17-19,51], and in pesticide spray workers [52].

Creatinine and uric acid concentrations in serum of treated rats were...
increased due to exposure to the tested insecticides (Figure 4a, b). These results are in agreement with those obtained by Issaa and Zidan [53] on abamectin and Mansour and Mossa [19] on chlorpyrifos. Both uric acid and creatinine are useful in early detection of nephrotoxicity induced by exogenous compounds, and are used as index of renal damage in living organisms [54]. Elevation of uric acid and creatinine concentrations in serum of treated male albino rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules. Impairment in kidney function could probably occur as a result of kidney oxidative damage. In fact, uric acid in blood is the end product of purine catabolism and can reduce oxidative stress by scavenging various reactive oxygen species (ROS) [55].

Biologically active substances (e.g., pesticides) enhance the formation of ROS which are responsible of inducing oxidative stress in the tissues and chronic permanent damage [3,14]. Such damage occurs in cases of excessive formation of ROS or insufficient of protective antioxidants [56]. Subsequently, this raised the concern to test alleviation of pesticide – oxidative stress by antioxidants. (In this respect, several studies have shown that zinc possesses antioxidant properties and thus can protect the cell from oxidative damage induced by certain xenobiotics [24]. Moreover, zinc is involved in sub-cellular metabolism. It is an important component of catalytic site(s) of various enzymes. Also, it plays an essential role in cellular glutathione regulation which is a vital process to cellular antioxidant defense [57]. Other elements such as copper [58] and selenium [59] have shown to play a protective role against oxidative stresses induced by several insecticides.

Although many studies have tested the ameliorative effect of some vitamins and natural products in CPF-induced oxidative stress, there are few studies on the ameliorative effect of zinc against oxidative stress induced by CPF in rat [18-19,27]. To the best of our knowledge, there are no similar studies on diazinon and its combination with chlorpyrifos with respect to co-administration with zinc. On the other hand, the obtained results regarding to ameliorative effect of zinc, adds further application to the previously investigated formula [17].

According to the data presented in Table 2, alteration in any biochemical parameter (Table 2) caused by the mixture (CPF+DIZ) was higher than alteration caused by CPF or DIZ alone; results which indicate an interactive effects between the mixture components. The mixture contained the sum amounts of both individual compounds; a matter which may suggest an antagonistic rather than additive effects.

In light of the data available in the literature, it’s worthy to mention that zinc plays an important role in protecting cell against ROS induced by OP pesticides, generally [18]. It interacts with cell membranes in order to stabilize them against damaging effects, including those caused by oxidative injuries. Apart from its direct antioxidant effect by occupying iron and copper binding sites on lipids, proteins and DNA, zinc also plays a structural role in maintaining the integrity of Cu-Zn-SOD as a cofactor [60], and in glutathione regulation which is vital to cellular antioxidant defense [24].

Results of the histopathological studies revealed that the tested insecticides alone or in combination caused toxic effects in tissues and increased injury in the organs such as liver, kidneys and testes of the treated rats (Figures 5-7). These injuries may be due to increasing of oxidative stress which causes organ injury. The observed changes in the overall histoarchitecture of these organs in response to CPF and DIZ could be due to their toxic effects primarily by the generation of ROS causing damage to the various membrane components of the cell [61]. It has been previously reported that during liver damage there was an observed decrease in antioxidant defenses in the liver [3], and cell damage exhibited good correlation with the enzyme leakage [62]. The hepatic function tests corroborated the histopathological lesions observed in the present study.

The tested insecticides caused significant increase in the serum creatinine level (Fig. 4a) and changes in relative kidney weights (Table 1). The significant rise in the serum creatinine level of male rats may refer to the impairment of the glomerular function and tubular damage in the kidneys (Fig. 6). Also, the increase in uric acid concentration (Figure 4b) in the sera of treated male rats compared with the control may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either overproduction or inability of excretion [63].

Testicular atrophy and degenerative changes of the seminiferous tubules have been reported in experimental animals with various insecticides. OP insecticides cause microtubule disruption of epithelium, and finally lead to tubular atrophy [64]. The histopathological changes observed in the testes (Figure 7) may indicate a reproductive damage. Such alteration may be caused by a direct effect of the pesticide on testicular Leydig and Sertoli cells, causing a decrease in testosterone production. In line with the current study, exposure to the pesticide ethylene dibromide has been reported to cause a significant reduction in sperm motility and viability, suggesting that exposure may affect accessory sex glands [65].

Conclusion

The results of the present study revealed that the acute oral toxicity of CPF to male rats was higher than that of DIZ, by a factor of magnitude approaching five folds. Deviation (i.e. % of change) of the values of the measured biochemical parameters in different insecticide treatments compared with control (normal) values (Table 2) gave an indication to the toxicity of the tested doses of CPF, DIZ and CPF+DIZ. Generally, both CPF and DIZ individually induced similar or slightly different alterations, while the mixture (CPF+DIZ) induced alterations higher than those recorded for each of the individual insecticide. However, with few exceptions, the amelioration indices (AIs) were closely around 1.0 which indicates high ameliorative effect of zinc supplementation with the pesticides. Also, the study has shown, for the first time the ability of zinc to ameliorate the oxidative stress induced by diazinon and its combination with chlorpyrifos. Zinc may therefore be useful as a powerful antioxidant agent against toxic damage induced by CPF, DIZ and their combination, especially in individuals who are occupationally exposed daily to low-doses of such pesticides.

Acknowledgment

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References


Citation: Mansour SAK, Abbassy MAL, Shaldam HA. Hepato-Renal Toxicity Induced by Chlorpyrifos, Diazinon and their Mixture to Male Rats with Special Concern to the Effect of Zinc Supplementation. J Toxicol Pharmacol 2017; 1:015.