Biochemical Alterations Induced by Subchronic Chlorpyrifos Exposure in Wistar Rats: Ameliorative Effect of Zinc

Suleiman Folorunsho Ambali[1], Ahmad Tijanni Abubakar[3], Mohammed Umoru Kawu[1], Chidiebere Uchendu[1], Muftau Shittu[1], and Suleiman Olawoye Salami[2]

[1]Department of Veterinary Physiology and Pharmacology,
[2]Department of Veterinary Anatomy
Ahmadu Bello University, Zaria, Nigeria
[3]Kwara State Minisry of Agriculture, Ilorin, Nigeria
Corresponding author: Dr. S.F. Ambali
Toxicology Unit,
Department of Veterinary Physiology and Pharmacology,
Ahmadu Bello University, Zaria, Nigeria
E-mail- fambali2001@yahoo.com; atunluse@gmail.com
Tel No: +234 8037015411

Abstract: Studies have shown that oxidative stress is partly involved in the molecular mechanism of chlorpyrifos-induced toxicity. The present study was aimed at evaluating the effect of zinc on alterations in biochemical changes induced by subchronic chlorpyrifos (CPF) exposure in Wistar rats. Forty adult Wistar rats of either sex used for the study were divided into 4 groups of 10 animals each. Group 1 was administered soya oil (2 ml/kg) while group II was given zinc gluconate (50 mg/kg). Rats in group III were administered chlorpyrifos (10.6 mg/kg~ 1/8th LD_{50}) only while those in group IV were pretreated with zinc gluconate (50 mg/kg) and then administerd with CPF (10.6 mg/kg), 30 min later. The regimens were administered orally via gavage for 8 weeks. The rats were evaluated for toxic signs, weekly body weight changes and death. The sera obtained from blood samples were analysed for the levels of electrolytes (Na^+, K^+, Cl^-), total proteins, albumin, globulin, glucose, urea, alanine aminotransferase (AST), alanine aminotransferase, alkaline phosphatase, creatine kinase and malonaldehyde (MDA). The liver was also examined for MDA concentration. The result showed that CPF caused alterations of these biochemical parameters, which were ameliorated by pretreatment with zinc.

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Key words: Organophosphate, chlorpyrifos, biochemical parameters, oxidative stress, amelioration, zinc

1. Introduction

Chlorpyrifos (CPF), a chlorinated broad spectrum organophosphate (OP) compound remains one of the most widely used insecticides globally in agriculture and public health (Saulsbury et al., 2009), despite restrictions placed on some of its domestic use by United States Environmental Protection Agency. Like many other OP insecticides, the mechanism of CPF toxicity is related to acetylcholinesterase (AChE) inhibition resulting in cholinergic manifestations (Eaton et al., 2008; Ambali et al., 2010b). However, toxicity occurs at doses that do not inhibit AChE (Chakraborti et al., 1993), prompting search for other molecular mechanisms involved in its toxicity. The induction of oxidative stress leading to celluar damage is been increasingly linked with CPF toxicity (Gultekin et al., 2001; Ambali et al., 2007;2010a-d, 2011; Verma et al., 2007).

Oxidative stress, which results from imbalance in the concentration of pro-oxidants and antioxidants in cells in favour of the former is known to cause cellular damage. The cell alleviates the damages caused by free radicals either by repairing the damage or neutralizing their effect before they could damage the tissue using the innate enzymatic and non-enzymatic antioxidant machineries. However, in situation of increased oxidative challenge such as those encountered in pesticide poisoning (Gultekin et al., 2001; Abdollahi et al., 2004) the innate antioxidant machineries in the body are overwhelmed resulting in oxidative stress. This compromises the functional status of the cells as a result of alterations in cellular integrity. In this type of situation, exogenous supplementation of antioxidants is recommended to boost the cellular antioxidant reserves. Many antioxidant compounds including vitamins C (Ambali et al., 2007; 2011a; El-Hossary et al., 2009) and E (Ambali, 2009; Ambali et al., 2010c) and co-administration of both vitamins (Ambali et al., 2010d) have shown promise in mitigating CPF-evoked oxidative damage. Our earlier study (Ambali et al., 2010a) and those conducted by other authors (Goel et al., 2005, 2007; Mansour and Mossa, 2010) have

shown the ability of zinc to mitigate toxicity induced by CPF. In furtherance of its antioxidant effect, the present study investigated the potentials of zinc in mitigating biochemical alterations induced by subchronic CPF exposure in Wistar rats. This is in view of the role of biochemical parameters as a tool in evaluating and predicting the health status of the individual.

2. Materials and Methods

2.1 Experimental animals

Forty young adult Wistar rats (12-14 weeks old) of either sex weighing 115-126g used for this study were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animal were housed in metal cages and fed on standard rat pellets, with water provided *ad libitum*.

2.2 Chemicals

Commercial grade CPF, TERMICOT® (Sabero Organics, Gujarat Limited, India), a 20% EC was obtained from a registered Agrochemical store in Zaria, Nigeria. It was reconstituted to 1% in soya oil prior to daily administration. Zinc gluconate tablets (50 mg/tablet, Nature Field, USA) was obtained from a Pharmaceutical store in Zaria, Nigeria. They were reconstituted in distilled water to appropriate dosing concentration prior to daily administration.

2.3 Animal treatment schedule

The rats were weighed and then divided at random into 4 groups with each group having 10 animals. Group I (S/oil) served as the control and was given only soya oil (2ml/kg). Group II (Zn) was administered zinc (50 mg/kg), while group III (CPF) was dosed with CPF only (10.6 mg/kg, ~1/8th LD_{50}). Group IV (Zn+CPF) was co-administered zinc (50 mg/kg) and CPF (10.6 mg/kg). The regimens were administered orally by gavage once daily for a period of 8 weeks. During this period, the rats were evaluated for toxic signs, body weight changes and death. At the end of the dosing period, the rats were sacrificed by severing the jugular vein after light chloroform anesthesia and blood samples were collected into test tubes, incubated for 30 min and then centrifuged at 800 x g for 10 min. The sera samples obtained as the supernatants were collected into clean test tubes and subsequently used for the evaluation of biochemical parameters. The procedures used in this study were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4 Serum biochemical analysis

The sera samples were evaluated for the concentrations of glucose, total proteins, albumin, electrolytes (Na^+, K^+ and Cl^-) and urea, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine kinase (CK) using an autoanalyzer (Bayer Clinical Chemistry Analyzer, Germany). Globulin was obtained by subtracting albumin concentration from the total serum protein, and consequently the albumin:globulin ratio was calculated.

2.5 Evaluation of serum and liver lipid peroxidation

The malonaldehyde (MDA) concentrations in the serum and the liver samples as an index of lipid peroxidation were evaluated using the double heating method of Draper and Hadley (1990).

2.6 Statistical Analysis

The data obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test using GraphPad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA (www.graphpad.com). Values of $P<0.05$ were considered significant.

3.0 Results

3.1 Clinical signs

Animals in the S/oil and Zn groups did not show any apparent sign of toxicity. Toxic signs observed in the CPF group include restlessness, mild diarrhea, lacrimation, congested ocular membranes, intermittent mild tremor, arched back, rough hair coat, huddling, and death (2 animals). Rats in the Zn+CPF group showed mild restlessness and huddling.

3.2 Effect of treatments on body weight changes

The effect of the treatments on body weight changes is shown in Figures 1 and 2. The dynamics showed a progressive increase in body weight throughout the dosing period in all the groups (Figure 1). There was a significant increase ($P<0.01$) in the body weight at termination compared to those obtained at the commencement of the study in all the groups. However, rats in CPF group showed the least increase (7%) in body weight changes when compared to those recorded for S/oil (26%), Zn (25%) or Zn+CPF (15%) group (Figure 2).

3.3 Effect of treatments on serum electrolytes concentration

There was no significant ($P>0.05$) change in the serum Na^+ ion concentration in between the groups. The Na^+ concentration in the CPF group decreased by 0.9% and 0.7%, respectively when compared to S/oil

and Zn+CPF groups, but it increased marginally by 0.3% compared to Zn group.

There was no significant (P>0.05) change in the serum K^+ concentration in between the groups. The K^+ concentration in the CPF group was comparatively higher than those in the S/oil (12%), Zn (12%) or Zn+CPF (4.4%) group (Figure 3).

The Cl^- concentration was not significantly (P>0.05) different in between the groups. The Cl^- concentration in the CPF group marginally decreased by 1.9%, 2.2% and 1.4% when respectively compared to S/oil, Zn and Zn+CPF groups (Figure 3).

3.4 Effect of treatments on glucose concentration

There was no significant (P>0.05) change in the glucose concentration in between the groups. The glucose concentration in the CPF group decreased by 9%, 7% and 15%, respectively relative to the S/oil, Zn and Zn+CPF groups (Figure 4).

3.5 Effect of treatments on protein concentrations

The total protein concentration in the CPF group was significantly (P<0.05) lower compared to S/oil and Zn group. Although there was no significant (P>0.05) change, the protein concentration in the CPF group was comparatively lower by 9% relative to the Zn+CPF group. The protein concentration in the Zn+CPF group was comparatively lower when compared to either S/oil (8.3%) or Zn (3.7%) group (Figure 5).

There was a significant (P<0.01) decrease in the albumin concentration in the CPF group compared to Zn group. There was no significant (P>0.05) change in the albumin concentration in the CPF group compared to either the S/oil or Zn+CPF group. However, the albumin concentration in the CPF group was comparatively lower than the value recorded in the S/oil (10%) and Zn+CPF (14%) groups. The albumin concentration in the Zn group was significantly higher compared to the S/oil (P<0.05) group but no significant change (P>0.05) relative to Zn+CPF group (Figure 5).

There was no significant (P>0.05) change in the globulin concentration in between the groups. However, the globulin concentration in the CPF group was comparatively lower relative to S/oil (21%), Zn (6%) or Zn+CPF (0.7%) group (Figure 5).

There was no significant (P>0.05) change in the albumin/globulin (A/G) ratio between the groups. However, the A/G ratio in the CPF group increased by 16% compared to the S/oil group but decreased by 14% and 9% compared to Zn and Zn+CPF groups, respectively (Figure 6).

3.6 Effect of treatments on serum urea concentration

The urea concentration in the CPF group was significantly (P<0.05) higher compared to Zn group. Although not significant (P>0.05), the urea concentration in the CPF group increased by 23% and 6% when respectively compared to S/oil and Zn+CPF groups (Figure 7).

3.7 Effect of treatments on activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase

There was a significant (P<0.01) increase in the activity of AST in the CPF group compared to S/oil, Zn or Zn+CPF group. However, there was no significant (P>0.05) change in the AST activity in Zn+CPF group compared to either the S/oil or Zn group (Figure 8).

The ALT activity in the CPF group was significantly (P<0.01) higher relative to the S/oil, Zn or Zn+CPF group. There was no significant (P>0.01) change in the ALT activity in the Zn+CPF group compared to either the S/oil or Zn group (Figure 8).

The ALP acitivity was significantly (P<0.01) higher in the CPF group compared to S/oil, Zn+CPF or Zn group. The ALP activity in the Zn+CPF group was significantly (P<0.05) higher compared to the Zn group but there was no significant (P>0.05) change compared to the S/oil group (Figure 8).

3.8 Effect of treatments on creatine kinase activity

There was no siginificant (P>0.05) change in the CK activity in between the groups. The CK activity in the CPF group was comparatively higher compared to S/oil (6.5%), Zn (10%) or Zn+CPF (7.4%) group (Figure 9).

3.9 Effect of treatments on serum and liver malonaldehyde concentrations

The MDA concentrations in both the serum and liver increased significantly (P<0.01) in the CPF group when compared to S/oil, Zn or Zn+CPF group (Figures 10 and 11). The liver MDA concentration in the Zn+CPF group was significantly (P<0.05) higher compared to the Zn group but no significant change (P>0.05) when compared to S/oil group. There was no significant (P>0.05) change in the serum concentration in the Zn+CPF group relative to either the S/oil or Zn group.

4. Discussion

The clinical signs observed in the CPF group were apparently due to inhibition of AChE resulting in accumulation of acetylcholine in the cholinergic receptors of the peripheral and central nervous systems (Eaton et al., 2008). Pretreatment with zinc has been shown by the present study to reduce these CPF-induced cholinergic manifestations and death. This may be partly due to its antioxidant properties and the role it plays in metabolic processes.

The present study also showed that subchronic CPF exposure caused a comparatively lower body weight gain compared to the other groups, despite the

fact that we did not observe appreciable differences in the level of food consumption in between the groups. This result agreed with findings from previous studies (Ambali et al., 2007; Ambali, 2009; Mansour and Mossa, 2010). The comparatively lower body weight gain by rats exposed to CPF may be due to the level of stress caused by the OP. Apart from oxidative stress, CPF has been shown to inhibit the activity of cholesteryl ester hydrolase (Civen et al., 1977), an enzyme that is essential in response to stress. Besides, CPF causes vacuolation of *zona fasciculata* of the adrenal cortex (Barna-Lloyd et al., 1990, Yano et al., 2000), the zone responsible for the elaboration of cortisol and corticosterone that are also essential in body's response to stress. Similarly, the cholinergic stress recorded in the CPF group may have contributed to the level of stress, hence the lower body weight gain. Therefore, the comparatively low body weight changes in the CPF group may have been due to toxic stress, oxidative stress and cholinergic stress. The improvement in body weight changes following zinc pretreatment agreed with previous findings (Goel et al., 2005; Mansour and Mossa, 2010). Apart from ameliorating the oxidative challenges, the involvement of zinc in the the regulation of metabolic processes as a functional component of about 200 enzymes involved in metabolism may have complemented the improvement in body weight gain (Ishikawa et al., 2002). Similarly, CPF, which has been reported to reduce zinc concentration in the body (Goel et al., 2007), a situation that has been associated with low body weight (Baltaci et al., 2005). Therefore, zinc supplementation may have boosted the body's reserve of the trace element thereby compensating for the CPF-induced decrease in its concentration, hence improvement in the weight gain.

Subchronic CPF exposure has also been shown by the present study not to significantly alter the serum electrolytes. This is in agreement with findings from our previous studies (Ambali et al., 2007, 2010d; Ambali, 2009). The apparent increase in serum K^+ in the CPF group signifies alteration in metabolic status of the animals. This relative hypokalaemia has been recorded in previous studies (Ambali et al., 2007, 2010d; Ambali, 2009) and may have resulted from the mild diarrhea recorded in the CPF group.

Although, there was no significant alteration in the glucose concentration in the CPF group, the fact that it was comparatively lower shows that this pesticide causes an apparent hypoglycemia. This contradicts OP-induced hyperglycemia reported by many authors (Ambali, 2009; Rahimi and Abdollahi; Ambali et al., 2011b) but agreed with the result obtained by Szabo et al. (1988). The relative hypoglycemia in the CPF group may be due to interference with liver function as a result of CPF-evoked hepatotoxicity, which has been demonstrated in the present study and other previous ones (Goel et al., 2005; Ambali et al., 2007; Ambali, 2009; Mansour and Mossa, 2010). The liver is the site responsible for glucose synthesis and interference with its functional capacity as a result of injury will compromise this function, hence the relative hypoglycemia recorded in the CPF group. The improvement in glucose concentration in Zn+CPF group may be due to the ability of the trace element to reduce hepatic oxidative damage provoked by CPF probably due to its anti-lipoperoxidative effect.

The present study also showed that subchronic CPF administration caused hypoproteinemia, which agrees with findings from previous studies (Kagan, 1971; Bomhard et al., 1981; Schilde and Bomhard, 1984; Szabo et al., 1988; Ambali, 2009). The low serum proteins, which is due to low albumin concentration may have resulted from impaired protein and albumin synthesis by the liver due to oxidative damage. Although, the A/G ratio in the CPF group was higher than those in the S/oil group, the apparently lower A/G ratio when compared to either Zn or Zn+CPF group further confirms its ability to induce relative hypoalbuminemia. The low serum albumin may have been due to its ability to bind the OP and subsquently hydrolyse it (Ortigoza-Ferado et al., 1984; Sultatos et al., 1984). Besides binding to OPs, albumin scavenges OP molecules and, therefore, reduces the amount available for reaction with AChE (Peeples et al., 2005). Therefore, the low albumin in the CPF group may have exacerbated its toxicity. Furthermore, albumin, an effective antioxidant (Roche et al., 2008) may have been used up due to CPF-evoked oxidative stress. CPF induced relatively lower serum globulin cocentration, apparently due to lymphocytic leukopenia that has been described in previous studies (Goel et al., 2006; Ambali et al., 2007; 2010a). Apoptotic damage of the immune cells has been described following pesticide exposure (Rabideau, 2001). Zinc supplementation has been shown in the present study to improve the total protein and albumin concentrations, in addition to increasing the A/G ratio. This may be due to its antioxidant properties, which help protect the liver from CPF-evoked oxidative damage. Similarly, this antioxidant property may have prompted low oxidative challenge thereby reducing the demand for the utilization of albumin as free radical scavenger, hence improving its concentration.

The significant increase in serum urea concentration in the CPF group is a demonstration of impairment of kidney function since the organ primarily excrete urea in the urine. This result agrees with our earlier findings (Ambali et al., 2007; 2010d). Repeated CPF exposure has been shown to cause glomeruli and tubular degeneration partly due to oxidative stress (Ambali, 2009), thereby impairing

renal functions. Pretreatment with zinc resulted in a slight decrease in urea concentration indicating its apparent protective effect. This protective effect may be due to its antioxidant properties, which protect the renal tissue from lipoperoxidative changes, thereby improveming renal functions.

The study also revealed a significant increase in the activities of AST, ALT and ALP in the group exposed to CPF only signalling hepatic damage. AST is a sensitive marker of liver damage, even if the damage is of a subclinical nature (Kauppinen, 1984; Meyer and Harvey, 1998). However, the activity of the enzyme is not limited only to the liver as it is also present in the brain, muscle and red blood cells (Ballanthyne, 1988). On the other hand, ALT is a more specific marker of hepatic injury (Ballantyne, 1988) and the increase in its activity in the CPF group indicates hepatic damage. ALT and AST are enzymes produced by the hepatocytes, where they are involved in the metabolism of amino acids and synthesis of proteins. In dying or damaged cells, these enzymes leak into the blood stream (Mansour and Mossa, 2010). The significant increase in ALP activity in the CPF group is also an indication of hepatotoxicity. However, the enzyme is not liver specific as it is also associated with muscle, bones and intestines. The elevation of AST, ALT and ALP activities elicited in the CPF group indicating hepatotoxicty has been reported in previous studies (Goel et al., 2005; Ambali, 2009; Mansour and Mossa, 2010). The elevation in the liver enzymes activities may be due to liver dysfunction with consequent reduction in their biosynthesis and altered membrane permeability permiting enzyme leakages into the serum (Mansour and Mossa, 2010). The liver is susceptible to damage because of direct exposure to toxic products due to its role in the detoxicification of metabolic by-products and xenobiotics.

Pretreatment with zinc was able to restore the activies of these enzymes almost to normal level recorded in the S/oil and Zn groups. This may be partly due to the antioxidant property of zinc that protect the hepatocytes from CPF-induced oxidative damage.

Chlorpyrifos, in the present study, induced a relative increase in serum CK activities in Wistar rats, indicating some level of muscular damage (Frieldman et al., 2003). Increase in CK activity and rhabdomyonecrosis of the skeletal muscle (Vanneste and Lison, 1993; Friedman et al., 2003; Lau et al., 2003), and cardiotoxicity (Agrawal et al., 2007) have been reported following OP exposure. We have also reported increased muscle lipoperoxidation and CK activities following repeated CPF exposure (Ambali, 2009). Pretreatment with zinc was able to mitigate the CK activity to near normal level, probably due to its antioxidant properties,which protect muscles from oxidative damage.

The study also showed an elevated level of MDA in the serum and liver of CPF group indicating increased lipoperoxidation. The level of MDA, a major oxidation product of peroxidized polyunsaturated fatty acids has been used as an indicator of lipoperoxidation (Kallender et al., 2004). Lipid peroxidation is one of the molecular mechanisms that has been implicated in CPF-induced toxicity (Gultekin et al., 2001; Goel et al., 2005; Ambali et al., 2010a-d, 2011a,b). By-products of lipid peroxidation cause profound alterations in the structural organization and functions of the cell membranes (Van Ginkel and Sevanian, 1994). CPF is lipophilic and may enhance lipid peroxidation by directly interacting with the cellular membranes (Hazarika et al., 2003). The overall effect of these is compromization of the structural integrity of the cytomembranes leading to alterations in cellular function. The altered biochemical parameters recorded in the group exposed to CPF only in the present study may be partly due to lipoperoxidative changes in some organs. Furthermore, CPF exposure has been associated with decrease in zinc concentration (Goel et al., 2007), which invariably results in lipid peroxidation (Sullivan et al., 1980).

Pretreatment with zinc reduced the liver and serum MDA concentrations, indicating amelioration of CPF-evoked lipoperoxidation. This demonstrates the antioxidant properties of zinc. Cellular zinc exists in only one redox state; thus, it cannot undergo redox reactions that are commonly responsible for the generation of reactive oxygen species (Zhou et al., 2007). Apart from its direct antioxidant effect via occupation of iron and copper binding sites on lipids, proteins and DNA thereby preventing hydroxyl radical formation near these structures (Powell, 2000; Prasad and Kucuk, 2002), zinc also plays a structural role in the maintenance of the integrity of Cu-Zn superoxide dismutase as a cofactor (Coudray et al., 1992; Sahin and Kucuk, 2003). Zinc is also involved in the regulation of glutathione that is vital to cellular antioxidant defense (Parrat et al., 1997). Furthermore, zinc stabilizes the thiol pool by protecting the sulfhydryl group, hence proteins from oxidation (Gibbs et al., 1985; Kruss et al., 1997; Verhaegh et al., 1998). The combined effect of these properties coupled with its role in metabolic reactions as a cofactor for many enzymes involved in metabolic processes may have contributed to the mitigation of cellualr injury evoked by CPF.

In conclusion, the present study has shown that repeated low level exposure to CPF alters the biochemical profiles of rats pointing to pathological changes in the liver, kidneys and muscles. Lipoperoxidative changes as shown by increased MDA concentration in the serum and liver of rats exposed to CPF may have been partly responsible for this organ

toxicity. Pretreatment with zinc has been shown by the present study to ameliorate the biochemical alterations evoked by repeated CPF exposure partly due to its antioxidant properties, which protect tissue from lipoperoxidative changes.

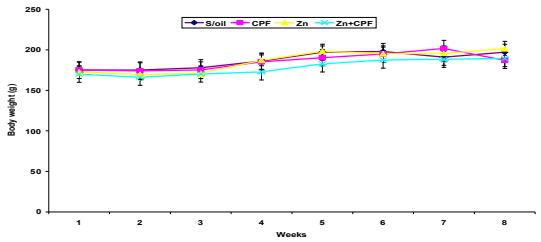

Figure 1: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos on dynamics of body weight changes in Wistar rats.

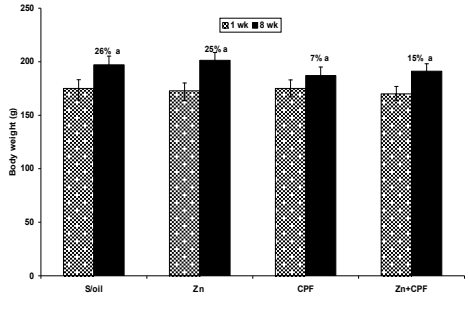

Figure 2: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on body weight changes at week 1 and week 8 in Wistar rats.[a]P<0.05 versus week 1; % expresses the difference in body weight between week 1 and week 8.

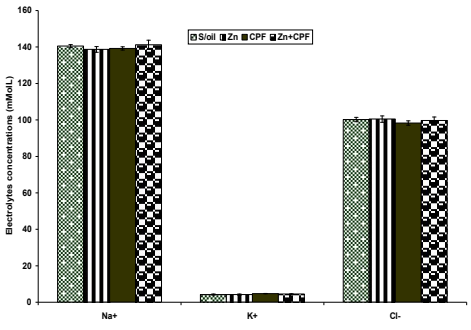

Figure 3: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos on serum electrolytes concentration in Wistar rats.

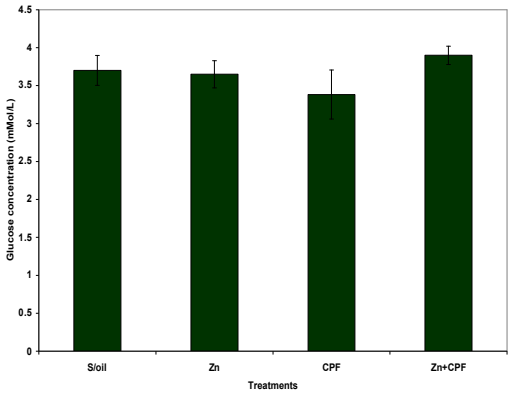

Figure 4: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos on serum glucose concentration in Wistar rats.

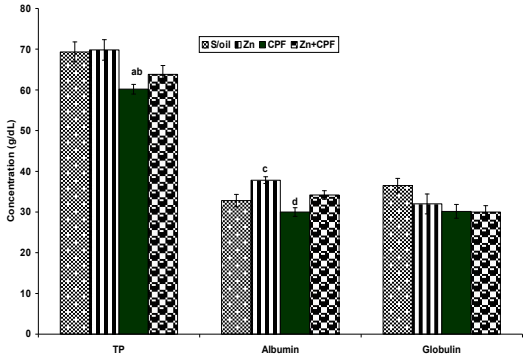

Figure 5: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos on serum total protein (TP), albumin and globulin concentrations in Wistar rats. [ab]P<0.01 versus S/oil and Zn, respectively; [c]P<0.05 versus S/oil; Zn and Zn+CPF groups, respectively and Zn+CPF groups, respectively

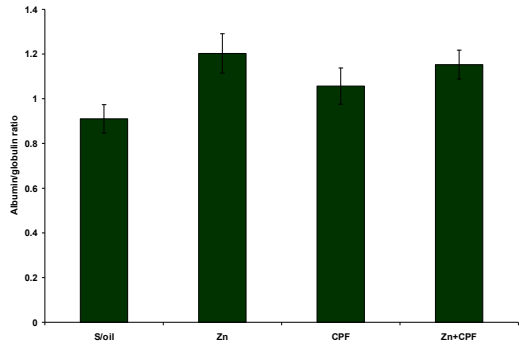

Figure 6: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos on albumin/globulin ratio

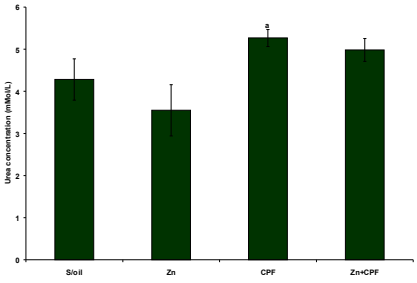

Figure 7: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on serum urea concentration in Wistar rats. [a]P<0.05 versus Zn group.

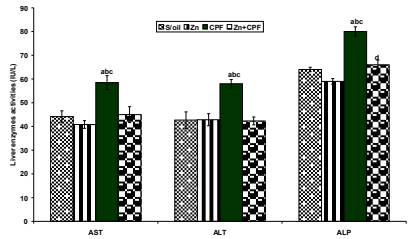

Figure 8: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on liver enzyme activities in Wistar rats**.** [abc]P<0.01 versus S/oil, Zn and Zn+CPF groups, respectively;[d]P<0.05 versus Zn group.

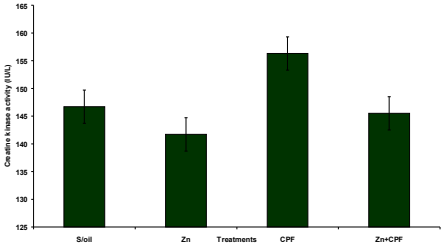

Figure 9: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on creatine kinase activities in Wistar rats.

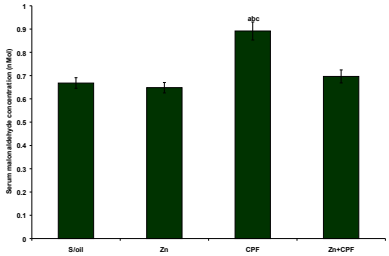

Figure 10: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on serum malonaldehyde concentration in Wistar rats. [abc]P<0.01 versus S/oil, Zn and Zn+CPF groups, respectively.

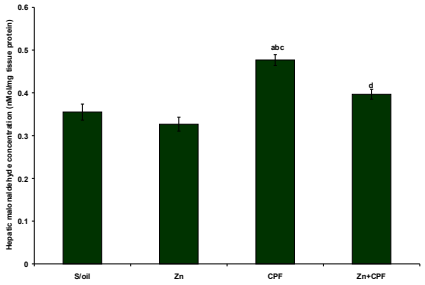

Figure 11: Effect of subchronic administration of soya oil (S/oil), zinc (Zn) and/or chlorpyrifos (CPF) on hepatic malonaldehyde concentration in Wistar rats.[abc]P<0.01 versus S/oil, Zn and Zn+CPF groups, respectively; [d]P<0.05 versus Zn group.

References

1. Saulsbury MD, Heyliger SO, Wang K, Johnson DJ. Chlopyrifos induces oxidative stress in oligodendrocyte progenitor cells. Toxicol 2009;259: 1–9.
2. Eaton DL, Daroff RB, Autrup H, Buffler P, Costa LG, Coyle J, Mckhann G, Mobley WC, Nadel L, Neubert D, Schukte-Hermann R, Peter S, Spencer PS. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol 2008; S2: 1-125.
3. Ambali SF**,** Idris SB, Onukak C, Shittu M, Ayo JO. Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats**.** Toxicol Ind Health 2010b; 26(9): 547-58.
4. Chakraborti TK, Farrar JD, Pope CN. Comparative neurochemical and neurobehavioral effects of repeated chlorpyrifos exposures in young rats. Pharmacol Biochem Behav 1993; 46: 219-24.
5. Gultekin F, Delibas N, Yasar S, Kilinc I. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch Toxicol 2001; 75(2): 88-6.
6. Ambali S, Akanbi D, Igbokwe N, Shittu M, Kawu M, Ayo J. Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. J Toxicol Sci 2007; 32(2): 111-20.
7. Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Anafi SB, Abdullahi A. 2010a Chlorpyrifos-induced alteration of hematological parameters in Wistar rats: Ameliorative effect of Zinc. Res J Environ Toxicol 2010a; 4(2): 55-66.
8. Ambali SF**,** Ayo JO, Ojo SA, Esievo KAN. Vitamin E protects rats from chlorpyrifos-induced increased erythrocyte osmotic fragility in Wistar rats. Food Chem Toxicol 2010c; 48: 3477-80.

9. Ambali S, Akanbi D, Shittu M, Giwa A, Oladipo OO, Kawu M, Ayo JO. Chlorpyrifos-Induced Clinical, Hematological and Biochemical Changes in Swiss Albino Mice- Mitigating effect by co-administration of vitamins C and E. Life Sci J 2010d; 7(3): 37-44.
10. Ambali SF, Ayo JO, Ojo SA, Esievo KAN. (2011a). Ameliorative effect of vitamin C on chlorpyrifos-induced increased erythrocyte fragility in Wistar rats. Hum Exp Toxicol 2011a;30 (1): 19-24.
11. Ambali SF, Shuaib K, Edeh R, Orieji BC, Shittu M, Akande MG. Hyperglycemia induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: Role of pancreatic lipoperoxidation and alleviating effect of vitamin C. Biol Med 2011b; 3(1): 6-14.
12. Verma RS, Mehta A, Srivastava N. *In vivo* chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pest Biochem Physiol 2007; 88: 191-96.
13. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Med Sci Monit 2004; 10(6): RA141-RA47.
14. El-Hossary GG, Mansour SM, Mohamed AS. Neurotoxic effects of chlorpyrifos and the possible protective role of antioxidant supplements: an experimental study. J Appl Sci Res 2009; 5(9): 1218-22.
15. Ambali SF. Ameliorative effect of vitamins C and E on neurotoxicological, hematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats, PhD Dissertation, Ahmadu Bello University, Zaria, 2009; 356 pp.
16. Goel A, Danni V, Dhawan DK. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem.-Biol Inter 2005;156: 131-34.
17. Goel A Dani V, Dhawan DK. Zinc mediates normalization of hepatic drug metabolizing enzymes in chlorpyrifos-induced toxicity. Toxicol Lett 2007;169: 26–33.
18. Mansour SA, Mossa AH. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pest Biochem Physiol 2010; 96(1): 14-23.
19. Guide for the care and use of laboratory animals, DHEW Publication No. (NIH) 85-23, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892, Revised 1985, .
20. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Meth Enzymol 1990; 186: 421-31.
21. Civen M, Brown CB, Morin RJ. Effects of organophosphate insecticides on adrenal cholesteryl ester and steroid metabolism. Biochem Pharmacol 1977; 26: 1901-07.
22. Barna-Lloyd T, Szabo JR, Davis NL. Chlorpyrifos-methyl (Reldan R) rat subchronic dietary toxicity and recovery study.Unpublished Report TXT: K-046193-026 from Dow Chemical, Texas, USA. Submitted to WHO by Dow Elanco,Indianapolis, USA, 1990.
23. Yano BL, Young JT, Mattsson JL. Lack of carcinogenicity of chlorpyrifos insecticide in a high-dose, 2-year dietary toxicity study in Fischer 344 rats. Toxicol Sci 2000; 53: 135-44.
24. Ishikawa Y, Kudo H, Suzuki S, Nemoto N**,** Sassa S, Sakamoto S**.** Down regulation by a low-zinc diet in gene expression of rat prostatic thymidylate synthase and thymidine kinase. Nutr Metab 2008; 5**:**12doi:10.1186/1743-7075-5-12.
25. Baltaci AK, Mogulkoc R, Halifeoglu, I. Effects of zinc deficiency and supplementation on plasma leptin levels in rats. Biol Trace Elem Res 2005; 104(1): 41-46.
26. Rahimi R, Abdollahi M. A review on the mechanisms involved in hyperglycaemia induced by organophosphorus pesticide. Pest Biochem Physiol 2007; 88: 115-21.
27. Szabo JR, Young JT, Granjean M. Chlorpyrifos: 13-week dietary toxicity study in Fisher 344 rats. Jackson Research Centre, Health and Environmental Sciences - Texas. Laboratory study No.: TXT: K-044793-071. Report dated December 28, 1988. Reviewed by PMRA, 1988
28. Kagan J. Topical questions about the toxicology of phosphorganic insecticides. Ernahrungsforschung 1971; 14: 503-14 (in German).
29. Bomhard E, Loser E, Schilde B. [E605– Methyl] (Methyl parathion) chronic toxicological studies in rats (2 year feeding trial) Wuppartal – Elberfeld Bayer AG, Institute of Toxicology IPCS -145, 1981: 155.
30. Schilde B, Bomhard E. E 605-methyl (Methyl parathion) supplementary histopathological test further to the 2-year feeding trials on rats. Addendum to report No. 9889.Wuppertal-Elberfeld, Bayer A. G., Institute of Toxicology (Unpublished Report No. 12559), 1984.
31. Ortigoza-Ferado J, Richter RJ, Hornung SK, Motulsky AG, Furlong CE. Paraoxon hydrolysis in human serum mediated by a genetically variable arylesterase and albumin. Am J Hum Gen 1984; 36: 295-05.
32. Sultatos LG, Shao M, Murphy SD. The role of hepatic biotransformation in mediating the acute toxicity of the phosphorothionate insecticide chlorpyrifos. Toxicol Appl Pharmacol 1984; 73: 60-8.
33. Peeples ES, Schopfer LM, Duysen EG, Spaulding R, Voelker T, Thompson CM, Lockridge O. Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry. Toxicol Sci 2005; 83: 303-12.

34. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. FEBS Lett 2008; 582: 1783–87.
35. Goel A, Danni V, Dhawan DK. Chlorpyrifbs-induced alterations in the activities of carbohydrate metabolizing enzymes in rat liver: The role of zinc. Toxicol Lett 2006; 163(3): 235-41.
36. Rabideau CL. Pesticide mixtures induce immunotoxicity: potentiation of apoptosis and oxidative stress. MSc Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 2001;170pp.
37. Kauppinen K. ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentration in plasma of normal and ketotic dairy cows. Zentralblat Veterinaärmed 1984; 31: 561-76**.**
38. Meyer DJ, Harvey JW. Evaluation of hepatobiliary system and skeletal muscle and lipid disorders. In: Meyer, D.J. and Harvey, J.W. (Eds.). Veterinary Laboratory Medicine Interpretation and Diagnosis 2nd Edition, WB Sanders, Philaldephia, London, Toronto, Montreal, Sydney, 1998: 157-87.
39. Ballantyne B. Xenobiotic-induced rhabdomyolysis. In: Ballantyne, B. (Ed.). Perspectives in Basic and Applied Toxicology, Butterworth and Co (Publishers) Ltd., 1988: 70-153.
40. Frieldman LS, Brautbar N, Barach P, Wolfe AH, Richhter ED. Creaine phosphate kinase elecations signaling muscle damage following exposures to anticholinesterases: 2 sentinel patients. Arch Env Health 2003; 58(3): 167-71
41. Vanneste Y, Lison D. Biochemical changes associated with muscle fibre necrosis after experimental organophosphate poisoning. Hum Exp Toxicol 1993;12(5):365-70.
42. Lau CL, Chung KL, Kam CW. Accidental organophosphate insecticide poisoning: a fatal child case with hazards to emergency department staff. Hong Kong J Emerg Med 2003;10: 248-52.
43. Agarwal B, Bhatnagar VK, Agarwal A, Agarwal U, Venkaiah K, Nigam S. K, Kashyap S K. Impairment in clinical indices in acute organophosphate insecticide poisoning patients in India . Int J Toxicol. 2007;4(1).
44. Kalender S, Kalender Y, Ogutcu A, Uzunhisarikli M, Durak D, Anikgoz F. Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. Toxicol 2004; 202: 227–35.
45. Van Ginkel G, Sevanian A. Lipid peroxidation induced membrane structural alterations, Meth Enzymol 1994; 233: 273-88.
46. Hazarika A, Sarka SN, Hajare S, Kataria M, Malik JK. Influence of malathion pretreatment on the toxicity of amilofos in male rats: a biochemical interaction study. Toxicol 2003; 185: 1-8.
47. Sullivan JF, Jetton MM, Hahn HK, Burch RE. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. Am J Clin Nutr 1980; 33: 51–6.
48. Zhou Z, Kang X, Jiang Y, Song Z, Feng W, Mcclain CJ, Kang YJ. Preservation of hepatocyte nuclear factor-4α is associated with zinc protection against TNF-α hepatotoxicity in mice. Exp Biol Med 2007; 232: 622–28.
49. Powell SR. The antioxidant properties of zinc. J Nutr 2000; 130: 1447S–54S.
50. Prasad AS, Kucuk O. Zinc in cancer prevention. Cancer Met Rev 2002; 21: 291–95.
51. Coudray C, Richard MJ, Laporte F, Faure P, Roussel AM, Favier AE. Superoxide dismutase and zinc status: a study in man and animals. J Nutr Med 1992; 3: 13-26.
52. Sahin A, Kucuk O. Zinc supplementation alleviates heat stress in laying Japanese quail. J Nutr 2003; 133: 2808–11.
53. Parrat MO, Richard MJ, Beani JC, Favier A. Involvement of zinc in intracellular oxidant/antioxidant balance. Biol Trace Elem Res 1997; 60: 187-04.
54. Gibbs PN, Gore MG, Jordan PM. Investigation of the effect of metal ions on the reactivity of thiol groups in human 5-aminolaevulinate dehydratase. Biochem J 1985; 225: 573-80.
55. Kraus A, Roth H, Kirchgessner M. Supplementation with vitamin C, vitamin E or β-carotene influences osmotic fragility and oxidative damage of erythrocytes of zinc-deficient rats. J Nutr 1997; 127: 1290-96.
56. Verhaegh G, Parat MO, Richard MJ, Hainut P. Modulation of p53 protein conformation and DNA binding activity by intracellular chelation of zinc. Mol Carcinog 1998; 21: 205-14.

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