



## Original Research

## Oxidative stress involvement in chronic chlorpyrifos-induced hepatocellular injury: Alleviating effect of vitamin C

\*<sup>a</sup>Ambali, S.F., <sup>b</sup>Shittu, M., <sup>a</sup>Akorede, G.J., <sup>a</sup>Olatunji, A., <sup>a</sup>Aremu, A., <sup>c</sup>Ibrahim, N.D.G. and <sup>d</sup>Ramon-Yusuf, S.B.

<sup>a</sup>Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria.

<sup>b</sup>Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria.

<sup>c</sup>Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria.

<sup>d</sup>National Universities Commission, Abuja, Nigeria.

## ARTICLE INFO

## Article history:

Received: December 9, 2017

Received in revised form: February 11, 2018

Accepted: February 27, 2018.

## Keywords:

Chlorpyrifos  
Hepatocellular injury  
Oxidative stress alleviation  
Vitamin C.

## ABSTRACT

**Introduction:** Chlorpyrifos (CPF), a phosphorothionate chlorinated organophosphate (OP) insecticide is widely used in agriculture and public health. Like other OP insecticides, its main mechanism of toxicity is the inhibition of acetylcholinesterase (AChE) leading to cholinergic syndrome. Since toxicity occurs at doses that do not inhibit AChE or long after its restoration, other mechanisms including the induction of oxidative stress have been widely implicated. The present study was aimed at evaluating the mitigating effect of vitamin C on CPF-induced hepatocellular injury in Wistar rats.

**Methods:** Twenty adult male Wistar rats were divided into 4 groups of five animals in each group. The four groups were exposed by gavage to soya oil (2 ml/kg), vitamin C (100 mg/kg), CPF (10.6 mg/kg-1/8<sup>th</sup> LD<sub>50</sub>) and vitamin C (100 mg/kg) + CPF (10.6 mg/kg; 30 min later), respectively for 17 weeks. The sera obtained from blood samples collected from the animals were analysed for the levels of total proteins, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase (GGT) while globulin concentration and albumin/globulin ratio were calculated. The liver homogenate was evaluated for the levels of malonaldehyde (MDA), superoxide dismutase (SOD) and catalase (CAT), and histological changes.

**Results:** The study showed that CPF altered the levels of the serum hepatic enzymes, hepatic MDA SOD and CAT, in addition to inducing hepatocellular degeneration. All these parameters were alleviated by pre-treatment with vitamin C.

**Significance:** CPF-induced hepatocellular injury which was partly due to oxidative changes was mitigated by vitamin C partly due to its antioxidative activity.

© 2018 Faculty of Veterinary Medicine, University of Ilorin, Nigeria. All rights reserved.

## Introduction

Organophosphate (OP) insecticides are one of the most widely used pesticides for agricultural and non-agricultural purposes accounting for about 50% of the global insecticide use (Casida and Quistad, 2004). The ubiquitous nature of their use has led to increasing propensity of their adverse effect on target and non-target species. Chlorpyrifos (CPF) [O,O-diethyl O-(3,5,6-trichloro-2-pyridinol) phosphorothionate] is a chlorinated OP insecticide that is widely used in agricultural, public health and domestic purposes, despite restrictions placed by United States Environmental Protection Agency on some of the latter applications. Like other OP insecticides, the mechanism of systemic toxicity of CPF resides in its ability to inhibit acetylcholinesterase (AChE) (Slotkin *et al.*, 2005) resulting in cholinergic toxicity (Eaton *et al.*, 2008). However, oxidative stress is one of the molecular mechanisms implicated in OP toxicity (Goel *et al.*, 2005; Ambali *et al.*, 2007; 2010; 2011a and b; Ambali and Ayo, 2011; Tanvir *et al.*, 2015). The body

is naturally endowed with enzymatic and non-enzymatic antioxidant machineries to combat the menace posed by moderate oxidative challenges. However, in severe oxidative challenges, the body's innate antioxidant machineries are overwhelmed that may prompt exogenous antioxidant supplementation. Antioxidants, including vitamin C, a water soluble antioxidant have been shown in several studies to mitigate oxidative challenges evoked by OP insecticides (Ambali *et al.*, 2007, 2011a,b; Aly *et al.*, 2010).

Due to its lipophilicity, CPF is easily absorbed following gastrointestinal, respiratory and dermal exposure from where it is preferentially deposited in the lipid rich internal tissue of the lungs, body fats, central and peripheral nervous systems, kidneys and liver. The liver is the main site of detoxification where metabolism occurs (Millward-Sadler, 1987). Furthermore, the liver is also the site for oxidative desulfuration of CPF to its more active metabolites, CPF-oxon, a more potent AChE inhibitor. Consequently, the liver is vulnerable to injury. The present study was therefore aimed at evaluating the alleviating effect of

\*Corresponding author Tel: +234 8037015411  
email addresses: [ambali.sf@unilorin.edu.ng](mailto:ambali.sf@unilorin.edu.ng); [fambali2001@yahoo.com](mailto:fambali2001@yahoo.com)

vitamin C on hepatocellular injury caused by chronic CPF exposure in Wistar rats.

## Materials and methods

### Chemical acquisition and preparations

Commercial grade chlorpyrifos (CPF) 20% EC, marketed as Termicot<sup>®</sup> (Sabero Organics, Gujarat limited, India), was prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria) to make 10% stock solution. A tablet (100 mg) of ascorbic acid (Med Vit. C<sup>®</sup>, Dol-Med Laboratories Limited, Lagos, Nigeria) was dissolved in 1 mL of distilled water to obtain 100 mg/mL suspension, daily prior to administration. All other reagents used in this study were of analytical grade and were obtained from Sigma Inc. (USA).

### Experimental animals

Twenty adult male Wistar rats weighing between 95 and 110g used for this study were obtained from the Laboratory Animal House of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize for two weeks in the laboratory prior to the commencement of the experiment. They were fed on standard rat chow and water was provided *ad libitum*.

### Animal treatments schedule

The rats were weighed and then assigned at random into 4 groups of 5 rats in each group. Group I (S/oil) served as the control group and were given only soya oil (2mL/kg b.w.) while group II (VC) was given vitamin C (100 mg/kg b.w.) and supplemented with soya oil (2 mL/kg b.w.). Group III (CPF) was administered with CPF only (10.6 mg/kg b.w.  $\sim 1/8^{\text{th}}$  LD<sub>50</sub> [Ambali, 2009]) while group IV (VC+CPF) was pre-treated with vitamin C (100 mg/kg b.w.), and then dosed with CPF (10.6 mg/kg b.w.), 30 minutes later. The different regimens were administered once daily by gavage for a period of 17 weeks. The study was carried out according to the specification of the Ahmadu Bello University Animal Research Committee and in compliance with the National Research Council on Guide for Care and Use of laboratory Animals (2011). At the end of the dosing period, the rats were sacrificed by severing the jugular vein after light ether anaesthesia. Blood samples were collected into heparinized sample bottles, left incubated on the shelf for 30 min, and thereafter centrifuged at 800 x g for 10 minutes.

### Evaluation of treatments on serum proteins and hepatic enzymes

The sera obtained were used to analyse for the concentrations of total proteins, albumin, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) using autoanalysers (Bayer Express Plus, Germany). The globulin concentration was calculated by subtracting albumin concentration from the total protein concentration. The albumin/globulin ratio was subsequently calculated.

### Evaluation of treatments on hepatic lipoperoxidation

The level of thiobarbituric acid (TBA) reactive substance, malonaldehyde (MDA) as an index of lipid peroxidation was evaluated on the liver sample using the method of Draper and Hadley (1990) as modified (Yavuz *et al.*, 2004). The principle of the method was based on spectrophotometric measurement of the colour developed during reaction of TBA with MDA. The MDA concentration in each sample was calculated by the absorbance coefficient of MDA-TBA complex  $1.56 \times 10^5/\text{cm/M}$  and expressed as nmol/mg of tissue protein. The protein concentration in the liver homogenates was evaluated using the Lowry method (Lowry *et al.*, 1951).

### Evaluation of treatments on hepatic superoxide dismutase activity

Superoxide dismutase (SOD) activity was evaluated using NWLSS<sup>™</sup> superoxide dismutase activity assay kit (Northwest Life Science Specialities, Vancouver, WA 98662) as stated by the manufacturer, expressed as nmol/mg tissue protein.

### Evaluation of treatments on hepatic catalase activity

Catalase (CAT) activity was evaluated using NWLSS<sup>™</sup> catalase activity assay kit (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) as stated by the manufacturer and expressed as nmol/mg tissue protein.

### Evaluation of treatments on hepatic acetylcholinesterase activity

Acetylcholinesterase activity was evaluated using the method of Ellman *et al.* (1961) with acetylthiocholine iodide as a substrate. Briefly, the liver sample of each animal was weighed and homogenized in a cold (0–4 °C) 20 mM phosphate buffer saline (PBS) incubated with 0.01M 5,5-dithio-bis(2-nitrobenzoic acid) in 0.1 M PBS, pH 7.0. Incubations were allowed to proceed at room temperature for 10 minutes. Then, acetylthiocholine iodide (0.075 M in 0.1 M PBS, pH 8.0) was added to each tube, and absorbance at 412 nm was measured continuously for 30 minutes using a UV spectrophotometer (T80<sup>+</sup> UV/VIS spectrometer<sup>®</sup>, PG Instruments Ltd, Leicestershire, LE 175BE, United Kingdom). The acetylcholinesterase activity expressed as IU/g tissue was calculated based on the rate of colour change per minute.

### Evaluation of the effect of treatments on hepatic histological changes

The liver samples of the rats were obtained and prepared for histological processing using the method described by Luna (1960). Briefly, the liver samples were fixed in 10% formalin, embedded in paraffin and cut to 5 mm. They were then stained with haematoxylin-eosin, examined under light microscope (x400) and lesions observed were recorded

### Statistical Analysis

All data were expressed as mean  $\pm$  SEM and then subjected to one-way analysis of variance followed by Tukey's multiple comparison test. Values of  $p < 0.05$  were considered significant.

## Results

### Effect of treatments on serum proteins

There was a significant decrease in the total protein concentration in the CPF group when compared to that of S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) or VC + CPF ( $p < 0.01$ ) group. There was no significant ( $p > 0.05$ ) change in the total protein concentration in the VC + CPF group relative to S/oil or VC group (Figure 1).

There was a significant ( $P < 0.05$ ) decrease in albumin concentration in the CPF group relative to that of S/oil or VC group. Although not significant ( $p > 0.05$ ), the albumin concentration in the VC + CPF group was relatively higher (19%) compared to CPF group. There was no significant ( $p > 0.05$ ) change in the albumin concentration in the VC + CPF group relative to that of S/oil or VC group (Figure 1).

There was no significant ( $p > 0.05$ ) change in the globulin concentration in the CPF group relative to S/oil, VC or VC + CPF group. However, the mean globulin concentration in the VC + CPF group was relatively higher compared to that recorded in the S/oil (18.2%), VC (10%) or CPF (11%) group (Figure 1).

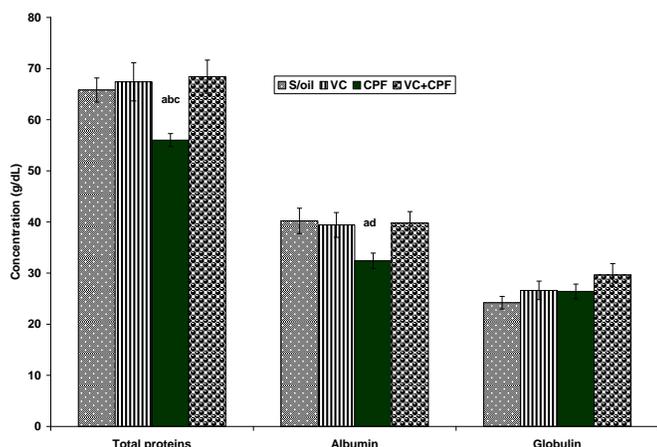


Figure 1. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on serum proteins concentration in Wistar rats.

<sup>a</sup> $p < 0.05$  vs S/oil group, <sup>bc</sup> $p < 0.01$  vs VC and VC + CPF groups, respectively; <sup>d</sup> $p < 0.05$  vs VC group

The albumin/globulin ratio was significantly ( $p < 0.05$ ) lower in the CPF group when compared to that recorded in the S/oil group. Although not significant ( $p > 0.05$ ), the mean albumin/globulin ratio relatively decreased by 50% in the CPF group compared to that obtained in the VC group. There was no mean difference in the albumin/globulin ratio in the CPF group relative to that of VC + CPF group (Figure 2).

### Effect of treatments on serum hepatic enzymes activities

There was a significant increase ( $p < 0.05$ ) in AST activity in the CPF group compared to that of S/oil, VC or VC + CPF group. There was no significant ( $p > 0.05$ ) change in the AST activity in VC + CPF group when compared to that in the S/oil or VC group (Figure 3).

The ALT activity in the CPF group was significantly higher in the CPF group when compared to that in the S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) or VC + CPF ( $p < 0.01$ ) group. There was no significant ( $p > 0.05$ ) change in AST activity in the VC + CPF group relative to that recorded in the S/oil or VC group (Figure 3).

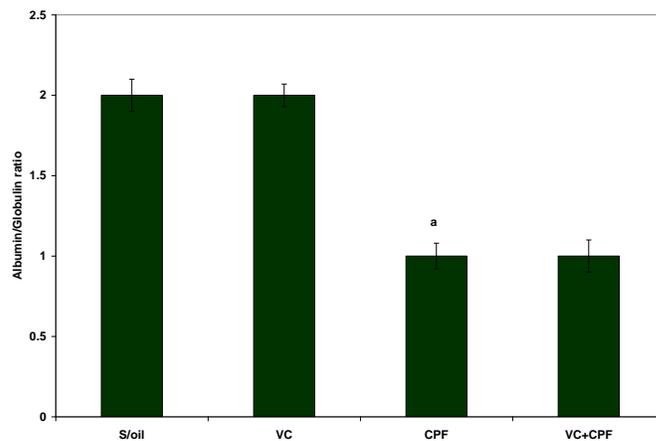


Figure 2. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on albumin/globulin in Wistar rats. <sup>a</sup> $p < 0.05$  vs S/oil group.

The ALP activity in the CPF group was significantly ( $p < 0.05$ ) higher relative to the S/oil or VC group. Although there was no significant ( $p > 0.05$ ) change in ALP activity in the CPF group compare to that recorded in the VC + CPF group, the ALP level obtained in the former group was comparatively higher (11%) than the latter. There was no significant ( $p > 0.05$ ) change in ALP activity in the VC + CPF group relative to S/oil and VC groups, respectively (Figure 3).

The GGT activity was significantly higher in the CPF group compared to that in the S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) and VC + CPF ( $p < 0.01$ ) groups, respectively. The GGT activity in the VC+CPF group was not significantly ( $p > 0.05$ ) different from that recorded in the S/oil or VC groups (Figure 3).

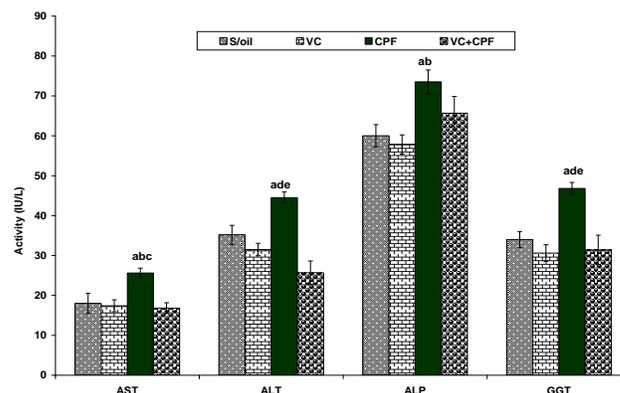


Figure 3. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on liver enzymes activities in Wistar rats. <sup>abc</sup> $p < 0.05$  vs S/oil, VC and VC + CPF groups, respectively; <sup>de</sup> $p < 0.01$  vs VC and VC + CPF groups, respectively.

*Effect of treatments on hepatic malonaldehyde concentration and activities of superoxide dismutase and catalase*

There was a significant increase in the hepatic MDA concentration in the CPF group relative to that in the S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) and VC + CPF ( $p < 0.05$ ) groups, respectively. There was no significant ( $p > 0.05$ ) change in the hepatic MDA concentration in the CPF group relative to that of the S/oil or VC group (Figure 4).

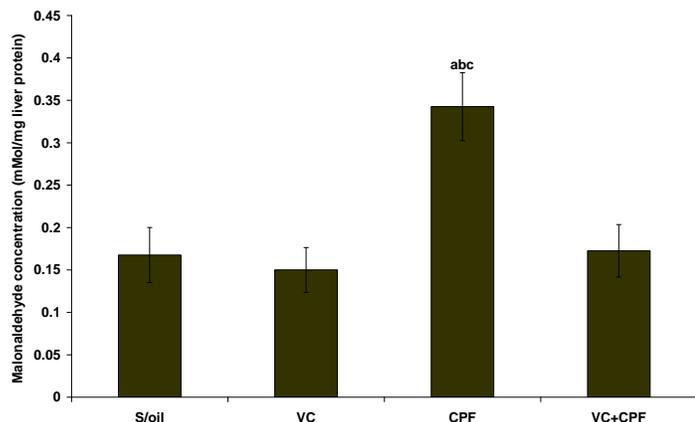


Figure 4. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on malonaldehyde concentrations in Wistar rats.

<sup>abc</sup> $p < 0.01$  vs S/oil, VC and VC + CPF groups, respectively.

There was a significant decrease in the hepatic SOD activity in the CPF group when compared to that recorded in the S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) and VC + CPF ( $p < 0.05$ ) groups, respectively. There was no significant ( $p > 0.05$ ) change in the hepatic SOD activity in the VC + CPF group relative to that recorded in the S/oil or VC group (Figure 5).

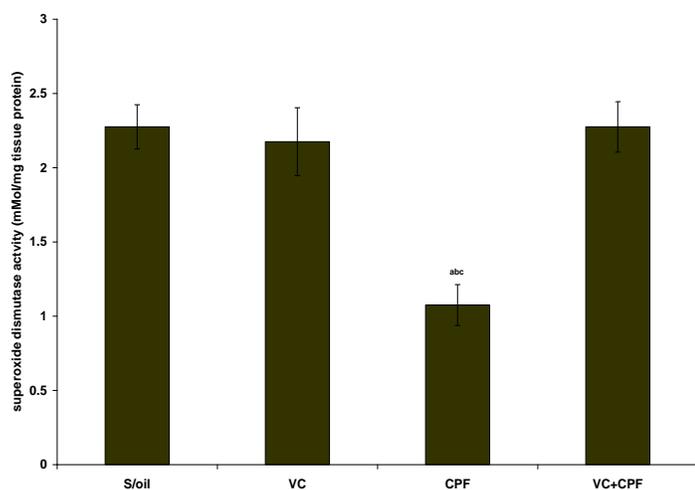


Figure 5. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on superoxide dismutase activity in Wistar rats.

<sup>abc</sup> $p < 0.01$  vs S/oil, VC and VC + CPF groups, respectively

The hepatic catalase activity in the CPF group was significantly lower relative to that recorded in the S/oil ( $p < 0.05$ ), VC ( $p < 0.01$ ) or VC+CPF ( $p < 0.05$ ) group. There was no significant ( $p > 0.05$ ) change in the VC + CPF group relative to that obtained in the S/oil ( $p > 0.05$ ) or VC ( $p > 0.05$ ) group (Figure 6).

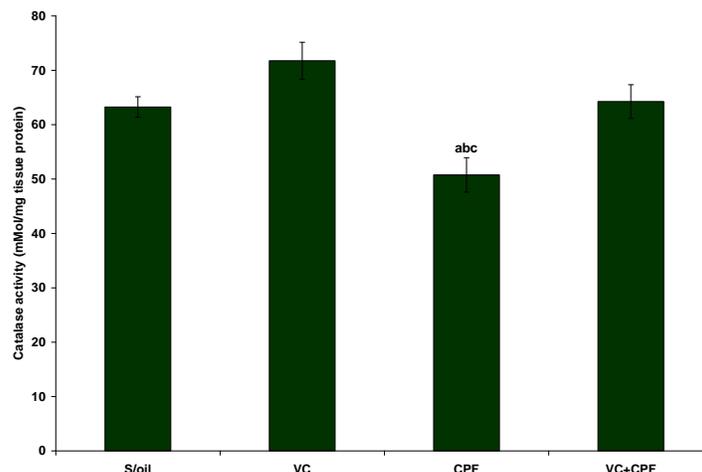


Figure 6. Effect of soya oil (S/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on liver enzymes activities in Wistar rats.

<sup>ac</sup> $p < 0.05$  vs S/oil and VC + CPF groups, respectively; <sup>p</sup> $p < 0.01$  vs VC group.

*Effect of treatments on hepatic histological changes*

The effect of treatments on hepatic histology is shown in Figure 7. There was widespread hepatocellular degeneration and sinusoidal dilatation in the CPF group. Sinusoidal dilatation was also recorded in the VC + CPF group. No apparent histological abnormality was recorded in the S/oil and VC groups.

**Discussion**

The increased hepatic MDA concentration and decreased SOD and catalase activities recorded in the CPF group is a further demonstration of the ability of CPF to cause oxidative stress. Oxidative stress, which results when there is imbalance in the level of prooxidants versus antioxidants in favour of the former, is known to cause cellular damage. The ability of CPF to cause hepatocellular oxidative changes has been demonstrated in many studies (Goel *et al.*, 2005; Aly *et al.*, 2010; Ambali *et al.*, 2010, 2011a, b). The increased hepatic MDA concentration and decreased SOD and catalase activities recorded in the CPF group in this study is a further demonstration of the ability of CPF to cause oxidative stress. Malonaldehyde is one of the products of membrane lipid peroxidation that has been shown to cause membrane damage, impaired membrane function, structural integrity, reduced membrane fluidity and inactivation of membrane-bound enzymes (Gutteridge and Halliwell, 2000; Shittu *et al.*, 2012). It has been used as a marker of oxidative stress.

Superoxide dismutase, a first line antioxidant enzyme dismutates the superoxide ( $O_2^-$ ) to hydrogen peroxide ( $H_2O_2$ ) and molecular oxygen. The  $H_2O_2$  is subsequently hydrolysed into water ( $H_2O$ ) and  $O_2$  by CAT, an enzyme whose activity was reduced in the CPF group in the present study. The decrease in CAT activity

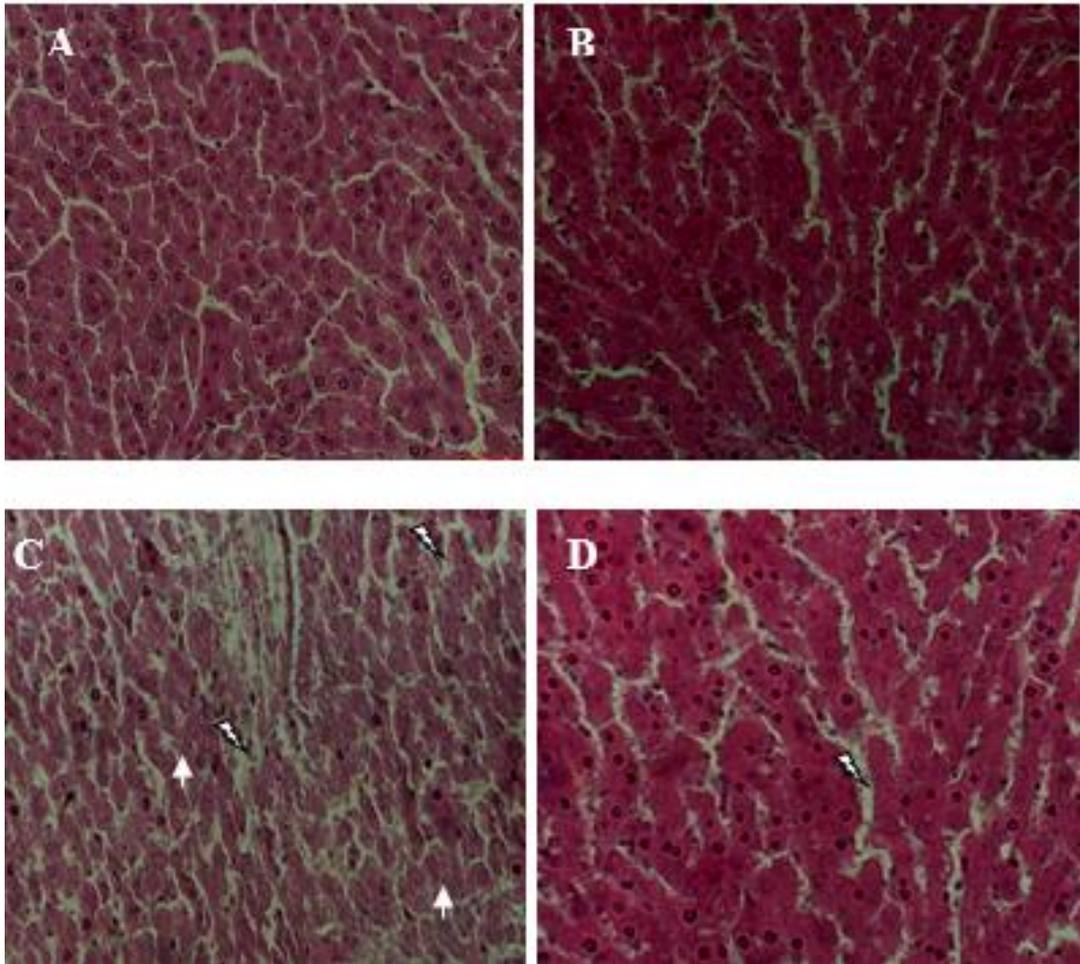


Figure 7. Histological changes in the liver of rats chronically exposed to Soya oil (A), vitamin C only (B), chlorpyrifos only (C), vitamin C+chlorpyrifos (D). Diffuse hepatocellular degeneration (arrow) and dilated sinusoids (lightning bolt). H&E x 400

may be linked to CPF-induced decrease in SOD activity, which eventually reduced the conversion of  $O_2^-$  to  $H_2O_2$ , the substrate for CAT. The increased prooxidant (MDA) concentration and the decreased activities of antioxidant enzymes (SOD and CAT) cause hepatocellular oxidative stress. Oxidative stress resulting from increased production of reactive oxygen species (ROS) in OP poisoning arises from the metabolism of the insecticide by cytochrome P450s (Lukaszewicz-Hussain, 2010). The cytochrome P450s are monooxygenases that catalyse oxidation by addition of one atom of molecular oxygen into the substrate (OP) by an electron transport pathway (Jakoby and Ziegler, 1990; White 1991; Chambers *et al.*, 2001). Moreover, the ability of CPF and some other OPs to inhibit mitochondrial adenosine triphosphate (ATP) production through the uncoupling of oxidative phosphorylation could also lead to the generation of ROS (Ishii *et al.*, 2004). Vitamin C was shown in the present study to decrease MDA concentration and increased SOD and CAT activities due to its antioxidant effect. The mechanism of free-radical scavenging ability of vitamin C may be due to its ability to abstract hydrogen from ascorbate, to become monodehydroascorbate, which soon gains another electron to become dehydroascorbate (Wilson, 2002). The ROS are reduced to water, while the oxidized forms of ascorbate are relatively stable, unreactive and does not cause cellular damage

(Krishnamoorthy *et al.*, 2007). Vitamin C has been previously shown to mitigate oxidative parameters in the liver of CPF-intoxicated mice (Aly *et al.*, 2010).

The significantly lower serum protein and albumin concentrations in the CPF group recorded in the present study have also been reported in previous studies (Peeples *et al.*, 2005; El-Banna *et al.*, 2009; Bayomy *et al.*, 2016). The significantly lower albumin/globulin ratio in the CPF group showed that the lower total proteins concentration in this group was due largely to a lower albumin concentration. The low protein and albumin concentrations may be partly due to impairment of liver synthesis consequent to hepatocellular degeneration. The ability of albumin to bind and, subsequently hydrolyse CPF-oxon has been demonstrated in previous studies (Ortigoza-Ferado *et al.*, 1984; Sultatos *et al.*, 1984). Indeed, Peeples *et al.* (2005) have proposed albumin as a biomarker of OP poisoning. Furthermore, lipid hydroperoxides, the lipid peroxidation products of polyunsaturated fatty acids (PUFA) which have been shown to cause protein degradation and denaturation (Chiba and Iwata, 2002) may have contributed to the low serum protein and albumin obtained in the CPF group in the present study.

Pre-treatment of the rats with vitamin C alleviated the deficit in total serum protein and albumin caused by CPF, partly through mitigation of hepatic degenerative changes. In addition, the

ability of vitamin C to increase the activity of paraoxonase (PON), especially PON I (Jarvik *et al.*, 2002), which aids in the detoxification of the OP compounds (Shih *et al.*, 1998) may have sparing effect on albumin since they compete for binding with the CPF and subsequent detoxification. The alleviation of oxidative changes in the liver may have reduced the production of lipid hydroxides, thereby limiting the degradation and denaturation of proteins.

The relative increase in globulin concentration in the CPF group relative to that of the control corroborates earlier observation by Subbotina and Bellonozhko (1968) following exposure to an OP compound, sevin. This may be due to induction of immunological abnormalities, since many pesticides have been shown to be toxic to the immune system through the induction of cytotoxicity (Corcoran *et al.*, 1994; Rabideau, 2001). Indeed, some of the chronic neurotoxic effects of OP insecticides may be due to their ability to induce the formation of antibodies to nervous tissue. Antibodies to myelin basic protein, neurofilament triplet protein, and glial fibrillary acidic proteins have all been exhibited after OP exposure (McConnel *et al.*, 1999). In addition, antibodies to smooth muscle, parietal cells, brush borders, thyroid follicles as well as antinuclear antibodies have been demonstrated in OP poisoning (Thrasher *et al.*, 1993). All these antibodies may be partly responsible for the apparent increase in globulin concentration observed in the present study. Pre-treatment with vitamin C further increased the CPF-induced hyperglobulinemia. This may be due to the ability of the vitamin C to stimulate the immune system by enhancing T-cell proliferation which may have ultimately assisted the B-cells to synthesize immunoglobulin (Campbell *et al.*, 1999; Naidu, 2003). Vitamin C has been shown to protect the immune system and improve natural killer cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity (Wintergerst *et al.*, 2006).

The central role of the liver in the detoxification of xenobiotics makes it highly susceptible to injury. Therefore, the extent of hepatic injury and consequent dysfunction are indirectly assessed via the measurement of the activities of serum enzymes (including ALT, AST and ALP). The significantly higher AST activity observed in rats chronically exposed to CPF compared to that of the control revealed its ability to cause liver damage. Although AST is less specific for hepatic injury, since its high level may also signal damages to the cardiac and skeletal muscles, red blood cells, kidney and brain tissue, it has been suggested that increased AST activity in the serum is a sensitive marker of hepatic lesion, even if the damage is of a subclinical nature (Kauppinen, 1984; Meyer and Harvey, 1998).

Similarly, chronic exposure to CPF only resulted in significant elevation of the activity of ALT, a more sensitive marker of liver damage (Ballantyne, 1988; Haschek *et al.*, 2010). This shows that chronic exposure to CPF caused damage to the hepatocytes. Alanine aminotransferase and AST are enzymes produced by the hepatocytes that are involved in the metabolism of amino acids and synthesis of proteins. In dying or damaged cells, these enzymes leak into the blood stream. Therefore, the observed elevation of these serum enzymes could be attributed to their release from the cytoplasm into the blood circulation after hepatic injury. The release of these enzymes could

further lead to hepatic necrosis and inflammatory reactions. The elevation in ALT activity level observed in the present study in rats chronically exposed to CPF agrees with the findings from previous studies in laboratory animals (Yoshida *et al.*, 1985; Tanvir *et al.*, 2015, 2016). Similarly, results obtained from a study involving pesticide sprayers have shown high liver enzyme activities in the serum of these subjects (Patil *et al.*, 2003). This result however contradicted the previous findings in rats (Szabo *et al.*, 1988; Barna-Lloyd *et al.*, 1990) and mice (Ambali *et al.*, 2007, 2011a). These studies reported low AST and ALT activities following repeated CPF exposure, although the implication of low activities of these enzymes is not clear. High ALT activity observed in the rats exposed to CPF only in the present study may be due to increased hepatic oxidative and degenerative changes. Indeed, CPF has been shown to cause reduction in the hepatic tissue contents of antioxidant vitamins C (Raina *et al.*, 2015) A and E (Spodniewska and Barski, 2016) due to the pro-oxidative properties of the pesticide. Furthermore, CPF, due to its lipophilicity, could accumulate in the liver to an appreciable extent causing direct cytotoxicity (Spodniewska and Barski, 2016).

The present study has shown that pre-treatment with vitamin C caused a relative decrease in the AST and ALT activities. This demonstrates a relative protective effect of antioxidant vitamin on CPF-induced liver damage. Histopathological findings in vitamin C-pre-treated group also revealed an improvement in hepatic histoarchitecture demonstrating hepatic protection. Therefore, it may be inferred that oxidative stress plays a significant role in CPF-induced liver and/or muscle damage, and that pre-treatment with antioxidant vitamin C ameliorated the damage.

The increased ALP activity in rats chronically exposed to CPF has been reported in previous studies (Goel *et al.*, 2005; Ambali *et al.*, 2010; 2011a, b; Tanvir *et al.*, 2015). Increased ALP activity is not only seen in biliary tract obstruction and hepatopathological changes, it is also associated with bone, placental, renal or intestinal damage. However, when juxtaposed with the increased GGT activity in the CPF group, it is most probable that the increased ALP activity was due to hepatobiliary injury. Gamma glutamyl transferase produced solely by the liver, is involved in transportation of amino acids and peptides into the cells. It is a good indicator of hepatobiliary disease (Stojević *et al.*, 2008) as it is not affected in bone pathology (Haschek *et al.*, 2010).

The restoration of AST, ALT, ALP and GGT activities in the group pre-treated with vitamin C demonstrated the protective effect of the antioxidant vitamin on hepatocellular injury provoked by CPF. The restoration of hepatic enzyme activities may have been due to reduction of CPF-provoked oxidative and cytotoxic changes in the hepatocytes because of the antioxidant and free radical scavenging properties of vitamin C.

The widespread hepatocellular degeneration and dilated sinusoids recorded in the CPF group, which may have been associated with oxidative injury and direct cytotoxicity evoked by the insecticide agrees with that recorded by previous studies (Tripathi and Srivastav 2010; Al-Shap *et al.*, 2015; Tanvir *et al.*, 2015; Ezzi *et al.*, 2016). Accumulation of ROS has long been shown to play a vital role in mediating apoptosis among differ-

ent cell types (Pierce *et al.*, 1991) via mitochondrial and non-mitochondrial dependent pathways (Sinha *et al.*, 2013). Pre-treatment with vitamin C was shown to mitigate hepatic histotoxicity induced by CPF apparently due to its antioxidant properties. Vitamin C may have neutralized the ROS that causes lipoperoxidation of the plasma and mitochondrial membranes, thereby stabilizing them, preventing the release of proapoptotic mediators.

### Conclusion

In conclusion, the present study has shown that chronic CPF exposure causes hepatocellular injury partly due to oxidative damage as revealed by biochemical and histological parameters. However, vitamin C alleviated the hepatocellular injury partly due to its antioxidant property. Therefore, individuals that are predisposed to exposure to CPF and other OP insecticides as a result of their occupation or the environment they live in may benefit from the hepatoprotective effect of vitamin C.

### Conflict of interest

The authors declare that they have no conflict of interest.

### References

- Al-Shap, N.A., El-Sheshtawy, S.N., Sobhy, H.M. and El-Ekhnawy, K.E. (2015) Influence of vitamin C on cytogenicity and biochemical parameters in chlorpyrifos intoxicated rats. *Egyptian Journal of Chemistry and Environmental Health*, 1(1): 331-348.
- Aly, N., EL-Gendy, K., Mahmoud, F., El-Sebae, A.K. (2010). Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. *Pesticide Biochemistry and Physiology*, 97:7-12.
- Ambali, S.F. (2009). Ameliorative effects of vitamins C and E on neurotoxicological, hematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats. PhD dissertation. Ahmadu Bello University, Zaria, Nigeria.
- Ambali, S., Akanbi, D., Igbokwe, N., Shittu, M., Kawu, M. and Ayo, J. (2007). Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. *Journal of Toxicological Sciences*, 32(2): 111-120.
- Ambali, S. Akanbi, D., Shittu, M., Giwa, A., Oladipo, O.O., Kawu, M. and Ayo, J.O. (2010). Chlorpyrifos-induced clinical, hematological and biochemical changes in Swiss albino mice- mitigating effect by co-administration of vitamins C and E. *Life Science Journal*, 7(3): 37-44.
- Ambali, S.F. Akanbi, D.O., Oladipo, O.O., Yaqub, L.S. and Kawu, M.U. (2011a). Subchronic chlorpyrifos-induced clinical, hematological and biochemical changes in Swiss albino mice: protective effect of vitamin E. *International Journal of Biology and Medical Research*, 2(2): 497-503.
- Ambali, S.F. and Ayo, J.O. (2011). Sensorimotor performance deficits induced by chronic chlorpyrifos exposure in Wistar rats: mitigative effect of vitamin C. *Toxicology and Environmental Chemistry*, 93(6): 1212-1226.
- Ambali, S.F., Abubakar, AT, Kawu, M.U., Uchendu, C. and Salami, S.O. (2011b). Biochemical alterations induced by subchronic chlorpyrifos exposure in Wistar rats: ameliorative effect of zinc. *Journal of American Science*, 7(9):73-81.
- Ballantyne, B. (1988). Xenobiotic-induced rhabdomyolysis. In: Ballantyne, B. (Ed.). *Perspectives in Basic and Applied Toxicology*. Butterworth and Co (Publishers) Ltd. pp 70-153.
- Barna-Lloyd, T., Szabo, J.R. and Davis, N.L. (1990). 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.
- Bayomy, M.M.F., Abdel Samie, H.A. and Gendia, S.E.M. (2016). Potential hepatoprotection exerted by ginseng against chlorpyrifos-induced hepatotoxicity in albino rats. *Journal of Bioscience and Applied Research*, 2(1): 51-63.
- Campbell, J.D., Cole, M., Bundittravorn, B. and Vell, A.T. (1999). Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. *Cell Immunology*, 194: 1-5.
- Casida, J.E. and Quistad, G.B. (2004). Organophosphate toxicology: Safety aspects of non-acetylcholinesterase secondary targets. *Chemical Research in Toxicology*, 17: 983-998.
- Chambers, J.E. R.L. Carr, S. Boone, H.W. (2001). Chambers, The metabolism of organophosphorus insecticides. *Handbook of Pesticide Toxicology*, Ed. II, Academic Press, USA. 919-927.
- Chiba, H. and Iwata, E. (2002). Studies on protein denaturation and enzyme inactivation caused by lipid hydroperoxides containing radicals. *Proceedings of the Japanese Academy*, 78 Serial B: 256-260.
- Corcoran, G.B., Fix, L., Jones, D.P., Moslen, M.T., Nicotera, P., Oberhammer, F.A. and Buttyan, R (1994). Apoptosis: Molecular control point in toxicity. *Toxicology and Applied Pharmacology*, 128: 169-181.
- Draper, H.H. and Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186: 421-431.
- Eaton, D.L., Daroff, R.B., Atrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., Neubert, D., Hermann, R.S. and Spencer, P.S. (2008). Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Critical Reviews in Toxicology*, S2: 1-125.
- El-Banna, S.G., Attia, A.M., Hafez, A.M. and Elkazaz, S.M. (2009). Effect of garlic consumption on blood lipid and oxidant/antioxidant parameters in rat males exposed to chlorpyrifos. *Slovakia Journal of Animal Science*, 3:111-117.
- Ellman, G.C., Courtney, K.O., Andres, V. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88-95.
- Ezzi, L., Salah, I.B., Haouas, Z., Sakly, A., Grissa, I., Chakroun, S., Kerkeni, E., Hassine, M., Mehdi, M. and Cheikh, H.B. (2016). Histopathological and genotoxic effects of chlorpyrifos in rats. *Environmental Science and Pollution Research International*, 23(5):4859-4867.

- Goel, A., Danni, V. and Dhawan, D.K. (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chemico-Biological Interactions*, 156: 131-134.
- Guide for the care and use of laboratory animals (2011). Committee for the Update of the Guide for the Care and Use of Laboratory Animals Institute for Laboratory Animal Research, National Research Council of the National Academies, National Academy Press, Washington DC, United States of America.
- Gutteridge, J. and Halliwell, B. (2000). Free radicals and antioxidants in the year 2000: a historical look to the future. *Annals of New York Academy of Science*, 899: 136–147.
- Haschek, W.M., Wallig, M.A. and Rousseau, C. (2010). Clinical Pathology. In: *Fundamental of Toxicologic Pathology*, second edition, pp. 43-65.
- Ishii, N., Senoo-Matsuda, N., Miyake, K., Yasuda, K., Ishii, T., Hartman, P.S. and Furukawa, S. (2004). Coenzyme Q 10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mechanism of Ageing and Development*, 125: 41-46.
- Jakoby, W.B. and Ziegler, D.M. (1990). The enzymes of detoxication, *Journal of Biological Chemistry*, 265: 29715–29718.
- Jarvik, G.P., Tsai, T.N., McKinstry, L.A., Wani, R., Brophy, V., Richter, R.J., Schellenberg, G.D., Heagerty, P.J., Hatsukami, T. and Furlong, C.E. (2002). Vitamin C and E intake is associated with increased paraoxonase activity. *Arteriosclerosis, Thrombosis and Vascular Biology*, 22: 1329 -1333.
- Kauppinen, K. (1984). ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentration in plasma of normal and ketotic dairy cows. *Zentralblatt Veterinärmedizin*, 31: 561-576.
- Krishnamoorthy, G., Venkataraman, P., Arunkumar, A., Vignesh, R.C., Aruldas, M.M. and Arunakarhan, J. (2007). Ameliorative effect of vitamins ( $\alpha$ -tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. *Reproductive Toxicology*, 23: 239-245.
- Lowry, H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurements with the folin phenol reagent. *Journal of Biological Chemistry*, 193: 265–275.
- Lukaszewicz-Hussain, A. (2010). Role of oxidative stress in organophosphate insecticide toxicity – Short review. *Pesticide Biochemistry and Physiology*, 98: 145–150.
- Luna, G.H. (1960). *Manual of the Histologic Staining Method of Armed Forces Institute of Pathology*. 35<sup>th</sup> Edition, McGraw-Hill Book Company, N.Y. Pp. 46.
- McConnell, R., Delgado-Tellez, E., Cuadra, R., Torres, E., Keifer, M., Almendárez, J., Miranda, J., El-Fawal, H.A., Wolff, M., Simpson, D. and Lundberg, I. (1999). Organophosphate neuropathy due to methamidophos: Biochemical and neurophysiological markers. *Archives of Toxicology*, 73: 296-300.
- Meyer, D.J. and Harvey, J.W. (1998). 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, Philadelphia, London, Toronto, Montreal, Sydney, Pp. 157-187.
- Millward-Sadler, G.H. (1987). The liver in systemic disease, in: D.G.D. Wight (Ed.), *Liver, Biliary Tract and Exocrine Pancreas*, Churchill Livingstone, Edinburgh, pp. 425–468.
- Naidu, K.A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal*, 2: 7-16.
- Ortigoza-Ferado, J., Richter, R. J., Hornung, S. K., Motulsky, A. G. and Furlong, C. E. (1984). Paraoxon hydrolysis in human serum mediated by a genetically variable arylesterase and albumin. *American Journal of Human Genetics*, 36: 295-305.
- Patil, J. A., Patil, A. J. and Govindwar, S. P. (2003). Biochemical effects of various pesticides on sprayers of grape gardens. *Indian Journal of Clinical Biochemistry*, 18: 16-22.
- Peeples, E.S., Schopfer, L.M., Duysen, E.G., Spaulding, R., Voelker, T., Thompson, C.M. and Lockridge, O. (2005). Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry. *Toxicological Sciences*, 83: 303-312.
- Pierce, G.B., Parchment, R.E. and Lewellyn, A.L. (1991). Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation*, 46:181–186.
- Rabideau, C.L. (2001). Pesticide mixtures induce immunotoxicity: Potentiation of apoptosis and oxidative stress. MSc Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 170pp.
- Raina, R., Baba, N.A., Verma, P.K., Sultana, M. and Singh, M. (2015). Hepatotoxicity induced by subchronic exposure of fluoride and chlorpyrifos in wistar rats: mitigating effect of ascorbic acid. *Biological Trace Element Research*, 166(2): 157-162.
- Shih, D.M., Gu, L., Xia, Y.R., Navab, M., Li, W.F., Hama, S., Castellani, L.W., Furlong, C.E., Costa, L.G., Fogelman, A.M. and Lusa, A.J. (1998). Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature*, 394 (6690): 284-287.
- Shittu, M., Ayo, J.O., Ambali, S.F., Fatihu, M.Y., Onyeansi, B.I. and Kawu, M.U. (2012): Chronic chlorpyrifos-induced oxidative changes in the testes and pituitary glands of Wistar rats: Ameliorative effect of vitamin C. *Pesticide Biochemistry and Physiology*, 102: 79-85.
- Sinha, K., Das, J., Pal, P.B. and Sil, P.C. (2013). Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Archive of Toxicology*, 87:1157–1180.
- Slotkin, T.A., Brown, K.K., and Seidler, F.J. (2005). Developmental exposure of rats to chlorpyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. *Environmental Health Perspectives*, 113:1291–1294.
- Spodniewska, A. and Barski, D. (2016). Concentration of hepatic vitamins A and E in rats exposed to chlorpyrifos and/or enrofloxacin. *Polish Journal of Veterinary Science*, 19(2): 371–378.
- Stojević, Z., Filipović, N, Božić, P., Tuček, Z. and David, J. (2008). The metabolic profile of Simmental service bulls. *Veterinarski Arhive*, 78(2): 123-129.

- Subbotina, S.G. and Belonozhko, G.A. (1968). The effect of Sevin on the thermoresistance and fractionary protein content of blood serum. *Gig. Primen Toxicol Pesticide Klin Otrav*, 442-444.
- Sultatos, L.G., Shao, M. and Murphy, S.D. (1984). The role of hepatic biotransformation in mediating the acute toxicity of the phosphorothionate insecticide chlorpyrifos. *Toxicology and Applied Pharmacology*, 73: 60-68.
- Szabo, J.R., Young, J.T. and Granjean, M. (1988). 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.
- Thrasher, J.D., Madison, R. and Broughton, A. (1993). Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. *Archives of Environment Health*, 48: 89-93.
- Tripathi, S. and Srivastav A.K. (2010). Liver profiles of rats after long-term ingestion of different doses of chlorpyrifos. *Pesticide Biochemistry and Physiology*, 97: 60-65.
- Tanvir, E., Afroz, R., Chowdhury, M., Khalil, I., Hossain, S., Rahman, A., Rashid, H. and Gan, S.H. (2015). Honey has a protective effect against chlorpyrifos-induced toxicity on lipid peroxidation, diagnostic markers and hepatic histoarchitecture. *European Journal of Integrative Medicine*, 7: 525-533.
- Tanvir, E.M., Afroz, R., Chowdhury, M., Gan, S.H., Karim, N., Islam, M.N. and Khalil, M.I. (2016). A model of chlorpyrifos distribution and its biochemical effects on the liver and kidneys of rats. *Human and Experimental Toxicology*, 35(9): 991-1004.
- White, R.E. (1991). The involvement of free radicals in the mechanisms of monooxygenases. *Pharmacology and Therapeutics*, 49: 21-42.
- Wilson, J.X. (2002). The physiological role of dehydroascorbic acid. *FEBS Letters*, 527: 5-9.
- Wintergerst, E.S., Maggini, S. and Hornig, D.H. (2006). Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Annals of Nutrition and Metabolism*, 50(2): 85-94.
- Yavuz, T., Delibao, N., Yıldırım, B., Altuntao, I., Candır, O., Cora, A., Karahan, N., Abruim, E. and Kutsal, A. (2004). Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. *Archives of Toxicology*, 78: 655-659.
- Yoshida, A., Kosaka, T., Miyaoka, T., Maita, K., Goto, S. and Shirasu, Y. (1985). Chlorpyrifos-methyl: 28-day oral toxicity study in mice. Unpublished Report No. GHF-R 80 from the Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by DowElanco, Indianapolis, USA.