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Full Length Research Article

Protective Effect of Methionine and Vitamin C on Hepatic Glucose Metabolism in Chlropyrifos-Exposed Male Wistar Rats

***S.T. Shittu, E.T. Akinlade, G.O. Isehunwa, S.A. Shittu, R.O. Oyelade and A.R.A. Alada**
Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[*Corresponding author; Email address: toyinsts@yahoo.com]

ABSTRACT
Chlorpyrifos exposure is associated with impairment in hepatic glucose metabolism. This study investigates the effect of methionine and/or vitamin C on hepatic glucose metabolism in chlorpyrifos-exposed male Wistar rats. Twenty-five male Wistar rats (120-180g) were grouped into 5 as I-V. Group I (control) received sunflower oil (1ml/kg) while II-V were orally administered chlorpyrifos (6.8mg/kg) followed by methionine (100 mg/kg), vitamin C (200 mg/kg) and a combination of both to III, IV and V, respectively for 28 days. Plasma was obtained for acetylcholinesterase activity and liver function test (AST, ALT, and ALP) while the liver was obtained for determination of glycogen content, hexokinase activity, lactate dehydrogenase activity (LDH), and markers of oxidative stress (MDA, SOD, and catalase). Hepatic MDA level increased in II and decreased in III-V compared with control. Increased LDH activity was observed in II relative to control. Glycogen content and hexokinase activities were decreased in II relative to control while they were increased in V compared with II. Acetylcholinesterase activity decreased in II compared with control and increased in V compared with II. In conclusion, methionine and vitamin C prevented chlorpyrifos-induced hepatic glycogen depletion and hexokinase inhibition through a mechanism that involves prevention of lipid peroxidation and promotion of acetylcholinesterase activity.
Keywords: Chlorpyrifos, Methionine, Vitamin C, Glycogen, Hexokinase activity, Liver

INTRODUCTION

Chlorpyrifos is a broad-spectrum organophosphorus (OP) insecticide applied worldwide for control of agricultural and structural pests and mosquitos. Human exposure occurs through residues in food, skin contact, and air dispersion (DuTeaux and Koshlukova, 2023). Approximately 2.3 million kilograms of chlorpyrifos were used annually between 2014 and 2018 for agricultural purposes in the United States (USEPA, 2020). While data on the volume of OP usage in Nigeria may be lacking, recent studies have documented their residues in foods (Ibrahim *et al*., 2018) and microenvironments such as offices, cars/buses, homes, and medical centers (Gbadamosi *et al*., 2023). Organophosphate compounds are capable of inducing both apoptosis and necrosis depending on the exposure levels. They inhibit mitochondrial enzymes, respiration, and ATP generation (Cocker *et al*., 2002; Karalliedde *et al*., 2003) and significantly cause DNA damage in the liver, brain, kidney, and spleen of rats (Ojiha *et al*., 2011). Although, CPF and other OPs act by irreversible inhibition of acetylcholinesterase activity (Jintana *et al.,* 2009), oxidative stress has also been implicated as an important mechanism of OP poisoning (Buyukokuroglu *et al*., 2008; Ndonwi *et al*., 2019). It is therefore not surprising that several antioxidants have been documented to mitigate OP toxicity *in vivo* (Yurumez *et al.,* 2007; Buyukokuroglu *et al.,* 2008; Cemek *et. al.,* 2009).

Altered glucose homeostasis has been documented in experimental animals following acute or chronic exposure to CPF ('Acker' and Nogueira, 2012; Hamza *et al*., 2014) and other OPs (Abdollahi *et al*., 2004; Rezg *et al*., 2006). For instance, in nulliparous female Wistar rats, gestational CPF exposure caused insulin resistance in both the mother and offspring exposed *in utero* with the effect persisting in the offspring up to adulthood (Ndonwi *et al*., 2020). The alterations in glucose metabolism include increased hexokinase activity, depletion of glycogen content, and increase in phosphoenolpyruvate carboxykinase (PEPCK) activity in rat liver upon exposure to OP (Abdollahi *et al*., 2004; Panahi *et al*., 2006). It is postulated that Chlorpyrifos-induced derailment in glucose metabolism may involve the stimulation of hepatic gluconeogenesis and glycogenolysis as well as activation of hypothalamus-pituitary-adrenal axis with resultant increased secretion of glucocorticoids leading to increased blood glucose in circulation (Rahimi and Abdollahi, 2007; Acker *et al*, 2012; Joshi and Rajini, 2012; Lukaszewicz-Hussain, 2013).

The greatest toxicological burdens of OPs are received by the liver where they like other xenobiotic substances, are metabolized (Vale, 1998; Cakici and Akat, 2013; Ezzi *et al*., 2016; Cobilinschi *et al*., 2020). Such toxicity is manifested by decreased methionine which is required in the synthesis of the precursor for antioxidant glutathione in the liver of mice exposed to CPF and its metabolites (Deng *et al*., 2016). It is therefore safe to postulate that methionine depletion contributes to the ability of chlorpyrifos to induce tissue oxidative stress particularly when methionine supplementation alone or in combination with vitamin C was found to be beneficial in conditions characterized by increased oxidative stress. For instance, L-methionine was demonstrated to prevent oxidative stress and mitochondrial dysfunction in an *in vitro* model of Parkinson's disease (Catanesi *et al*., 2021). Also, methionine and vitamin C supplementation was documented to improve performance, liver enzymes, thyroid hormones, plasma antioxidant activity, and intestinal morphology of broilers exposed to oxidative stress (Erfani *et al*., 2021). It is however not known if methionine supplementation alone or in combination with vitamin C during CPF exposure may have any effect on hepatic glucose metabolism. The current study was

therefore designed to investigate the effect of methionine and/or vitamin C on glucose metabolism in the liver of CPF-exposed male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty- five adult male Wistar rats (120-180 g) obtained from the Central Animals house of the College of Medicine, University of Ibadan, Ibadan Nigeria were used for this study. They were kept in the Postgraduate Animal house of the Department of Physiology, University of Ibadan, Ibadan, Nigeria under standard laboratory conditions and housed in well-ventilated plastic cages at room temperature (28-30°C) and under controlled light cycles (12-h light/12-h dark). The animals were allowed free access to standard rat chow (Vitalfeeds®, UAC Food Plc) and water. Prior to the experiment, they were acclimatized for two weeks. All animal handling and experimentation were following the guidelines of the University of Ibadan Animal Ethical Committee which was based on the NIH guideline for the use of animals for experiment.

Experimental Design

The animals were randomly divided into five groups (n=5) and orally treated for 28 days as follows:

I. Control: administered sunflower (1ml/kg b.wt) which was the vehicle for CPF.
II. Chlorpyrifos (CPF): received 6.8 mg/kg b.w of Chlorpyrifos (Suleiman *et al*., 2010)
III. Chlorpyrifos and methionine (CPF+Meth): received 6.8mg/kg b.w of Chlorpyrifos followed by 100 mg/kg Methionine (Patra *et al* ., 2001)
IV. Chlorpyrifos and vitamin C (CPF+Vit C): received 6.8 mg/kg b.w of chlorpyrifos followed by 200 mg/kg Vitamin C
V. Chlorpyrifos, Methionine, and Vitamin C (CPF+Meth+Vit C): received 6.8 mg/kg b.w of Chlorpyrifos followed by methionine (100 mg/kg) and vitamin C (200 mg/kg)

Fasting Blood Glucose Determination

At the end of the 28 day exposure, fasting blood glucose level was determined following an overnight fast from blood obtained from the tail using a glucometer (ACCUCHEK, Roche Diagnostic®, Germany).

Sample Collection and Biochemical Analysis

Blood, collected via ocular puncture into heparinized bottles and liver was obtained for biochemical analysis. The liver was divided into 2 portions; the first portion (1 g) was used immediately for glycogen determination while the second portion was homogenized in phosphate-buffered saline (PBS, pH 7.4), centrifuged at 4 ^{0}C and the supernatant was stored at -20 ^{0}C until used for biochemical analysis.

Determination of Acetylcholinesterase Activity*:* Acetylcholinesterase activity was determined in the blood by the method of Ellman *et al*., (1961) based on the ability of the enzyme in the sample to liberate thiocholine from acetylthiocholine (ATI) which is then reacted with dithiobisnitrobenzoic acid (DTNB) to yield a yellow chromogen whose intensity can be determined by spectrophotometer at a wavelength of 412nm. Briefly, 100 µl of blood is added to a test tube containing 1.4 ml of 0.1 M phosphate buffer and mixed. It was poured into a cuvette followed by the addition of 50 µl of 0.01 M DTNB prepared in 0.1 M phosphate buffer. The initial absorbance at 412 nm was recorded then 50 µl of 0.028 M ATI was added and the absorbance after 2 minutes was recorded. The difference in the initial and final absorbance (ΔA/min) was determined per unit of time and the acetylcholinesterase activity was calculated as follows:

$$\text{acetylcholinesterase activity } (\mu mol/min/ml) = \frac{\Delta A/min}{1.36\ X\ 10^4}$$

where $1.36\ X\ 10^4$ is the extinction coefficient of the yellow chromogen formed.

Liver Function Tests: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were determined in the plasma using commercially available kits (Fortress Diagnostics®, United Kingdom) according to the manufacturer's descriptions.

Determination of Glycogen Content: Liver glycogen contents were determined by the Anthrone method as previously reported (Shittu *et al*., 2018). Briefly, 1 g liver was digested in 10 ml of 30 % KOH over heat until the tissue dissolved. It was cooled and 4 ml was obtained for washing by adding 5 ml of 95 % ethanol, centrifuged at 4000 rev/min for 5 minutes and drained. The precipitate was reconstituted with 0.5 ml of distilled water and rewashed to obtain a white precipitate which was reconstituted with 2 ml distilled water. In a glass test tube, 0.5 ml of the reconstituted glycogen was pipetted followed by stepwise addition of 0.5 ml concentrated HCl, 0.5 ml 88 % formic acid, and 4 ml anthrone reagent (added slowly to minimize frothing) then mixed thoroughly. It was incubated at 100 ^{o}C for 10 minutes to obtain a blue-coloured solution. Absorbance of the solution was recorded at 630 nm against a reagent blank. Several dilutions of 0.2 mg/mL of glycogen standard were similarly treated to obtain a standard curve from which the glycogen concentrations (mg/ml) of the samples were determined. The glycogen content /100 g tissue was calculated as follows:

$$= glycogen\ concentration\ X\ \frac{10}{4}\ X\ \frac{2}{0.5}\ X\ \frac{100}{liver\ weight}$$

Determination of Lactate Dehydrogenase Activity (LDH)*:* The supernatant was used to determine hepatic LDH activity using commercially available kits (Fortress Diagnostics®, United Kingdom) according to the manufacturer's description.

Determination of Hepatic Hexokinase Activity*:* Hexokinase activity was determined in the PBS homogenized liver samples using the Branstrup *et al*

(1957) method as previously reported (Shittu *et al*., 2018). Briefly, 2 mL of a buffer containing 0.0025 M glucose, 0.0025 M $MgCl_2$, 0.01 M K_2HP0_4, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane (pH 8) was pipetted into a test tube followed by 0.1 ml of 0.18 M ATP solution and 0.9 ml of distilled water. The mixture was preheated in water for 5 minutes at 38 ^{0}C, then 1 mL of liver homogenate's supernatant was added and the initial glucose concentration was determined immediately. The mixture was then incubated at 38 ^{0}C for 30 minutes and the final glucose concentration was determined. The difference in the level of glucose was calculated and hexokinase activity was expressed as activity/mg.pr. All assays were carried out in duplicates. In this assay, glucose was assayed using a commercially available Glucose GOD-PAP kit (Fortress Diagnostic®, United Kingdom).

***Determination of Oxidative Stress Markers**:*

Malondialdehyde (MDA) was determined according to the method described by Hagège *et al*. (1995). Briefly, 0.5ml of the supernatant was pipetted into 1ml of TCA-TBA-HCL solution (15 g of trichloroacetic acid and 0.375 g of thiobarbituric acid dissolved in 100 ml of 0.25 N hydrochloric acid) and incubated for 15 minutes in boiling water (100℃). After cooling, the mixture was centrifuged at 1000 g for 10 minutes and supernatant was read at 535 nm against the blank. The malondialdehyde concentration of the sample can be calculated using the extinction coefficient of $1.56\times10^5 m^{-1}cm^{-1}$.

MDA concentration = $\frac{O.D \times V \times 1000}{a \times v \times l \times Y}$

Where O .D = absorbance of sample test at 535 nm; V= total volume of the reaction=1.5 ml; a= molar estimation coefficient of product= $1.56\times10^5 m^{-1}cm^{-1}$; l= light path=1 cm; v= volume of sample used = 0.5 ml.

Superoxide Dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972). Briefly, 0.2 ml of the supernatant (test) or distilled water (reference) was added to 2.5 ml of 0.05 M Carbonate buffer (pH 10.2) was then added and incubated at room temperature. 0.3 ml of 0.3 mM adrenaline solution was added to the test and each of the reference solutions was mixed by inversion and read using the spectrophotometer at 420nm within 3 minutes.

% Inhibition= $\frac{Abs(sample)-ABS(reference)}{ABS(reference)} \times 100$

1 unit of SOD activity was taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome. Thus, SOD activity (Unit/ml) = $\frac{\%\ \text{Inhibition}}{50\%}$

Catalase activity was determined using a method based on the reaction of undecomposed hydrogen peroxide with ammonium molybdate to produce a yellowish color (Góth, 1991). Briefly, 0.2 ml of supernatant was incubated with 1 ml of substrate solution (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37 °C for three minutes. The reaction was stopped with 1 ml of 4% ammonium molybdate in 12.5mM H_2SO_4 and read at 305nm wavelength.

Statistical Analysis

Data obtained were analyzed using a one-way analysis of variance and presented as mean ± standard error of the mean. Statistical significance was determined at $p<0.05$ using the Tukey Posthoc test (GraphPad Prism Statistical Package 8.0.2, USA).

RESULTS

Effect of Methionine and/or Vitamin C on Fasting Blood Glucose in Chlorpyrifos-Exposed Rats

Fasting blood glucose level showed no significant change in the chlorpyrifos-exposed group (82.14 ± 2.89 mg/dl) and all treated groups relative to control (90.57 ± 4.34 mg/dl) (Figure 1).

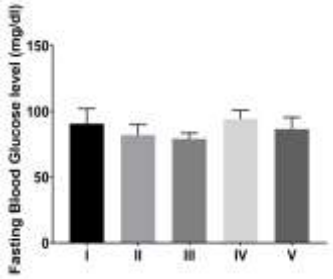


Figure 1: Effects of methionine and/or vitamin C on fasting blood glucose level in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF+Meth, IV=CPF+Vit C, V=CPF+Meth+Vit C

Effect of Methionine and/or Vitamin C on Plasma Acetylcholinesterase (AchE) Activity

As shown in Figure 2, acetylcholinesterase activity was significantly reduced in the CPF group (0.121 ± 0.005 µmoles/min/ml) compared with the control (0.147 ± 0.006 µmoles/min/ml). Treatment with methionine (0.095 ± 0.007 µmoles/min/ml) or vitamin C (0.097 ± 0.002 µmoles/min/ml) in groups III and IV further decreased the AchE activity when compared with group II. However, the AchE activity of group V (0.132 ± 0.003 µmoles/min/ml) rats treated with a combination of methionine and vitamin C was significantly increased when compared with the CPF-only exposed group II rats.

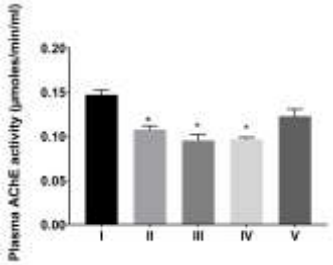


Figure 2: Effect of methionine and/or vitamin C on plasma AChE in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF+ Meth, IV= CPF + Vit C, V = CPF+ Meth+ Vit C *p <0.05 Vs control

Effect of Methionine and/or Vitamin C on Hepatic Glycogen Content in Chlorpyrifos-Exposed Rats

Hepatic glycogen content was significantly depleted in group II (36.50 ± 5.24 mg/100g tissue) compared to control (77.99 ± 3.79 mg/100g tissue). Vitamin C administration in group IV (67.0 ± 7.20 mg/100g tissue) and in combination with methionine in group V (51.17 ± 5. 68 mg/100g tissue) caused significantly increased hepatic glycogen content when compared with group II (36.50 ± 5.24 mg/100g tissue) while methionine alone had no effect on hepatic glycogen content in group III (39.74 ± 2.59 mg/100g (Figure 3).

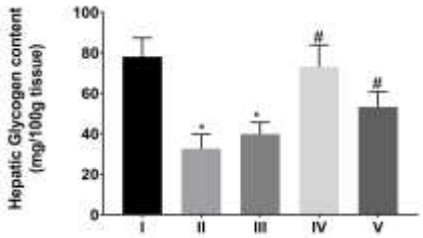


Figure 3: Effect of methionine and/or vitamin C on hepatic glycogen content in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF+ Meth, IV = CPF+ Vit C, V= CPF+ Meth + Vit C. *p<0.05 Vs Control, #p<0.05 Vs CPF

Effect of Methionine and/or Vitamin C on Hepatic Hexokinase Activity in Chlorpyrifos-Exposed Rats

Hepatic hexokinase activity was significantly decreased in the chlorpyrifos-exposed group (0.01 ± 0.006 activity/mg.pr) compared to the control (0.05 ± 0.01 activity/mg.pr). The observed decrease was not prevented by treatment with methionine or vitamin C only however, combined treatment with methionine and vitamin C in group V significantly increased the activity when compared with group II (Figure 4)

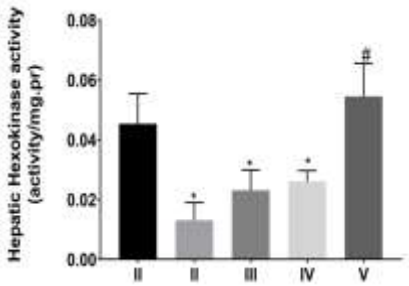


Figure 4: Effect of methionine and/or vitamin C on hepatic hexokinase activity in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF + Meth, IV=CPF +Vit C, V=CPF+ Meth + Vit C. *p<0.05 Vs Control, #p<0.05 Vs CPF

Effect of Methionine and/or Vitamin C on Hepatic Lactate Dehydrogenase Activity in Chlorpyrifos-Exposed Rats

As shown in Figure 5, hepatic lactate dehydrogenase activity was significantly increased in group II (283.5 ± 14.96 activity/mg.pr) compared with the control (187.6 ± 17.97 activity/mg.pr). The LDH activities in the methionine and/or vitamin C-treated groups were not significantly different from the control group.

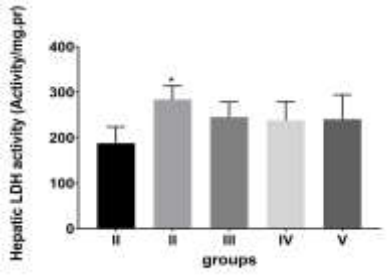


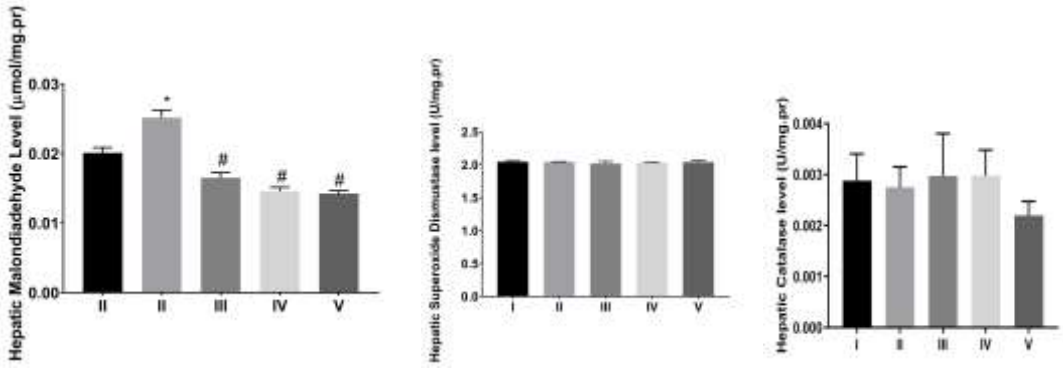
Figure 5: Effect of methionine and/or vitamin C on hepatic LDH activity in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF+ Meth, IV=CPF+ Vit C, V=CPF+ Meth+ Vit C. *p<0.05 Vs Control.

Effect of Methionine and/or Vitamin C on Hepatic Oxidative Stress Biomarkers in Chlorpyrifos-Exposed Rats

The effect of the treatments on oxidative stress biomarkers, MDA, SOD, and Catalase are shown in Figure 6. Exposure to Chlorpyrifos significantly caused elevation in the liver MDA level of group II (0.025 ± 0.001µmol/l) compared to the control (0.020 ± 0.0007µmol/l). Methionine, vitamin C and the combination of methionine and vitamin C treatments significantly decreased MDA levels in groups III (0.017 ± 0.0008µmol/l), IV (0.015 ± 0.0006µmol/l) and V (0.014 ± 0.0004µmol/l), respectively when compared with group II (0.025 ± 0.001µmol/l) as well as the control group (0.020 ± 0.0007µmol/l). Superoxide dismutase and catalase activities were however not statistically different across all groups.

Effect of Methionine and/or Vitamin C on Liver Function Test in Chlorpyrifos-Exposed Rats

As shown in Table 1, there was no significant effect of the exposure to chlorpyrifos in liver aspartate aminotransferase activity in group II (81.85 ± 1.31 U/L) relative to control (83.81 ± 1.31 U/L). However, treatment with methionine alone or in combination with vitamin C in groups III (89.81 ± 2.63 U/L) and V (92.6 ± 2.24 U/L) caused significantly increased AST level compared with group II compared to group II (81.85 ± 1.31 ng/ml). Alanine transaminase and alkaline phosphatase activity were not different across all groups.



A **B** **C**

Figure 6: Effect of methionine and/or vitamin C on (A) Hepatic MDA level, (B) SOD and (C) catalase activity in chlorpyrifos-exposed rats. I=Control, II=CPF, III=CPF+ Meth, IV=CPF+ Vit C, V=CPF+ Meth+ Vit C. *p<0.05 Vs Control, #p<0.05 Vs CPF

Table 1. Effect of methionine and/or vitamin C on liver function test in chlorpyrifos-exposed rats

PARAMETERS	I	II	III	IV	V
AST (U/L)	83.81 ± 0.60	81.85 ± 1.31	89.81 ± 2.63#	88.31 ± 1.35	92.6 ± 2.24*#
ALT(U/L)	75.05 ± 1.17	76.88 ± 1.98	77.52 ± 1.96	75.24 ± 1.04	81 ± 4.46
ALP(U/L)	17.49 ± 0.29	17.45 ± 0.18	17.37 ± 0.20	15.57 ± 0.45	17.02 ± 0.17

I=Control, II=CPF, III=CPF+ Meth, IV=CPF+ Vit C, V=CPF+ Meth+ Vit C. *p<0.05 Vs Control, #p<0.05 Vs CPF

DISCUSSION

This study investigated the effects of Chlorpyrifos on hepatic glucose metabolism and the roles of methionine, vitamin C or the combination of both may play in the observed effects. Chlorpyrifos and other organophosphates are potent inhibitors of acetylcholinesterase (Jintana *et al.,* 2009*),* accordingly, acetylcholinesterase activity was significantly inhibited in the chlorpyrifos-exposed rats of this study. While acetylcholinesterase inhibition was not prevented by the administration of methionine or vitamin C alone in the chlorpyrifos rats, such inhibition was absent in animals treated with their combination. The lack of effect of these antioxidants on acetylcholinesterase activity when administered singly in the current study is not surprising given that earlier studies have documented that individual antioxidants may be less effective in conditions characterized by severe oxidative stress when used alone (Osada *et al*., 1986; Flora *et al.,* 2003; Ugbaja *et al*., 2017). In fact, the further inhibition of acetylcholinesterase activity by individual administration of vitamin C or methionine to the chlorpyrifos-exposed rats was similar to the observation of Ugbaja *et al*. (2017) who reported that vitamin C or Vitamin E caused further decreased acetylcholinesterase activity in aluminum iodide exposed rats. Vitamin C when used singly has been shown to be prooxidative (Halliwell, 1996) which is prevented when used in combination with another antioxidant such as methionine or vitamin E (Ming *et al*., 2006). Also, chlorpyrifos is associated with methionine depletion which tends to reduce endogenous antioxidant production (Deng *et al*., 2016). It is therefore conceivable that methionine supplementation along with vitamin C will stimulate endogenous antioxidant production which will inhibit the prooxidant effect of vitamin C thereby contributing to the observed synergistic beneficial effect on acetylcholinesterase activity in the chlorpyrifos-exposed rats.

In the present study, exposure to chlorpyrifos had no effect on the fasting blood glucose level. Previous studies had also shown no alteration in glucose level (Ambali *et al*., 2011; Acker and Nogueira, 2012) or hypoglycemia (Tang *et al*., 2002; Wang *et al*., 2009; Akhtar *et al*., 2009; Mohamed, 2014) following exposure to chlorpyrifos. Moreover, there was no significant change in the treated groups when compared with control and CPF-exposed groups which is in agreement with an earlier finding that showed glucose level remained unaltered following treatment with the effect of Vitamin C in chlorpyrifos-exposed broiler chickens (Kammon *et al*., 2011).

Hepatic glycogen depletion had been documented in chlorpyrifos-exposed rats (Goel *et al*., 2006) and in like manner, hepatic glycogen content of the CPF rats of this study was significantly reduced. Other organophosphates such as carbamate and organochlorine agents have also been documented to cause hepatic glycogen depletion (Rambabu and Rao, 1994; Goel *et al*., 2006). Compromised hepatic glycogenesis with increased glycogen phosphorylase activity was shown to mediate this depletion (Abdollahi *et al*., 2004; Goel *et al*., 2006). Glycogen phosphorylase is the enzyme that cleaves the glycogen molecules at their

glycosidic links to produce glucose-1-phosphate in an ATP-independent reaction (Liu *et al.,* 2010), therefore increase in its activity leads to glycogen depletion. A recent study showed that CPF exposure for two months in rats caused increased cortisol, triiodothyronine, thyroxine, and thyroid-stimulating hormone (Cobilinschi *et al*., 2021). These hormones, through their permissive effect on catecholamines, may induce glycogenolysis. For instance, the thyroid hormone increases the expression of beta-adrenoceptors (Ojamaa *et al*., 2000) which mediate the catecholamine effect on hepatic tissue (Barth *et al*., 2007). Epinephrine acting through the beta-adrenoceptor results in increased cAMP which activates the cAMP-dependent protein kinase which phosphorylates several intercellular enzymes involved in glycogenolysis and gluconeogenesis (Abdollahi *et al*., 2004). Treatment with Vitamin C alone and in combination with methionine significantly prevented chlorpyrifos-induced hepatic glycogen depletion. Such prevention of hepatic glycogen depletion by vitamin C following chlorpyrifos exposure has been documented in freshwater fish (Narra *et al*., 2015).

The observed inhibition of hexokinase activity in the chlorpyrifos-exposed rats is indicative of a repressed glycolytic process which is essential in the conversion of glucose to glucose-6-phosphate. Such significant inhibition of hexokinase activity following chlorpyrifos exposure had earlier been reported in rats (Goel *et al*., 2006). Similarly, the observed increased hexokinase activity in the methionine and vitamin C-treated group is consistent with the earlier study (Goel *et al*., 2006) that used zinc as an antioxidant. Although methionine or vitamin C had no effect on hexokinase activity individually in this study, the effect produced by their combination further underscores the earlier reported synergistic effect of the two antioxidants on chlorpyrifos-induced toxicity (Cemek *et al*., 2010).

Lactate Dehydrogenase enzyme (LDH) activity can be used as an indicator for cellular damage and cytotoxicity of toxicants. Elevation in the activity indicates cell lysis and death and the switching of the cell from aerobic glycolysis to anaerobic respiration (Kendig and Tarloff, 2007). In this present study, LDH activity was significantly increased in CPF-exposed groups and this could be a result of the increased production of superoxide anions and hydroxyl radicals thereby causing oxidative damage to the cell membrane and increased permeability of the membrane. This result is in accordance with a study by Tilak *et al*. (2005) which reported that chlorpyrifos mediated an increase in LDH after exposure to its sublethal concentrations in freshwater fishes. However, treatment with methionine and/or Vitamin C showed a slight reduction in the activity due to the scavenging abilities of the antioxidants to remove the overproduced superoxide anions and hydroxyl radicals.

Malondialdehyde level is a marker of lipid peroxidation which leads to oxidative damage of polyunsaturated fatty acids and cellular membranes thereby causing reduced membrane fluidity and inactivity of membrane-bound enzymes (Goel *et al*., 2005; Demir *et al*., 2011). Chlorpyrifos had been documented to induce lipid peroxidation and elevate MDA production (Gultekin *et al*., 2000) accordingly; MDA was significantly elevated in the CPF-exposed rats. The increased lipid peroxidation caused by Chlorpyrifos may be a result of its direct interaction with the cellular plasma membrane due to its lipophilic nature (Hazarika *et al*., 2003). The increased MDA level was however absent in the group treated with methionine, vitamin C, and their combination. The ameliorative effect observed in the treated groups is consistent with their known properties (El-Gendy *et al*., 2010, Rajak *et al*., 2022). Such amelioration of CPF-induced elevation of MDA level by antioxidants had been documented earlier by several workers (Ogutcu *et al*., 2006, Ambali *et al*., 2011, Ambali and Aliyu, 2012).

Various defensive mechanisms are involved in combating the toxic effects of reactive oxygen species caused by toxicants. Superoxide dismutase and Catalase are the most important ones which involve the catalytic conversion of superoxide radicals to hydrogen peroxide and molecular oxygen while catalase converts hydrogen peroxide into water and molecular oxygen respectively and hence catalyzes its removal (Schneider and de Oliveira, 2004). The activities of SOD and CAT were not significantly affected by exposure to chlorpyrifos in all treated groups in this present study. The observed lack of effect on catalase activity was also observed by Oruc *et al*. (2010) in diazinon, another OP pesticide.

The activity of liver AST, ALT, and ALP enzymes in this present study was not significantly altered in the CPF-exposed groups and treated groups. This is consistent with a study by Kammon *et al*. (2011) which showed no significant changes in serum levels of these liver function markers in broiler chickens administered chlorpyrifos.

CONCLUSION

The study showed that exposure of rats to chlorpyrifos caused a decrease in the activities of AChE, hepatic hexokinase activity, depletion of hepatic glycogen, and elevation of LDH and MDA levels. Administration of vitamin C and methionine prevented the effects of chlorpyrifos on hepatic hexokinase and reduced levels of MDA. The study therefore concluded that administration of vitamin C and methionine prevented chlorpyrifos-induced oxidative stress and alteration in hepatic glucose metabolism. Future studies on the molecular interactions between vitamin C, methionine, and chlorpyrifos on hepatic glucose metabolism will throw more light on the findings of the current study.

REFERENCES

Abdollahi, M., Donyavi, M., Pournourmohammadi, S. and Saadat, M. (2004). Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats

following subchronic exposure to malathion. *Comparative Biochemistry and Physiology Part C*, **137**:343-7.

Acker, C.I. and Nogueira, C.W. (2012). Chloropyrifos acute exposure induces hyperglycemia and hyperlipemia in rats. *Chemosphere,* **89**:602–608.

Akhtar, N., Srivastava, M.K. and Raizada, R.B. (2009). Assessment of chlorpyrifos toxicity on certain organs in rat, Rattus norvegicus. *Journal of Environmental Biology,* **30**(6):1047–1053

Ambali, S.F. and Aliyu, M.B. (2012). Short-term sensorimeter and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats: ameliorative effect of vitamin E. *Pharmacologia,* **3**(2): 31-38.

Ambali, S.F., Akanbi, D.O., Oladipo, O.O., Yaquba, L.S. and Kawu, M.U. (2011). Subchronic chlorpyrifos-induced clinical, hematological and biochemical changes in Swiss Albino Mice: protective effect of vitamin E, *International Journal of Biological & Medical Research*, **2**(2):497 – 503.

Barth, E., Albuszies, G., Baumgart, K., Matejovic, M., Wachter, U., Vogt, J. and Calzia, E. (2007). Glucose metabolism and catecholamines. *Critical Care Medicine,* **35**(9): S508-S518.

Branstrup, N., Kirk, J.E. and Bruni, C. (1957). Hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individual of various ages. *Journal of Gerentology,* **12**, 166-170.

Buyukokuroglu, M.E., Cemek, M., Yurumez, Y., Yavuz, Y. and Aslan, A. (2008). Antoxidative role of melatonin in organophosphate toxicity in rats. *Cell Biology Toxicology,* **24**, 151–158.

Cakici, O. and Akat, E. (2013). Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. *Analytical and quantitative cytology and histology*, **35**(1):7–16.

Catanesi, M., Brandolini, L., d'Angelo, M., Benedetti, E., Tupone, M.G., Alfonsetti, M., Cabri, E., Iaconis, D., Fratelli, M., and Cimini, A. (2021). L-Methionine Protects against Oxidative stress and mitochondrial dysfunction in an in vitro model of Parkinson's disease. *Antioxidants*, **10**(9):1467.

Cemek, M., Buyukben, A., Buyukokuroglu, M.E., Aymelek, F. and Tur, L. (2010). Protective roles of vitamin E (α-tocopherol), selenium and vitamin E plus selenium in organophosphate toxicity in vivo: a comparative study. *Pesticide Biochemistry and Physiology*, **96**: 113-118.

Cobilinschi, C., Tincu, R.C., Băetu, A.E., Deaconu, C.O., Totan, A., Rusu, A. and Grințescu, I. M. (2021). Endocrine disturbances induced by low-dose organophosphate exposure in male wistar rats. *Acta Endocrinologica (Bucharest)*, **17**(2): 177.

Cobilinschi, C., Ţincu, R.C., Cobilinschi, C.O., Neagu, T.P., Becheanu, G., Sinescu, R.D., Checheriţă, I.A., Grinţescu, I.M., Lascăr, I. (2020). Histopathological features of low-dose organophosphate exposure. *Romanian Journal of Morphology and Embryology*, **61**(2):423-432.

Cocker, J., Mason, H.J., Garfit, S.J. and Jones, K., (2002). Biological monitoring of exposure to organophosphate pesticides. *Toxicology letters*, **134**, 97–103.

Demir, F., Uzun, F.G., Durak, D. and Kalender, Y. (2011). Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pesticide Biochemistry and Physiology*, **99**(1): 77-81.

Deng, Y., Zhang, Y., Lu, Y., Zhao, Y. and Ren, H. (2016). Hepatotoxicity and nephrotoxicity induced by the chlorpyrifos and chlorpyrifos-methyl metabolite, 3,5,6-trichloro-2-pyridinol, in orally exposed mice. *Science of the Total Environment,* **544**:507–514.

DuTeaux, S. and Koshlukova, S.E. (2023). Chlorpyrifos. Reference Module in Biomedical Sciences, Elsevier. Retreived September 20, 2023 from https://doi.org/10.1016/B978-0-12-824315-2.00746-6.

El-Gendy, K.S., Aly, N.M., Mahmoud, F.H., Kenawy, A. and El-Sebae, A.K.H. (2010). The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. *Food and chemical Toxicology*, **48**(1): 215-221.

Ellman, G.L., Courtney, K.D., Andres, V. And Featherstone, R.M., (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7**: 88–95.

Erfani, M., Eila, N., Zarei, A. and Noshary, A. (2021). The effects of vitamin C and methionine hydroxyl analog supplementation on performance, blood performance, blood parameters, liver enzymes, thyroid hormones, antioxidant activity of blood plasma, intestine morphology, and HSP70 gene expression of broilers under heat stress. *Tropical Animal Health and Production,* **53**(2): 296.

Ezzi, L., Belhadj Salah, I., Haouas, Z., Sakly, A., Grissa, I., Chakroun, S., Kerkeni, E., Hassine, M., Mehdi, M. and Ben Cheikh, H. (2016). Histopathological and genotoxic effects of chlorpyrifos in rats. *Environmental Science and Pollution Research International*, **23**(5):4859–4867.

Flora, S.J., Pande, M. and Mehta, A. (2003). Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. *Chemico-Biological Interactions*,**145**(3):267–280

Gbadamosi, M.R., Ogunneye, A.L., Al-Omran, L.S., Abdallah, M.A. and Harrad, S. (2023). Presence, source attribution, and human exposure to organophosphate esters in indoor dust from various microenvironments in Nigeria, *Emerging Contaminants*, **9**(2):100208.

Goel, A. and Dhawan, D.K. (2001). Zinc supplementation prevents liver injury in chlorpyrifos-treated rats. *Biological Trace Element Research*, **82:**:185-200.

Goel, A., Dani, V. and Dhawan, D.K. (2006). Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood. *Biometals.* **19**: 483-492.

Goel, A., Dani, 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**(2-3):131–40.

Góth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*,**196**(2-3):143-151.

Gultekin, F., Ozturk, M. and Akdogan, M. (2000). The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). *Archives of Toxicology*, **74**(9):533-8.

Hagège, D., Feutry, S., Krsnik-Rasol, M., Poder, D. and Menez, J.F. (1995). Estimation of free and bound MDA in plant extracts: comparison between spectrophotometric and HPLC methods. In: Plant lipid metabolism. *Springer, Dordrecht,* pp 259–261

Halliwell, B. (1996). Vitamin C: antioxidant or pro-oxidant in vivo? *Free Radical Research*, **25**(5):439–454.

Hamza, R.Z., Diab, A.E.A. and Abd El-Aziz, E.A. (2014). Hyperglycemic effect of chlorpyrifos, profenofos and possible ameliorative role of propolis and ginseng. *Scientia Agriculturae*, **5**:9–14.

Hazarika, A., Sarkar, S.N., Hajare, S., Kataria, M. and Malik, J.K. (2003). Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. *Toxicology*, **185**(1-2):1-8.

Ibrahim, G.E., Yakubu, N., Nnamonu, L. and Yakubu, M.J. (2018). Prevalence of organophosphorous pesticide residues in pumpkin, spinach and sorrel leaves grown in Akwanga, Nasarawa State, Nigeria. *Journal of Environmental Protection*, **9**: 516-524.

Jintana, S., Sming, K., Krongtong, Y. and Thanyachai, S. (2009). Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. *International Archives of Occupational and Environmental Health*, **82**: 833-842.

Joshi, A.K. and Rajini, P.S. (2008). Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: role of gluconeogenesis. *Toxicology:* **257**:40-5.

Joshi, A.K.R. and Rajini, P.S. (2012). Hyperglycemic and stressogenic effects of monocrotophos in rats: evidence for the involvement of acetylcholinesterase inhibition. *Experimental and Toxicologic Pathology,* **64**(1-2):115-120.

Kammon, A.M., Brar, R.S., Sodhi, S., Banga, H.S., Singh, J. and Nagra, N.S. (2011). Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. *Open Veterinary Journal*, 1(1):21-7.

Kammon, A.M., Brar, R.S., Sodhi, S., Banga, H.S., Singh, J. and Nagra, N.S. (2011). Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. *Open Veterinary Journal*, **1**(1):21-7.

Karalliedde, L., Edwards, P. and Mars, T.C. (2003). Variables influencing the toxicity of organophosphates in humans. *Food and Chemical Toxicology,* **41**:1–13.

Kendig, D.M. and Tarloff, J.B. (2007). Inactivation of lactate dehydrogenase by several chemicals: implications for in vitro toxicology studies. *Toxicology in Vitro*, **21**(1): 125-132.

Lima, P.L., Benassi, J.C., Pedrosa, R.C., Dal Magro, J., Oliveira, T. B. and Wilhelm Filho, D. (2006). Time course variations of DNA damage and biomarkers of oxidative stres in tilapia (Oreochromis niloticus) exposed to effluents from a swine industry. *Archives of Environmental Contamination and Toxicology,* **50**:23–30.

Liu, C.M., Zheng, Y.L., Lu, J., Zhang, Z.F. and Fan, S.H. (2010). Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. *Environmental Toxicology and Pharmacology*, *T* **29**: 158–166.

Lukaszewicz-Hussain, A. (2013). Serum glucose concentration in subacute intoxication with chlorpyrifos-organophosphate insecticide. *Medycyna Pracy*, **64**(4):527–31

Ming, Z., Fan, Y.J., Yang, X., and Lautt, W.W. (2006). Synergistic protection by S-adenosylmethionine with vitamins C and E on liver injury induced by thioacetamide in rats. *Free Radical Biology and Medicine*, **40**(4):617-624.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase *Journal of Biological Chemistry*, **247**(10):3170–3175.

Mohamed, F.E-T. (2014). Toxicological effects of short-term feeding with chlorpyrifos and chlorpyrifos-methyl insecticides on adult albino rats. *Middle East Journal of Agriculture Research,* **3**(2):208–220

Narra, M.R., Rajender, K., Reddy, R.R., Rao, J. V. and Begum, G. (2015). The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish Clarias batrachus. *Chemosphere*, **132**, 172-178.

Ndonwi, E.N., Atogho, B.T, Yimagou, E.L., Shinkafi, T.S., Nanfa, D., Balti, E.V., Routray, I., Mahmood, A., Katte, J.C., Mbanya, A., Matsha, T., Mbanya, J.C., Shakir, A. and Sobngwi, E. (2019). Gestational exposure to pesticides induces oxidative stress and lipid peroxidation in offspring that persist at adult age in an animal model. *Toxicological Research*, **35**(3): 241–248.

Ndonwi, E.N., Atogho-Tiedeu, B., Lontchi-Yimagou, E., Shinkafi, T.S., Nanfa, D., Balti, E.V., Katte, J.C., Mbanya, A., Matsha, T., Mbanya, J.C., Shakir, A. and Sobngwi, E. (2020). Metabolic effects of exposure to pesticides during gestation in female Wistar rats and their offspring: a risk factor for diabetes? *Toxicological Research*,.**36**(3):249-256.

Nicholson, J.K., Lindon, J.C. and Holmes, E. (2008). 'Metabonomics': under- standing the metabolic responses of living systems to patho- physiological stimuli via multivariate statistical analysis of bio-logical NMR spectroscopic data. *Xenobiotica*, **29**(11):1181–1189.

Ogutcu, A., Uzunhisarcikli, M., Kalender, S., Durak, D., Bayrakdar, F. and Kalender, Y. (2006). The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E. *Pesticide Biochemistry and Physiology*, **86**: 93–98.

Ojamaa, K., Klein, I., Sabet, A. and Steinberg, S.F. (2000). Changes in adenylyl cyclase isoforms as a mechanism for thyroid hormone modulation of cardiac β-adrenergic receptor responsiveness. *Metabolism*, **49**(2): 275-279.

Ojiha, A., Yaduvanshi, S.K., Pant, S.C., Lomash, V. and Srivasta, N. (2011). Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. *Environmental Toxicology*, **28**(10):543-552

Oruc, E.O. (2010). Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in Oreochromis niloticus exposed to chlorpyrifos. *Pesticide Biochemistry and Physiology*, **96**:160–166.

Osada, J., Aylagas, H., Sanchez-Vegazo, I., Gea, T., Millan, I., Palacios-and Alaiz, E. (1986). Effect of *S*-adenosyl-L-methionine on thioacetamide-induced liver damage in rats. *Toxicology Letters*, **32**(1–2):97–106

Panahi, P., Vosough-Ghanbari, S. and Pournourmohammadi, S. (2006). Stimulatory effects of malathion on the key enzymes activities of insulin secretion in langerhans islets, glutamate dehydrogenase and glucokinase. *Toxicology Mechanisms and Methods* **16** (4): 161–167.

Patra, R.C., Swarup, D. and Divedi, S.K. (2001). Antioxidant effects of alpha tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology*, **162**:81-88.

Rahimi, R. and Abdollahi, M. (2007). A review on mechanisms involved in hyperglycemia induced by organophosphorus insecticides. *Pest. Biochem. Physiol*. **88**, 115–121.

Rajak, P., Roy, S., Ganguly, A., Mandi, M., Dutta, A., Das, K. and Biswas, G. (2022). Protective potential of vitamin C and E against organophosphate toxicity: current status and perspective. *Journal of Ecophysiology and Occupational Health*, **22**(3):141-154.

Rambabu, J.P., Rao, M.B. (1994). Effect of an organochlorine and three organophosphate pesticides on glucose, glycogen, lipid, and protein contents in tissues of the freshwater snail Bellamya dissimilis (Muller). *Bulletin of Environmental Contamination and Toxicology*, **53**: 142–148.

Rezg, R., Mornagui, B., El-Arbi, M., Kamoun, A., El-Fazaa, S. and Gharbi, N. (2006). Effect of subchronic exposure to malathion on glycogen phosphorylase and hexokinase activities in rat liver using native PAGE. *Toxicology,* **223**:9-14..

Rezzi, S., Ramadan, Z., Fay, L.B. and Kochhar, S. (2007). Nutritional meta- bonomics: applications and perspectives. *Journal of Proteome Research*, **6**(2):513–525.

Schneider, C.D. and Oliveira, A.R.D. (2004). Oxygen free radicals and exercise: mechanisms of synthesis and adaptation to the physical training. *Revista Brasileira de Medicina do Esporte*, **10**: 308-313.

Shittu, S.T., Oyeyemi, W.A., Shittu, S.A. and Lasisi, T.J. (2018). Ocimum gratissimum inhibits glycogen phosphorylase activity without changes in hepatic nuclear factor kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) in streptozotocin-induced diabetic rats. *Nigerian Medical Practitioner*, **73**(1-2):10-17.

Tang, J., Cao, Y., Rose, R.L. and Hodgson, E. (2002). In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. *Chemico-Biological Interactions*, **141**(3):229–241.

Tilak, K.S., Veeraiah, K. and Rao, D.K. (2005). Biochemical changes induced by chlorpyrifos, an organophosphate compound in sublethal concentrations to the freshwater fish Catla catla, Labeo rohita and Cirrhinus mrigala. *Journal of Environmental Biology*, **26**(2 Suppl), 341-347.

USEPA (2020). Chlorpyrifos: Proposed Interim Registration Review Decision Case Number 0100. Docket Number EPA-HQ-OPP-2008-0850. US EPA, Washington, DC Retrieved September 20, 2023 from https://www.epa.gov/sites/default/files/2020-12/documents/chlorpyrifos_pid_signed_120320.pdf.

Ugbaja, R.N., Olaniyan, T.O., Afolabi, O.K., Onunkwor, B.O., Dosumu, O.A., Akinhanmi, T.F., Tiamiyu, A.Y. and Aliyu-Banjo, N.O. (2017). Ionoregulatory disruption and acetylcholinesterase activity in aluminium toxicity: effects of vitamins C and E. *Nigerian Journal of Physiological Sciences*, **32**(2):207-212.

Vale, J.A. (1998). Toxicokinetic and toxicodynamic aspects of organophosphorus (OP) insecticide poisoning. *Toxicology Letters*,**102-103**:649–652.

Wang, H.P., Liang, Y.J., Long, D.X., Chen, J.X., Hou, W.Y. and Wu, Y.J. (2009). Metabolic profiles of serum from rats after subchronic exposure to chlorpyrifos and carbaryl. *Chemical Research in Toxicology*, **22**(6):1026–1033.

Yurumez, Y., Cemek, M., Yavuz, Y., Birdane, Y.O. and Buyukokuro˘glu, M.E. (2007). Beneficial effect of N-acetylcysteine against organophosphate toxicity in mice. *Biological and Pharmaceutical Bulletin*, **30** (3):490–494.