Journal of
Medical Sciences

Deleterious Effect of Chlorpyrifos and Cypermethrin on Oxidative Stress Enzymes and Biochemical Indices of Male Albino Rats

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This study was carried out to evaluate the effects of chlorpyrifos and cypermethrin, singly and in combination on oxidative stress enzymes and biochemical indices of male albino rats. 35 male albino rats weighing 170-200 g were divided into seven groups (A-G) of five rats each in a Completely Randomized Design. Rats in group A served as the control and received feed and distilled water only. Groups B and C received 5 and 10 mg of chlorpyrifos; groups D and E received 5 and 10 mg of cypermethrin, while groups F and G received 5 and 10 mg of combination (chlorpyrifos+cypermethrin), respectively. Treatment was administered via oral gavage and lasted for 65 days. At the end of treatment, the rats were sacrificed and blood samples were analyzed for oxidative stress enzymes and biochemical indices. On the one hand, result obtained for oxidative stress enzymes revealed that pesticide treatment significantly ($p<0.05$) reduced the levels of superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) activity and increased lipid peroxidation (MDA) level in rats treated with chlorpyrifos only. On the other hand, result for biochemical indices revealed that aspartate transaminase (AST) increased significantly ($p<0.05$) in the group of rats treated with 10 mg of chlorpyrifos and 5 mg of cypermethrin and reduced in those treated with 5 mg of chlorpyrifos and 10 mg of cypermethrin. Alkaline phosphatase (ALP) level reduced significantly in all the groups except for the group that received 5 mg of chlorpyrifos. While, alkaline transaminase (ALT) level increased significantly in some treated rats especially in the group that received 10 mg of the combination. Present findings therefore demonstrate that chlorpyrifos and cypermethrin could induce oxidative stress and alter the levels of some liver marker enzymes in male rats.

Key words: Chlorpyrifos, cypermethrin, deleterious effects, oxidative stress, albino rats

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INTRODUCTION

Globally, the improvement of crop yield depends on the crop genotype, fertility of soil as well as the application of pesticides. The widespread application of pesticides, though effective in their intended use has been suspected to be harmful to animals and humans. Pesticides are designed to kill specific pests, however, a large percentage reach a destination other than their target as their residues are present in air, water, soil and even food (Saeed *et al.*, 2005).

Currently, a lot of pesticides are used for agricultural, domestic and veterinary purposes. Chlorpyrifos and cypermethrin are among pesticides commonly used by farmers in Nigeria. Chlorpyrifos, o, o-diethyl-o-(3, 5, 6-trichloro-2-pyridyl) phosphorothionate is a broad spectrum organophosphate utilized extensively to control agricultural and residential pests throughout the world (Whyatt *et al.*, 2004). It is an irreversible inhibitor of acetyl cholinesterase in the central and peripheral nervous systems that causes the accumulation of acetylcholine which in turn results in neurotoxicity in animals and humans (Eaton *et al.*, 2008). The nervous system is the primary target because acetyl cholinesterase catabolizes acetylcholine, thereby terminating its synaptic functions (Eaton *et al.*, 2008).

Similarly, cypermethrin is a type II pyrethroid insecticide that is widely used in agriculture and presumed to be environmentally safe (Shukla *et al.*, 2002). It is a fast acting neurotoxin and is known to cause free radical mediated tissue damages (Patel *et al.*, 2006). Like all other pyrethroid, cypermethrin kills pests by interacting with sodium channels in nerve cells through which sodium enters the cell in order to transmit nerve signals. These channels can remain open for a second instead of the normal period of a few milliseconds after a signal has been transmitted. Human exposure to cypermethrin is reported to be mainly occupationally (Sankar *et al.*, 2010). In a study, populations exposed to cypermethrin in cotton fields showed adverse effects such as severe dizziness, eyes, skin and nervous disorders, congenital defects and neonatal lethality. Cypermethrin can also elicit a range of immunotoxic and genotoxic effects as well as reproductive toxicity in various experimental systems (Yousef *et al.*, 2003).

The combination of pesticides with different mode of action is fast gaining popularity in pest control programmes because such applications result in broader spectrum activity with better efficacy in pest control (Babu *et al.*, 2010). Consequently, the combination of these chemicals exhibits a different toxicological profile compared with toxicity of the pesticides singly (Babu *et al.*, 2010). Although combination of pesticides is beneficial in combating pest resistance, this combination poses a new challenge since the resultant effects of such interactions are unknown (Banke *et al.*, 2014).

Pesticides may induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity (Sharma *et al.*, 2005). Oxidative stress is an imbalance between the formation of Reactive Oxygen Species (ROS) and antioxidant defense mechanisms (Galle, 2001). Reactive oxygen species are related to oxidative stress. Studies have shown that over production of ROS can further aggravate oxidative stress and trigger health conditions such as heart diseases, diabetes, liver injuries, cancer and aging (Mansour and Mossa, 2011; Bokov *et al.*, 2004; Klaunig and Kamendulis, 2004). Maintaining the balance between ROS and antioxidant enzymes like Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and malondialdehyde (MDA), is an important mechanism for preventing damages arising from oxidative stress. It is suggested that this balance is crucial in preventing pesticides toxicity (Mansour and Mossa, 2011; Jaeschke, 2000).

This study was therefore carried out to evaluate the effects of chlorpyrifos and cypermethrin, singly and in combination, on oxidative stress enzymes and biochemical indices of male albino rats.

MATERIALS AND METHODS

Experimental materials: The experiment was carried-out in the Animal house of Genetics and Biotechnology Department, University of Calabar, Calabar. Chlorpyrifos and cypermethrin were purchased from Department of Agrochemicals, Ministry of Agriculture, Calabar, Nigeria.

Experimental animals/procedure: Thirty five mature male albino rats of body weight ranging from 170-200 g were purchased from the Department of Physiology, University of Calabar, Calabar. They were kept in aluminum cages covered with wire mesh. They were fed with growers mash and allowed unrestricted access to clean water. The rats were allowed to acclimatize for 1 week. They were handled in accordance with the guidelines for care and use of laboratory animals as modified by the Animal Genetics research committee of the Department.

After acclimatization, the rats were divided randomly into seven groups (A-G) of five animals each. Group A served as the control and received feed and water only. Rats in groups B and C were administered 5 and 10 mg kg^{-1} b.wt of chlorpyrifos, respectively, groups D and E received 5 and 10 mg kg^{-1} b.wt of cypermethrin, respectively and then groups F and G received 5 and 10 mg kg^{-1} b.wt of chlorpyrifos+cypermethrin respectively. The animals were treated via oral gavage for 65 successive days and then sacrificed under chloroform anesthesia 24 h after the last dose was administered.

Evaluation of biochemical indices: Blood samples collected were analyzed for aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphate (ALP).

ALT and AST were estimated using Reitman and Frankel (1957) method. A 0.5 mL of AST/ALT substrate was collected

into a test tube using a pipette and warmed for 5 min. before adding 0.1 mL of serum. The mixture was incubated at 37°C and then 0.5 mL of 2, 4-dinitrophenylhydrazine was added to the mixture. The ALT and AST were determined based on colorimetric measurement of pyruvate and oxaloacetahydrazone formed at wavelength of 510 nm. ALT and AST were expressed in micro per liter.

For ALP, 1 mL of was collected into a test tube and placed in a water bath at 37°C for 3 min. The 0.1 mL of serum was added, mixed gently and incubated for 15 min. The 0.1 mL of NaOH was added to stop the reaction. The ALP was measured using a colorimeter at 510 nm wavelength and expressed in micro per liter.

Determination of oxidative stress enzymes: Glutathione Peroxidase (GPx) was assessed according to the method of Paglia and Valantine (1967), using the Fortress diagnostic kit. GPx catalyses the oxidation of glutathione and then the oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. The NADPH is oxidized to NADP and decrease in absorbance at 340 nm is measured and expressed in micro per gram Hb.

For superoxide dismutase (SOD), packed erythrocytes were obtained from blood sample and washed four times with 5 mL of 0.9% saline solution and centrifuged at 3500 rpm for 10 min. The cells were lysed with ice cold distilled water and centrifuged twice to obtain erythrocyte membrane and hemolysate. The cells were further treated with chloroform and ethanol and used to determine SOD enzyme activity which was expressed in µmol.

Catalase activity was determined according to the method of Aebi (1984). The method is based on the decomposition of H_2O_2 by catalase. Sample containing catalase was incubated in H_2O_2 and then mixed with 4-aminophenozone and 3, 5-dichloro-2-hydrobenzenesulfonic acid and catalysed by horseradish peroxidase. The resulting quinoneimine dye was measured at 510 nm and expressed in nmol.

Malondialdehyde (MDA) is the end product of lipid peroxidation. MDA was determined by the method of Draper and Hadley (1990). The principle of this method is based on fluorometric measurement of the colour produced during the reaction of Thiobarbituric Acid (TBA) with MDA. Absorbance was measured using a spectrophotometer at 532 nm. The concentration of MDA was calculated and expressed in nmol mL^{-1}.

Statistical analysis: All data obtained were subjected to analysis of variance (ANOVA) using PASW (version 18.0). Least Significant Difference (LSD) was used to separate the means of groups that were significant at p<0.05.

RESULTS

Biochemical indices: The result revealed that AST increased in rats administered pesticide treatment (Table 1). The highest of these was observed at 5 mg of cypermethrin (66.90 µL) while combination of the two pesticides did not show any significant difference (p>0.05) at 5 and 10 mg concentration. ALP was significantly higher in rats administered 5 mg of chlorpyrifos (132.45 µL) than those in the control (124.27 µL). There was no significant difference (p>0.05) in ALT level among rats in the control group (21.41 µL), 5 mg of chlorpyrifos (23.63 µL), 10 mg of cypermethrin (22.28 µL) and 5 mg of the combination (21.71 µL).

Oxidative stress enzymes: The levels of SOD, GPx and CAT activity in treated rats generally reduced significantly (p<0.05) as the concentration of pesticide increased (Table 2). The level of SOD was reduced most in rats treated with 10 mg of cypermethrin (64.00 µmol) compared to those in the control (178.65 µmol) while rats administered 10 mg of chlorpyrifos and 5 mg of the combination treatment showed no significant difference (p>0.05). Similarly, at 10 mg of chlorpyrifos and 10 mg of combination, there was no significant difference (p>0.05) in CAT activity of rats. The result also revealed that

Table 1: Biochemical indices of control and pesticide treated rats

Parameters	A	B	C	D	E	F	G
AST (µL^{-1})	53.87±0.49[c]	32.55±1.06[e]	59.49±1.38[b]	66.90±1.09[a]	41.41±0.99[d]	55.29±1.08[c]	53.53±1.24[c]
ALP (µL^{-1})	124.27±2.22[b]	132.45±1.18[a]	93.55±0.92[d]	83.68±1.43[e]	76.02±1.48[f]	103.41±1.11[c]	66.24±1.64[g]
ALT (µL^{-1})	21.41±1.02[c]	23.63±1.24[c]	33.42±0.48[b]	36.54±0.82[b]	22.28±1.02[c]	21.71±0.94[c]	42.38±1.27[a]

Values are presented as Mean±SEM, Means followed by the same case letter along the horizontal array indicate no significant difference (p>0.05), A: Control (0 mg kg^{-1} b. wt), B and C: 5 and 10 mg kg^{-1} body weight of chlorpyrifos respectively, D and E: 5 and 10 mg kg^{-1} body weight of cypermethrin respectively, F and G: 5 and 10 mg kg^{-1} body weight of combination, respectively

Table 2: Oxidative stress enzymes of control and pesticide treated rats

Parameters	A	B	C	D	E	F	G
SOD (µmol^{-1})	178.65±0.37[a]	124.67±0.88[b]	118.47±0.58[c]	68.33±0.88[e]	64.00±1.15[f]	115.33±1.45[c]	109.67±1.45[d]
GPx (µgHb^{-1})	78.15±0.18[a]	42.67±0.64[b]	38.90±0.40[c]	10.93±0.15[e]	8.57±0.32[f]	10.63±0.19[e]	12.00±0.15[d]
CAT (nmol)	1.38±0.22[ab]	1.25±0.03[abc]	0.94±0.03[d]	1.47±0.09[a]	1.03±0.09[cd]	1.12±0.06[bcd]	0.94±0.03[d]
MDA (nmol)	1.79±0.02[c]	3.83±0.44[b]	6.11±0.48[a]	0.90±0.06[de]	1.34±0.06[cd]	0.40±0.06[e]	0.63±0.03[de]

Values are presented as Mean±SEM, Means followed by the same case letter along the horizontal array indicate no significant difference (p>0.05), A: Control (0 mg kg^{-1} b. wt), B and C: 5 and 10 mg kg^{-1} body weight of chlorpyrifos respectively, D and E: 5 and 10 mg kg^{-1} body weight of cypermethrin, respectively, F and G: 5 and 10 mg kg^{-1} body weight of combination, respectively

lipid peroxidation (MDA) increased in rats treated with 10 mg of chlorpyrifos (6.11 nmol), while the least MDA level was recorded in rats administered 5 mg of combination (0.4 nmol).

DISCUSSION

Free radicals generation explains the toxicity of numerous xenobiotics such as pesticides and some of these free radicals interact with tissue components resulting in dysfunctional conditions (Mansour and Mossa, 2009; Mossa *et al.*, 2013). In fact, oxidative damage arising from excessive production of Reactive Oxygen Species (ROS) has been associated with defective organs and the inhibition of enzymes involved in the removal of free radicals, thus leading to lipid peroxidation, alteration in gene expression and cell death (Halliwell and Gutteridge, 2000; Mossa *et al.*, 2013). The SOD, CAT and GPx are known to play a vital role in scavenging ROS. The SOD catalyzes the destruction of superoxide radicals to H_2O_2 while CAT and GPx reduce H_2O_2 into water and oxygen to prevent oxidative stress and maintain cell homeostasis.

In the present study, the levels of SOD, GPx and CAT activity in rats reduced significantly as the concentration of pesticide increased. The change in SOD, GPx and CAT could be in response to increased oxidative stress. When oxidative stress has been established, the defense capacity against ROS becomes weak and reduces the intracellular concentration of lipid peroxidation and alters antioxidant enzymes activity. The change in the activity of antioxidant enzymes have been reported to be an indicator of oxidative stress (Mossa *et al.*, 2013).

Lipid peroxidation has been suggested as one of the mechanisms involved in pesticide induced toxicity (Mossa *et al.*, 2013). Malondialdehyde (MDA) level in chlorpyrifos treated rats was significantly higher than those in the control. This suggests an increase in the production of free radicals in the exposed rats. Highly reactive oxygen metabolites especially hydroxyl radicals act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid per-oxidation product. Previous studies indicate that insecticides in both *in vivo* and *in vitro* test (Mossa *et al.*, 2012; Mansour *et al.*, 2009), alter the enzyme activities associated with antioxidant defense mechanisms. The result of this study corroborates the findings of Abbassy *et al.* (2014), who reported a significant decrease in SOD, GPx and CAT activity and an increase in MDA level of rats treated with lambda cyhalothrin.

Liver is a target organ and plays a major role in the detoxification of many endogenous and exogenous compounds. It is actively involved in metabolism and biotransformation of toxic compounds and as such, any injury or impairment could cause severe health problems. Liver biomarker enzymes such as AST, ALT and ALP have been associated with liver dysfunction and damage. Hayes *et al.* (1989) reported that one of the indicators of liver damage and dysfunction is increase in the activities of AST and ALT in the serum. They are involved in amino acids catabolism and biosynthesis. The ALP on the other hand catalyzes the hydrolysis of a wide variety of phosphoric acid esters in alkaline medium. This study revealed that the treatment significantly increased the levels of AST and ALP in rats. The AST level increased most in rats administered 5 mg of cypermethrin, while ALP was significantly higher in rats treated with 5 mg of chlorpyrifos.

In conclusion, this study demonstrates that chlorpyrifos and cypermethrin induced oxidative stress and altered the level of some liver enzymes in male albino rats. This is evident upon the increase in lipid peroxidation (MDA), AST and ALP levels and a decrease in the levels of SOD, GPx and CAT activity. However, further studies should be carried out to assess the effects of these pesticides on a molecular level.

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