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Chlorpyrifos: Toxicological effects on *Clarias gariepinus* and its amelioration by *Blighia sapida* seeds' extract

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Abstract

Chlorpyrifos; CPF (O, O-diethyl O-3, 5, 6–trichloro-2-pyridyphosphorothioate) is commonly used for the control of pests and insects in agricultural fields and surrounding freshwater reservoirs. This study was carried out to find out the ameliorative potentials of ***Blighia sapida* seeds** on ***Clarias gariepinus*** exposed to chlorpyrifos. The Acute toxicity assay was to determine the 96 h Lethal Concentration (LC_{50}) values of organophosphate pesticide, which was conducted with definitive test in a semi-static system in the laboratory using the standard methods. Range finding test was carried out prior to determination of the concentrations of the test (chlorpyrifos) solution for definitive test. The experiment was conducted in plastic containers containing 10L of non-chlorinated and aerated water. The acute study; A set of 15 fish specimen were randomly exposed to organophosphate concentrations; 1ml of stock solution was added to B_1 and B_2, 2ml to C_1 and C_2, 3ml to D_1 and D_2, 4ml to E_1 and E_2 while 5ml was added to F_1 and F_2. The exposure was for 7 days. For chronic study; one tenth of LC_{50} (0.12ml/L) = 0.012ml/L was used

for Groups B-E while group A is the positive control with 0.00ml/L of Chlorpyrifos (i.e. the group was free of chlorpyrifos), Group B was made the negative control, fishes in this group, which were also exposed to 0.012ml/L chlorpyrifos, were only fed with normal fish feed i.e. no *Blighia sapida* seeds' extract was added, to serve as the negative control. The exposure was for 28 days and the fishes in groups C to E were fed with feeds, formulated using *Blighia sapida* seed. Results obtained from this study revealed that chlorpyrifos caused considerable alterations, i.e. increase in enzymes' activities (ALT, AST, ALP) as well as alterations in other metabolites (urea, albumin, protein and glucose) in the serum, liver and kidney of all ***Clarias gariepinus*** in the negative group which are fishes exposed to the insecticide; chlorpyrifos is suspected to have induced some tissue damages in the *C. gariepinus*, that may result in enzymes and metabolites leakages into the serum. *Blighia sapida* seeds' extract (at 25%, 50% and 75%) reduced some of the adverse effects of chlorpyrifos in *C. gariepinus* exposed and it was therefore concluded to possess ameliorative effects against animals' exposure to chlorpyrifos pesticides. Therefore, this chemical should be handled with care as usual and lots of caution must still be taken to prevent or reduce its entrance or leakage into the aquatic environment. *Blighia sapida* seeds' extract is therefore recommended for use to ameliorate any possible toxic effects of chronic chlorpyrifos exposure.

Keywords

Chlorpyrifos, *Blighia sapida*, Amelioration, *Clarias gariepinus*, Pesticides

Introduction

Pesticide is defined by United Nations Environment Programme as any substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are routinely employed to protect crops from various pathogens (UNEP, 2005). Pesticides are of vital importance in the fight against diseases, for the production and storage of food being widely used for pest control in agriculture, in gardening, at homes and in soil treatment (Janssen, 1997; Crespo-Corral *et al.*, 2008). They constitute a versatile class of compounds used as insecticides, fungicides, nematicides, acaracides, molluscicides, sprout inhibitors or herbicides. Widespread use of pesticides is

now a worldwide phenomenon (Omitoyin *et al.*, 2006). It has been estimated that only about one percent of applied pesticides get to the target and that the remaining 99% contaminates the environment (Lawson *et al.*, 2011). Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Lawson *et al.*, 2011). Chlorpyrifos (O,O-diethyl O-3,5,6–trichloro-2-pyridyphosphorothioate) is an organophosphorous pesticide commonly known as cholinesterase inhibitor and may cause disorders in animals's physiology, by inhibiting the enzyme, acetylcholinesterase (AChE), which modulates the amount of neurotransmitter, acetylcholine (John, 2007).

The contamination of surface waters by insecticides is known to have negative effects on the survival, growth and reproduction of aquatic organisms such as fishes (Banaee *et al.*, 2011). The use of such pesticides has increased the mortality rate in aquatic animals, such as fishes which has drawn attention to the problems caused by these pesticides. Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they gain entrance into the organs of fishes (John, 2007; Banaee *et al.*, 2011).

Over the years, medicinal plants were used as strong antioxidant to scavenge free radicals generated in living systems exposed to pollutants such as pesticides (Banaee *et al.*, 2011). A plant, *Blighia sapida*, commonly known as Ackee (English), *Gwanja Kusa* (Hausa; Northern Nigeria), *Isin* (Yoruba; Western Nigeria), *Okpu* (Igbo; Eastern Nigeria) and *Yila* (Nupe; North Central Nigeria) is a soap berry plant of the family Sapindaceae. In folk medicine, *B. sapida* extracts are commonly used in developing countries to treat a wide range of disease conditions such as dysentery and yellow fever, its use in the management of eye sore, burns, wounds, skin sore, etc. has also been reported (Etukudo, 2003). The present study was therefore carried out to determine the ameliorative potentials of *B. sapida* seeds on some biochemical indices monitored in *Clarias gariepinus* exposed to various concentrations of chlorpyrifos.

Materials and Methods

Experimental Animals

Juvenile African catfish (*Clarias gariepinus*) were purchased at *Oja Oba*, in Ilorin, Kwara state. They were identified and authenticated at the Department of Zoology and acclimatized for two weeks in the Zoology laboratory of the University of Ilorin. They were fed with conventional fish feed during the period of acclimatization.

Animal Grouping

Three hundred fishes were used in this research, where the first one hundred and sixty-five experimental animals (*Clarias gariepinus*) were grouped into 11 of 15 each for the acute exposure with the tags A (control), B_1, B_2, C_1, C_2, D_1, D_2, E_1, E_2, F_1, and F_2 while another one hundred and thirty-five were grouped into 9 for the chronic exposure which were labeled A (positive control), B_1, B_2 (negative control), C_1, C_2 (25% *Blighia sapida* seeds' extract), D_1, D_2 (50% *Blighia sapida* seeds' extract) and E_1, E_2 (75% *Blighia sapida* seeds' extract)**.**

Chemicals and Reagents

The assay kits for alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CREA), urea, and albumin (ALB) were products of Randox Laboratories Limited, Antrim, United Kingdom. All other reagents and chemicals used were of analytical grade and supplied by Sigma Aldrich Inc., St. Louis, USA.

Sample Collection and Extraction

Ripe *Blighia sapida* seeds were collected in Ilorin using sickle to pluck the fruits. The seeds were identified and authenticated at the Herbarium of the Department of Plant Biology. The seeds were removed or detached from the splitted-open fruits using hand and knife. The arils were detached from the seeds. Seeds were then removed from the matured fruits. The seeds were sun-dried for a month, after which they were milled using attrition mill. The milled *B. sapida* seeds were extracted in petroleum ether for 12-16 hours. The *B. sapida* seed oil, which is less dense than the solvent (petroleum ether), was then decanted and the residue solvent in the *B. sapida* seed was evaporated. The defatted *Blighia sapida* seed cake was packed in polythene bags and autoclaved at 121^{o}C and 15*psi* for 15 minutes to remove any microorganism, which could be present in the cake. The cooled *B. sapida* seed cake was inoculated with *Aspergillus niger* which was collected

from the Microbiology Department Laboratory, University of Ilorin and incubated at room temperature. In about 7 days, the fungus (*Aspergilus niger*) had colonized the substrate and the growth was terminated by oven drying the cake at 70^{o}C for 24 hours.

Administration of Chlorpyrifos and Toxicity Assay

Acute toxicity assay was to determine the 96 h LC_{50} values of organophosphate pesticide, which was conducted with definitive test in a semi-static system in the laboratory using the standard methods. The range finding test was carried out prior to determination of the concentrations of the test solution for definitive test. The experiment was conducted in plastic containers containing 10L of non-chlorinated and aerated water. A set of 15 fish samples were randomly exposed to organophosphate concentrations in duplicate for 7, 14 and 28 days exposure time for the two sets of fish that were taken at random. Another set of 15 fish were simultaneously maintained in well aerated borehole water as control.

For Acute administration: A stock solution of 2ml of chlorpyrifos in 100ml of distilled water was prepared for the acute test. Eleven sets of plastic container were filled with 10 litres of tap water each and were labelled A (control), B_1, B_2, C_1, C_2, D_1, D_2, E_1, E_2, F_1, and F_2. The container labelled A did not contain any chemical (chlorpyrifos) being the positive control while the other containers have different quantities of the stock solution. 1ml of stock solution was added to B1 and B_2, 2ml to C_1 and C_2, 3ml to D_1 and D_2, 4ml to E_1 and E_2 while 5ml was added to F_1 and F_2. The exposure was for 7 days.

For Chronic administration: From the calculation for acute, a stable concentration was reached for chronic exposure which was One tenth of LC_{50} (0.12ml/L) = 0.012ml/L, this concentration was thereafter used for groups B-E while group A was the positive control with 0.00ml/L of chlorpyrifos, group B was the negative control, fishes in this group, which were also exposed to 0.012ml/L chlorpyrifos, were only fed with normal fish feed i.e. no *Blighia sapida* seeds' extract was added, to serve as the negative control group. The exposure was for 28 days and the fishes in groups C to E were fed with feeds, formulated using *Blighia sapida* seed at 25%, 50% and 75% respectively.

Daily exposure to chlorpyrifos and feeding with the formulated *B. sapida* seed feed: The amelioration attempt, which involves the introduction of the *B. sapida* seeds' extract through their feeds during the course of the chronic exposure, varies per group. The extract was introduced by mixing it with their feeds at 25%, 50% and 75% for groups B, C and D.

Blood Collection and Isolation of Tissues of Interest

The fishes were earlier kept without food for twelve hours before the exposure to chlorpyrifos. The fishes were sacrificed on the seventh day of exposure (acute exposure) and twenty-eight day (chronic exposure). They were sacrificed and blood samples were collected via jugular vein puncture into EDTA-coated sample bottles and the blood samples were allowed to clot. The blood samples were centrifuged and 1ml of the serum was collected in appropriately labelled sterile bottles containing 4 ml of 0.9% saline solution. The fishes were then dissected and organs of interest (kidneys, heart and liver) were collected, weighed and kept in 5ml of 0.25M sucrose solution in preparation for homogenization and centrifugation

Biochemical Parameters

The activities of alanine aminotransferase (ALT) (E.C. 2.6.1.2) by Reitman and Frankel (1957), aspartate aminotransferase (AST) (E.C. 2.6.1.1) by Reitman and Frankel (1957), alkaline phosphatase (ALP) (E.C.3.1.3.1) by Wright *et al*., 1972, and the concentrations of Albumin (ALB) by Doumas *et al.*, 1971, Creatinine (CREA) and urea were assayed using the Randox assay kits (Randox Laboratory Limited) by Bartels and Bohmer (1972). The total protein content of the serum and homogenates was determined using the Biuret reagent by Gornall *et al.*, 1949. All measurements were done using a spectrophotometer (Bausch and Lomb, Rochester, NY, USA).

Statistical Analysis

All numerical data were subjected to statistical analysis. The groups mean ± S.E.M were calculated for each data and significant differences evaluated using one way analysis of variance (ANOVA). Post-test analysis were done using the Duncan Multiple Range at 99.5% significance level (α=0.05) where n = 300.

Results

Table 1 shows the AST Activity (U/I) in the serum and selected tissues of cat fish exposed to various concentrations of chlorpyrifos. The AST activity was significantly higher ($p<0.05$) in the serum of negative control (68.41) fish when compared with the positive control (39.56). However, there was a significant reduction ($P<0.05$) in AST activities in the serum of fishes fed with the *B. sapida* seeds' extract at various concentrations (42.90, 44.06 and 45.65), which compares favourably with the positive control as well as a reduced serum activity when compared with the negative control (68.41).

The AST activity was significantly higher ($p<0.05$) in the liver of negative control fishes (63.48) when compared with the positive control (47.68). Also, there were significant reductions in the AST activities of the liver of the experimental fishes compared with the negative control. However, there was no significant difference among the experimental fishes in groups C, D and E (50.43, 52.46, and 54.93) and the positive control (47.68).

The AST activity was significantly higher ($p<0.05$) in the kidney of negative control fishes (64.49) when compared with the positive control (45.07). Also, there were significant reductions ($p<0.05$) in the AST activities of the kidney of the experimental fishes (42.61, 44.49 and 41.01) compared with the negative control (64.49).

Table 2 shows the ALT Activity (U/I) in serum, liver and heart of fishes exposed to chlorpyrifos. In the serum, there was a significant increase ($p<0.05$) in the ALT activity of the negative control *C. gariepinus* (36.69) compared with the positive control (28.33). The ALT activities of *B. sapida* seeds' extract fed fishes (24.52, 22.32 and 28.27) have no significant difference ($P>0.05$) in ALT activities when compared with the positive control (28.33).

In the liver, there was a significant increase ($p<0.05$) in the ALT activities of the negative control (38.69) compared with the positive control (29.29). However, there was no significant difference ($P>0.05$) in ALT activity between the *B. sapida* seeds' extract fed fishes and the positive control. The *B. sapida* seeds' extract was able to reduce the initial effects of the chlorpyrifos.

In the heart, there was a significant increase ($p>0.05$) in the ALT activity of the negative

control (45.30) compared with the positive control (32.02).

Table 3 shows the ALP Activity (U/I) in serum, liver and heart of fishes exposed to solution of chlorpyrifos. In the serum, there was a significant increase ($p<0.05$) in the ALP activity of the negative control *C. gariepinus* (6742) compared with the positive control (2475). In the *B. sapida* seeds' extract fed fishes there was no significant differences in the ALP activities (2584, 2458 and 2205) ($p>0.05$) when compared with that of the positive control (2475). They compared favourably with the control group.

Table 4 shows the urea concentration in serum, liver and heart of fishes exposed to solution of chlorpyrifos. In the serum, liver and kidney, there was no significant difference ($p<0.05$) in the urea concentration in all groups but the urea concentrations in the serum, liver and kidney of the negative control fishes were higher compared with that of all other groups. .

Table 5 shows the albumin concentration in serum and liver of fishes exposed to solution of chlorpyrifos. In the serum and liver, there was no significant difference ($p<0.05$) in the albumin concentrations in all the *B. sapida* seeds' extract fed groups except with the negative control when compared with the positive control.

Table 6 shows the protein concentration in serum and liver of fishes exposed to solution of chlorpyrifos. In the serum and liver, there was a significant increase ($p<0.05$) in the protein concentration of the negative control group (13.580) compared with the positive control (9.593) and the other *B. sapida* seeds' extract fed groups.

Table 7 shows the glucose concentration in the serum of fishes exposed to a solution of chlorpyrifos. There was no significant difference ($p<0.05$) in the glucose concentration in all the groups when compared with the positive control group.

Table 1: Ameliorative effect of *B. sapida* seeds' extract formulated diet on AST activity (U/I) in the serum and selected tissues of *Clarias geriepinus* exposed to Chlorpyrifos concentrations

	AST Activity (U/I)		
Group	Serum	Liver	Kidney
A (Positive Control)	39.56±1.959[a]	47.68±1.426[a]	45.07±1.514[a]
B (Negative Control)	68.41±2.614[b]	63.48±1.150[b]	64.49±4.875[b]
C (*B. sapida* 25%)	42.90±2.030[ab]	50.43±3.045[a]	42.61±0.904[a]
D (*B. sapida* 50%)	44.06±1.384[ab]	52.46±3.630[a]	44.49±2.763[a]
E (*B. sapida* 75%)	45.65±0.666[ac]	54.93±5.803[a]	41.01±0.949[a]

Values are expressed as mean ± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p< 0.05$).

Table 2: Ameliorative effect of *B. sapida* seeds' extract formulated diet on ALT activity (U/I) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	ALT Activity (U/I)		
Group	Serum	Liver	Heart
A (Positive Control)	28.33±8.336[a]	29.29±1.555[a]	32.02±1.209[a]
B (Negative Control)	36.69±4.528[b]	38.69±3.843[b]	45.30±2.599[b]
C (*B. sapida* 25%)	24.52±4.725[a]	25.83±2.718[a]	32.02±0.297[a]
D (*B. sapida* 50%)	22.32±1.291[a]	31.34±3.003[a]	31.13±2.270[a]
E (*B. sapida* 75%)	28.27±2.114[a]	27.74±4.870[a]	27.98±1.445[a]

Values are expressed as mean± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p< 0.05$).

Table 3: Ameliorative effect of *B. sapida* seeds' extract formulated diet on ALP activity (U/I) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	ALP Activity (U/I)		
Group	Serum	Liver	Kidney
A (Positive Control)	2475±043.74[a]	1994±262.40[a]	2113±055.20[a]
B (Negative Control)	6742±066.81[b]	5800±248.00[b]	5808±313.70[b]
C (*B. sapida* 25%)	2584±197.90[a]	1835±565.90[a]	2525±139.10[a]
D (*B. sapida* 50%)	2458±204.30[a]	2003±409.90[a]	2551±152.20[a]
E (*B. sapida* 75%)	2205±131.50[a]	2626±240.90[a]	2668±153.10[a]

Values are expressed as mean± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p< 0.05$).

Table 4: Ameliorative effect of *B. sapida* seeds' extract formulated diet on urea concentration (mg/ml) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	Urea concentration (mg/ml)		
Group	Serum	Liver	Kidney
A (Positive Control)	2.186±0.07[a]	3.504±0.14[a]	2.011+0.01[a]
B (Negative Control)	2.902±0.38[a]	3.853±0.26[ab]	3.563±0.31[b]
C (*B. sapida* 25%)	2.826±0.11[a]	2.413±0.11[c]	2.162±0.04[a]
D (*B. sapida* 50%)	2.304±0.14[a]	2.422±0.09[c]	2.024±0.01[a]
E (*B. sapida* 75%)	2.247±0.07[a]	2.389±0.24[c]	2.266±0.03[a]

Values are expressed as mean± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p < 0.05$).

Table 5: Ameliorative effect of *B. sapida* seeds' extract formulated diet on albumin concentration (g/dl) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	Albumin concentration (g/dl)	
Group	Serum	Liver
A (Positive Control)	5.509±0.04003[a]	5.559±0.01559[a]
B (Negative Control)	1.626±0.02709[b]	1.752±0.03200[b]
C (*B. sapida* 25%)	5.460±0.01618[a]	5.491±0.02051[a]
D (*B. sapida* 50%)	5.510±0.02544[a]	5.532±0.02367[a]
E (*B. sapida* 75%)	5.446±0.01178[a]	5.819±0.1535[a]

Values are expressed as mean± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p < 0.05$).

Table 6: Ameliorative effect of *B. sapida* seeds' extract formulated diet on protein concentration (mg/ml) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	Protein concentration (mg/ml)	
Group	Serum	Liver
A (Positive Control)	9.593±0.62[a]	8.283±1.81[a]
B (Negative Control)	13.580±0.84[d]	12.860±0.96[c]
C (*B. sapida* 25%)	10.070±0.42[c]	8.113±0.73[a]
D (*B. sapida* 50%)	8.470±0.46[b]	8.197±1.19[a]
E (*B. sapida* 75%)	9.593±0.58[a]	9.963±0.72[b]

Values are expressed as mean ± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p < 0.05$).

Table 7: Ameliorative effect of *B. sapida* seeds' extract formulated diet on glucose concentration (mg/ml) in the serum and selected tissues of *Clarias geriepinus* exposed to chlorpyrifos concentrations

	Glucose (mg/dl)
Group	Serum
A (Positive Control)	0.2553±0.1353[a]
B (Negative Control)	0.3057±0.09913[ab]
C (*B. sapida* 25%)	0.2293±0.05834[a]
D (*B. sapida* 50%)	0.1393±0.03435[a]
E (*B. sapida* 75%)	0.1377±0.03318[a]

Values are expressed as mean ± SEM of two replicates (15 per group) and those with different superscripts within the group are significantly different at ($p < 0.05$).

Discussion

Alanine Aminotransferase (ALT) and Aspartate amino Transaminase (AST) Activities (U/I) in Serum and Tissues of *Clarias gariepinus* Exposed to Concentrations of Chlorpyrifos and *B. sapida* Formulated Feed

Enzyme activities are considered as sensitive biochemical indicators showing effects that occur in the animals studied and they are important parameters for monitoring the presence of toxicants (Velmurugan *et al.,* 2008). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver marker enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes (Rao, 2006). ALT plays an important role in transporting amino group in a non-toxic form via a pathway called glucose alanine cycle in liver (Arshad *et al.,* 2007). The present results revealed a significant increase in the liver ALT activity in chlopyrifos exposed fish throughout the experimental periods. Morowati (1997) recorded that, the elevation of ALT activity appears to reflect an acute hepatic disease more specifically than using AST values. A significant increase in the AST activity of fish exposed to the high sublethal concentration of chlorpyrifos was recorded in the liver, serum and kidney. De Aguiar *et al.* (2004) attributed the increase observed in the liver AST to mitochondrial membrane damage. While Arshad *et al.* (2007) revealed that the increased level in liver AST may be due to enzyme induction as a result of insecticide stress or due to the

adverse effect of the insecticide on the oxidation process in the Kreb's cycle.

There was a significant increase in ALP Activity in chlorpyrifos exposed groups. In the other hand, studies carried out by Das *et al.* (2004) showed that there was an elevation in activity of ALP of Indian major carps exposed to nitrite toxicity and suggested that the elevation of the transferase was as a result of the diversion of the alpha amino acids in the TCA cycle as keto-acids to augment energy production. A significant increase in enzyme activities (AST, ALT and ALP) in the liver of *C. gariepinus* exposed to monocrotophose, methl-parathion and dimethoate given orally for 90 days was reported by Kaur and Dhanju (2004) and they stated that such increase is an indication of cellular toxicity of these organophosphates.

Increase in blood urea in the experimental fish is due to the inability of damaged kidney to filter urea up to normal levels. The alteration of blood urea in freshwater fish, *Mystus vittatus* was investigated after chronic exposure to metasystox. It has been reported that blood urea showed similar increasing trend, which was in agreement to the present study (John, 2007). Similar findings were also reported in *Jundia ramdia quelen* after sublethal toxicity of cypermethrin where blood urea was used as a diagnostic feature for renal function test haematological and serum biochemical values (Borges *et al*., 2007) were also studied, however present investigation was supported by studying the blood biochemistry of *Channa punctatus* (Bloch.) after Nuvan toxicity (Rani and Gautam, 2009). They reported a similar increasing trend in serum urea and attributed this to the renal damage as a result of Nuvan toxicity. Depletion of serum urea was observed in *Clarias gariepinus* after paraquat dichloride toxicity (Ogamba *et al*., 2011), however elevated levels of serum urea were recorded in *Clarias albopunctatus* due to roundup toxicity (Okonkwo *et al*., 2013) and in *Clarias gariepinus* after *Jatropa* extract toxicity (Ogoruvwe *et al*., 2012).

Remarkable decrease in serum albumin (hypoalbuminemia) in the chlorpyrifos exposed fish can be attributed to the liver damage. The reduced level of albumin was observed in the Rock fish exposed to cypermethrin (Jee *et al.*, 2005). Depletion of serum albumin was found in Nile Tilapia, *Oreochromis mossambicus* exposed to benomyl (Min and Kang, 2008). Recently, a similar result of decreasing serum albumin

was observed in *Channa punctatus* under indofil toxicity (Sharma *et al.,* 2009), the same trend was recorded in *Channa punctatus* exposed to sub-lethal concentration of Nuvan (Rani and Gautam, 2009). A similar finding was also observed recently in *Clarias gariepinus* (Ogamba *et al.,* 2011).

Proteins are good indicators of metabolic activity of cells (Arshad *et al.*, 2007). There was a significant increase in the liver protein concentration in chlorpyrifos exposed groups and in other groups. Gill *et al.*, 1990 recorded that, the sub-lethal concentration of organophosphate phosphamidon increased total proteins in the liver of freshwater fish, *Puntius conchonius*, which are needed for repair of damaged cell organelle and tissue regeneration. Hazarika *et al.*, 2003 and Kalender *et al.*, 2005 revealed that, insecticides cause disorder in protein metabolism leading to either decrease or increase in protein content. This must be due to alteration in either protein synthesis and/or degradation (Koll *et al.,* 2003).

Conclusion

The present work indicates that chlorpyrifos causes considerable alterations in enzymes' activities of exposed fishes, *Blighia sapida* seeds' extract (at 25%, 50% and 75%) reduced some of the adverse effects of chlorpyrifos in *C. gariepinus* exposed and it was therefore concluded to possess ameliorative effects against animals' exposure to chlorpyrifos pesticides. This pestiside should be handled with care as usual and lots of caution must still be taken to prevent or reduce its entrance or leakage into the aquatic environment. *Blighia sapida* seeds' extract is therefore recommended for use to ameliorate any possible toxic effects of chronic chlorpyrifos.

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