OXIDATIVE STRESS BIOMARKERS, HAEMATOLOGICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN THE AFRICAN CATFISH, *Clarias gariepinus* EXPOSED TO AN ORGANOPHOSPHATE PESTICIDE, FENTHION

BY

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BEING A PROJECT SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE IN FISHERIES BIOLOGY IN THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF NIGERIA, NSUKKA

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TOPIC

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CERTIFICATION

Somdare, Peace Onas, a postgraduate student in the Department of Zoology and Environmental Biology, with registration number; PG/M.Sc./13/65268 has satisfactorily completed the requirements of course and research work for the degree of Master of Science (M.Sc.) in Fisheries Biology.

The research work embodied in this thesis report is original and has not been submitted in part or full for any other diploma or degree of this University or any other University.

…… ……

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(External Examiner) Date

DEDICATION

This thesis is dedicated to my mother; Mrs Biyantu Somdare whose love and care I cannot repay.

ACKNOWLEDGEMENTS

I would like to start by appreciating God Almighty for seeing me through my postgraduate programme.

I would also like to express my heart-felt gratitude to my supervisor, Dr C.D. Nwani whose encouragements, guidance and support during the course of the research enabled me to develop an understanding of the subject.

I offer special regards and blessings to family and friends for invaluable support during my research.

ABSTRACT

In the present study, specimens of *Clarias gariepinus* having mean weight and standard length of 197.38 ± 2.34 g and 27.36 ± 0.23 cm respectively were exposed to an organophosphate pesticide, fenthion. The median lethal concentration value (LC_{50}) of fenthion and the effects of sub-lethal concentrations of the pesticide on the oxidative stress biomarkers, haematological parameters and histopathological changes in the blood and tissues of *Clarias gariepinus* were determined. The 24, 48, 72 and 96 hour LC_{50} values (with 95% confidence limits) estimated by probit analysis were found to be 61.987, 50.318, 45.056 and 39.968 mg/L, respectively. Significant differences ($p < 0.05$) at different durations of LC50 were observed. A concentration-dependent increase and time-dependent decrease was observed in mortality rate. Behavioural responses such as hyperactivity, erratic swimming, skin discoloration, jerky movement, followed by exhaustion and death were observed during the acute toxicity test. Fish were exposed to 2.0, 4.0 and 8.0 mg/L corresponding to $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ respectively, of 96 hour LC_{50} of fenthion for 21 days and 7 days recovery to evaluate the haematological parameters, enzyme activities and histopathological changes. There was no significant difference between the condition factors of the treated fish and the control ($p > 0.05$) but values of hepatosomatic index of the treated groups were reduced compared to the control though the reduction was not significant ($p > 0.05$) except on day 21 of the exposure. The liver and gill tissues were sampled on day 1, 7, 21 and during the 7 days recovery for analysis of the different parameters. Induction of oxidative stress in the liver and gill tissues of *Clarias gariepinus* were evidenced by increased lipid peroxidation (LPO) levels ($p < 0.05$), such that increased LPO was higher in fish exposed to the highest concentration of fenthion. The values of glutathione (GSH) activities were not significant ($p > 0.05$) except on day 1 of the exposure where there was significant increase ($p < 0.05$) in GSH activities in liver of fish exposed to the different concentrations of fenthion compared to the gills. Slight decrease in the activity of glutathione reductase (GR) was observed in the liver and gill tissues of the treated fish compared to the control but the reduction was not significant ($p > 0.05$). Increase in GR activities was observed in post-exposure (7 days recovery). There was a general reduction in glutathione peroxidase (GPx) activities but not significant ($p > 0.05$) except on days 1 and 7 where liver tissues exposed to the highest concentration of fenthion differed significantly compared to the gill tissues ($p < 0.05$). Increase in catalase (CAT) activities was both time and concentration dependent in all the tissues. CAT activity increased significantly in the liver of fish exposed to the highest concentration compared to gill tissues ($p < 0.05$) except on day 7. Superoxide dismutase (SOD) activities in the liver decreased from day 1 of the exposure to day 7 (though not significant). SOD activity slightly increased from day 14 of the exposure to post exposure (though not significant) except on day 21 where the liver tissues significantly increased compared to the gill tissues ($p < 0.05$). The exposure of fish to fenthion revealed that the pesticide had adverse effects on various blood parameters. Red blood cell (RBC) counts of the treated fish were lower compared to the control ($p < 0.05$). Packed cell volume (PCV) of the treated fish were lower than that of the control but the difference was not significant ($p > 0.05$) except in fish exposed to 8.0 mg/L on day 1 of the exposure ($p < 0.05$).

Haemoglobin concentration (Hb) in fish decreased after the exposure though not significant ($p > 0.05$) but fish exposed to the highest concentration had significant increase in Hb at days 1 and 21 of the exposure. Significant increase in white blood cell (WBC) counts was also observed ($p < 0.05$). No significant difference ($p > 0.05$) in erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) was observed in all the concentrations during the experiment. Changes in white blood cell differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils) revealed that no significant difference ($p > 0.05$) was observed in the parameters except in monocytes. The Histopathological effects of fenthion on the liver and gill tissues were also studied. The liver and gill tissues of the control exhibited quite a normal architecture. The main histopathological changes caused by fenthion in the liver of fish exposed to different concentrations were disarray of hepatic chords, vacuolation, sinusoid enlargement and necrosis. Liver suffered from toxicity as hypertrophy of hepatocytes and necrosis. These hepatic alterations were more evident in fish exposed to the highest concentration of fenthion. Alterations in gill structure exposed to the highest concentration were oedema, lifting of lamellar epithelia, destruction of gill architecture and lamellar fusion. Gill disorder and fusion of the secondary lamellar were pronounced in all treatments. The present study revealed that sub-lethal concentrations of fenthion resulted in the alterations of the studied parameters. Histopathogical changes observed during the period of the experiment as well as the recovery shows that fenthion-induced oxidative stress is irreversible.

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AT	Adipose Tissue
AF	Application Factor
ANOVA	Analysis of Variance
C	Cartilage
CAT	Catalase
CBS	Congestion of Blood Spaces
CBV	Clogged Blood Vessels
CCV	Congestion of Central Vein
CF	Condition Factor
CPT	Congestion of Portal Triad
CV	Central Vein
CTSL	Curved Tip of Secondary Lamella
DHC	Disarray of Hepatic Chords
DL	Deformed Lamella
EDTA	Ethylenediaminetetraacetic Acid
EBA	Efferent Branchial Artery
EEL	Extensive Epithelial Lifting
EL	Epithelial Lifting
ES	Extravasated Sinusoids
ESL	Erosion of Secondary Lamella
FTL	Fusion of the Tips of Lamella
GPx	Glutathione Peroxidase
GSH	Glutathione
GR	Glutathione Reductase
H	Hepatocytes
Hb	Haemoglobin
HL	Hypertrophy of Lamella
HSI	Hepatosomatic Index
IS	Intercellular Spaces
KC	Kuffer Cells
LC_{50}	Median Lethal Concentration
LF	Lamella Fusion
LOEC	Lowest Observed Effect Concentration
LPO	Lipid peroxidation
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume

MDA	Malondialdehyde
MFC	Macro and Micro Fatty Change
N	Necrosis
NOEC	No Observed Effect Concentration
O	Oedema
OPIs	Organophosphate Insecticides
OSE	Onset of Sinusoid Enlargement
PCV	Packed Cell Volume
PEL	Partail Epithelial Lifting
pH	Potential of Hydrogen
PGL	Primary Gill Lamella
PL	Primary Lamella
PBCs	Proliferation of Blood Cells
RBC	Red Blood Cell
RH	Ruptured Hepatocytes
ROS	Reactive Oxygen Species
S	Sinusoids
SL	Secondary Lamella
SOD	Superoxide Dismutase
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TL	Tips of Lamella
V	Vacuolation
WBC	White Blood Cell

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CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Living systems encounter a variety of stresses during their continuous interaction with the environment. Environmentally-induced stresses frequently activate the endogenous production of reactive oxygen species (ROS), most of which are generated as side products of tissue respiration. Hence, constant exposure to stressors may enhance ROS-mediated oxidative damage. Increased number of agricultural and industrial wastes enter aquatic environment and cause changes in aquatic organisms (Stoliar and Lushchak, 2012). Some of them directly enhance ROS formation whereas others act indirectly, for example, by binding with cellular thiols and reducing antioxidant potential.

Fresh water fish diversity is threatened by a number of environmental stressors including contaminants and nutrient loading, overharvesting, habitat degradation and climate change (Dudgeon *et al*., 2006; Jelks *et al*., 2008). The discharge of pesticides into water bodies is of accelerating concern and is likely to continue and possibly increase in the near future (Lucero *et al*., 2000). The use of pesticides has been recognized as part of agricultural practices globally (Nwani *et al*., 2011). For centuries, pesticides have been used to enhance the production of food by controlling disease vectors (Amin and Hashem, 2012). Although the use of pesticides is of great importance, there are problems associated with it (Quinn, 2007). The aquatic organisms are vulnerable to contamination as run-offs from farms and industries end up in water bodies (Botelho *et al*., 2009) and accumulate as huge amount of residues in the environment, thereby causing a substantial hazard in the environment due to its uptake and accumulation in the food chain and drinking water. The washing of packaging materials and application equipments often carried out on the banks of water bodies help in scaling up their contamination potential (Trovo

et al., 2005). Furthermore, residues from pesticides and associated human activities such as deforestation, release of domestic, hospital and industrial effluents may contribute to a large buildup and discharge into the aquatic environment (Nwani *et al*., 2014). In the water, the molecules of pesticides may bind to the materials in suspension, accumulate in the sediment or pose a considerable risk to non-target organisms (Miles and Pfeuffer, 1997). They could also be absorbed by these animals through inhalation, orally or skin absorption with attendant physiological responses that may have effect on behaviour, morphological, enzymological, biochemical and antioxidant responses (Jordan *et al*., 2013).

Fish are particularly threatened by water pollution even though they have antioxidant defense system. Upon exposure to pollutants, free radicals such as superoxide (O_2^-), hydroxyl radicals (OH^*) and hydrogen peroxide (H_2O_2) are generated (Nwani *et al*., 2014). Under normal circumstances, there is a balance between the amount of free radicals generated in the body and the antioxidant defence systems that are able to cater for these free radicals (Kiew and Don, 2012). Problems would only arise when the amount of free radicals is beyond the normal physiological level induced by the environmental condition or produced within the body (Nose, 2002). The free radicals at excess reacts with biological macromolecules to increase the level of lipid peroxidation, protein denaturation and alterations in the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), Glutathione reductase (GR) and Glutathione Peroxidase (GPx). These antioxidant enzymes are involved to counteract the toxicity of ROS (Orbea *et al*., 2002) thereby preventing the cells and tissues from oxidative damage. Excessive ROS offset the antioxidant capacity and build up oxidative stress. Pesticide-induced oxidative stress has been a focus of toxicological research in the last decade as a possible mechanism of toxicity (Akhgari *et al*., 2003; Ciccheti and Argentin, 2003; Abdollahi *et al*.,

2004). Several circumstances promote the antioxidant defence responses in fish (Trendazor *et al*., 2006). Factors intrinsic to the fish itself as well as environmental factors such as type of diet, daily or seasonal changes in temperature, dissolved oxygen, toxins present in the water, pathogens or parasites can fortify or weaken antioxidant defences (Felton, 1995; Martinez-Alvarez, *et al*., 2005).

Haematological alteration is a good method for rapid evaluation of the chronic toxicities of compounds (Jaya and Ajay, 2014). A thin epithelial membrane separates fish blood from the water and any unfavourable change in the water body is reflected in the blood (Kori-Siakpere and Ubogu, 2008). Therefore, observations of different haematological parameters would provide a better understanding in diagnosing the effect of environmental stress on an animal and in fact, would give an insight into changes induced in the circulating fluid thereby offering a valuable knowledge on the correct monitoring of fish health status (Lazar and Lazar, 2012).

Histopathological studies could help to establish causal relations between contaminant exposure and various biological responses (Boran *et al*., 2012) and have proved to be a sensitive tool used in detecting direct effects of chemical compounds within target organs of fish in laboratory experiments (Altino and Capkin, 2007; Capkin *et al*., 2009). An organ can function normal only when its structure is normal but any structural damage to it is likely to affect the function of that organ (Muralidharan, 2014b). A study on histopathology would provide a very important and useful data concerning changes in cellular or sub cellular structure of an organ much earlier than external notification. One of the advantages of using histopathological biomarkers in environmental studies is that it allows the examination of target organs (Gernhofer *et al*., 2001). Also, the alterations found in these organs are easier to identify than functional ones (Fanta *et al*., 2003). These alterations serve as warning signs of damage to the well-being of an

organism. Studies have been conducted on histopathological changes in the gills, liver and kidney of fish exposed to pesticides which have been reported to cause pathological alterations in the exposed fish (Auta, 2001). Histopathological changes have been used as important biomarkers in environmental monitoring that allows examining specific target organs (Devi and Mishra, 2013) such as liver and the gills.

Fenthion is one of the most widely used organophosphate insecticides and avicide in agriculture and public health for controlling many sucking and biting pests (Cong *et al*., 2009; Sevgiler and Uner, 2010). While it is effective as an insecticide, it is moderately toxic to mammals and highly toxic to birds through dermal contact and inhalation (Vlastos and Ganidi, 2004). Fenthion is characterized as a cholinesterase inhibitor as well as a lipophylic compound that is bioaccumulated in the fat tissues of animals (Vlastos and Ganidi, 2004). According to Environmental Protection Agency (2003) and Pest Management Regulatory Agency (2004), all Fenthion formulations have been banned in the United States and Canada. However, it is still produced in some countries such as China and India and the application of these insecticides is ongoing in Nigeria.

Biomarkers are key molecular or cellular events that link a specific environmental exposure to a health outcome (National Institute of Environmental Health, 2014). In other words, they are measurable indicators of some biological state or condition. The use of fish as biomarkers for assessing the effect of pollution is of increasing importance and permits early detection of aquatic environmental problems (Lorez-Barea, 1990; Van-Der Oost *et al.,* 2003). Studies on pesticide-induced effects on various antioxidant enzymes in fish and other aquatic organisms can provide useful information about the ecological consequences of pesticide use (Kavitha Venkateswara-Rao, 2008). Gills are important in respiration, osmoregulation, acid-base

balance and excretion of nitrogenous wastes in fish and they serve as the first area of contact of the animal with the external environment (Deb and Das, 2013). The liver is the main organ of detoxification due to its lipophilicity (Ali *et al*., 2009). Therefore, these tissues can be used in determining biomarkers of oxidative stress such as changes in antioxidant enzymes activity or the degree of accumulation of damaged molecules. This can offer an early warning sign for exposure to redox-active xenobiotics. Enzyme analyses are becoming increasingly important in the determination of toxic effects of chemical pollutants in environmental toxicology (Muralidharan, 2014a) since alterations in enzyme activity with respect to environmental change are used as stress indicators.

1.1.2 Statement of the problem

Aquatic environments are a home to a vast diversity of organisms ranging from prokaryotes to higher vertebrates. They are also of great importance to humans, providing essentials such as water and food as well as transportation, economic opportunities, recreation, etc. Unfortunately, these environments also act as sinks for a great variety of anthropogenic pollutants, many of which are toxic. Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue due to the extent of damage caused to the aquatic organisms as the orderly balance in their physiological process is constantly under attack by the environmental adversities and the disruption in the natural food chain (Amin and Hashem, 2012; Nwani *et al*., 2013a; Muralidharan, 2014a). Most of the studies on oxidative stress in fish focused on toxicological aspects, such as effects of xenobiotics on antioxidant-enzyme activities, induction of biotransformation processes as well as on the intensity of lipid peroxidation and other biomarkers of oxidative damage (Winston and Giulio, 1991). Despite these parameters having been used as biomarkers for contaminants, a review of related literature revealed no clear

trend since fish response depends on several variables such as the species, tissues, the antioxidant parameters itself, time of exposure and contaminant concentration, besides the other physiological and environmental changes mentioned above.

Studies have demonstrated that fenthion induce DNA damage and should be considered potentially hazardous to humans (Wu *et al*., 2011). Recent research by Kanter and Celik (2012) revealed that exposure of frogs to fenthion induce an increase in melondialdehyde (MDA), and antioxidant defence systems (ADS). Israel and Sam (2012) reported biochemical changes in some tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion. Altuntas and Delibas (2002) reported that fenthion caused liver damage in Wister albino rats and may be one of the molecular mechanisms involved in fenthion-induced toxicity. Extensive works on the effect of fenthion on liver function of *Cyprinus carpio* have been performed (Gopi, 1993; Kitamure *et al*., 2003). The pigments of *Cyprinus carpio* exposed to fenthion have been studied (Muralidharan and Pillai, 2012). However, basic research that provides evidence for the oxidative stress biomarkers, haematological parameters and histopathological changes in the African catfish, *Clarias gariepinus* exposed to fenthion is lacking.

1.1.3 Justification of the study

The need to improve agriculture and public health standards has necessitated the widespread use of pesticides but these pesticides are drained into the natural habitat of fishes. Many of the pesticides are not biodegradable and due to accumulation, can enter into food chain and ultimately affect human and animal health as some of them are toxic. It is believed that fish possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species (Al-Akel *et al*., 2010; Alkahem *et al*., 2011). Therefore, it is important to study the toxic effects of pesticides on fish since they constitute an

important link in food chain and their contamination by pesticides affect the aquatic system. The study attempts to investigate the oxidative stress biomarkers, haematological parameters and histopathological changes in *Clarias gariepinus* exposed to fenthion as studies on oxidative stress in fish have opened a number of research lines on fish physiology in recent years. The study will provide information concerning the response of antioxidant defenses, haematological alterations and histopathological changes under different circumstances as well as regulatory mechanisms of these responses which will no doubt benefit aspects related to fish farming and production. The findings will also be beneficial as it will serve as a baseline for data assessment of health status of the catfish, *Clarias gariepinus* as well as provide governing bodies with information useful in management of aquatic ecosystems, information on which to base regulations concerning usage and handling of chemical compounds and essentially help to protect aquatic ecosystems from the negative effects of anthropogenic activities.

1.1.4 Objectives of the study

The objectives of this study are to;

- determine the median lethal concentration (LC_{50}); no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) of fenthion in *Clarias gariepinus*.
- determine the safe levels of fenthion in *C. gariepinus*.
- observe the behavioural responses of *C. gariepinus* associated with fenthion exposure.
- investigate the effects of fenthion on lipid peroxidation in *C. gariepinus*.
- investigate the effects of fenthion on the activities of some selected antioxidant enzymes as biomarkers of oxidative stress in different tissues of *C. gariepinus.*

- investigate the changes in haematological parameters in *C. gariepinus* exposed to fenthion.
- evaluate the changes in condition factor and hepatosomatic index of *Clarias gariepinus* exposed to fenthion.
- assess the changes in physico-chemical parameters of the test water.
- investigate the histopathological changes in the gill and liver tissues of *Clarias gariepinus* exposed to fenthion.

1.2 LITERATURE REVIEW

1.2.1 Pesticides

Pesticides are organic compounds manufactured and used for the control of pests (Ortiz-Hermandez and Sanchez-Salines, 2010). Pesticides can be defined as any chemical substance or mixture of substances intended for preventing, destroying, repelling or mitigating effects of any pest of plants and animals (Agarry *et al.,* 2013). The main groups of pesticides are fungicides, herbicides, weedicides, rodenticides and insecticides (Sharma *et al*., 2014). Among these classes of pesticides, insecticides are widely used in agriculture to control insect pests. Pesticides are categorized based on their chemical structures (Sternerson, 2004) and the various categories are often used against a number of pests (Quinn, 2007) or weeds in modern agriculture technology for high yield and management of public health (Yadav *et al*., 2010). Some of the categories include; organochlorine, organophosphate, carbamate, synthetic pyrethroids, among others (Natala and Ochoje, 2009; Naqvi *et al*., 2011; Nsikak and Aruwojoye, 2011). Pesticides enter water bodies directly or indirectly resulting in contamination of various aquatic ecosystems.

The effects of insecticide pollution on non-target organisms in the environment can be studied by detecting changes in organisms at the physiological, biochemical or molecular levels, providing “early warning” signs in monitoring environment quality (Sharma *et al*., 2014). These sensitive early warning biomarkers measure interactions between environmental xenobiotics and biological effects. Inhibition and induction of these biomarkers is a reliable approach of measuring potential impacts of pollutants on environmental organisms (Rendo `n-von Osten *et al*., 2005).

The number of pesticide in use has been on the increase since World War II (Alavi *et al*., 2008). In Nigeria, the use of pesticide has been on the increase ever since its introduction in the

early fifties for cocoa production (Agarry *et al*., 2013). It has been estimated that about 125,000 to 130,000 metric tons of pesticides are applied every year in Nigeria (Asogwa and Donga, 2009). Many of these compounds will linger in the environment for many years to come due to their environmental persistence. Humans as well as animals are inevitably exposed to pesticides, through occupational use or environmental contamination. Pesticides act selectively against certain organisms without adversely affecting others. Absolute selectivity, however, is difficult to achieve (Bolognesi, 2003).

1.2.2 Organophosphate (OP) insecticides

OP compounds have largely been used as pesticides in many parts of the world. These chemicals are readily available because of insufficient regulations to control their sale (Jeyarathnam, 1990).

OP are phosphorus-containing insecticides whose insecticidal qualities were first observed in Germany during World War II in the study of the extremely toxic OP nerve gases (sarin, soman, and tabun) (Cabello *et al*., 2001). The use of OP increased not only in agricultural environments (Fensake *et al*., 2002; Koch *et al*., 2002) but also significantly in residential and urban settings (Lu *et al*., 2001; Berkowitz *et al*., 2003). OP insecticides have been considered as alternatives to organochlorine insecticides due to their broad-spectrum pesticidal properties and relatively shorter persistence after applications (Sharma *et al*., 2005). OP agents in addition to their intended effects like control of insects or other pests are sometimes found even to affect non-target organisms including humans (Chaudhuri *et al*., 1999; Burns *et al*., 2013).

The effects of various OP insecticides in humans and animals include cholinergic and non-cholinergic biological disturbances (Gordon and Mack, 2003; Jain *et al*., 2009). The widespread use of organophosphate insecticides (OPIs) has long been shown to exert deleterious effects on living organisms (Gultekin *et al*., 2001) as they are powerful inhibitors of acetylcholinesterase (AChE). AChE is an enzyme that breaks the neurotransmitter acetylcholine (ACh), which activates choligenic neurons, the nerve cell that control the signals in the peripheral nervous system, brain and spinal cord (Bakry *et al*., 2006). If ACh is not inactivated immediately after it has done its job, the neuron becomes over stimulated leading to increased secretions, sensory and behavioural disturbances, uncoordination, depressed motor function, respiratory depression, convulsion and death (Reigart and Roberts, 1999). Recovery depends on the manufacture of more AChE. AChE is also involved in various clinical effects such as neck muscle weakness and diarrhoea which can occur due to organophosphate poisoning in humans (Serdar and Gibson, 1985; Grimsley *et al*., 1997). Measurement of the activity of this enzyme in aquatic animals does not only offer a means of detecting serious pollution by AChE agents but has the potential for indicating the extent of poisoning the animals. Through inhibition of AChE, OP poisoning is characterized by the clinical picture of acute cholinergic crisis. Other manifestations are intermediate neurotoxic syndrome and delayed polyneuropathy (Aardema *et al*., 2008). At high doses, OPs are irreversible inhibitors of AChE, producing accumulation of acetylcholine in cholinergic nervous system. However, little is known regarding possible toxic effects of exposure to low doses of OP compounds (Ceh and Majdic, 2010). Thus, in addition to AChE inhibition being the principal mode of action of OP pesticides, increased LPO has also been implicated in mediating OP-induced toxicity in animals (Kaur and Dhanju, 2004).

1.2.3 History and composition of organophosphate (OP) compounds

OP compounds were first developed by Schrader shortly before and during the Second World War. They were first used as an agricultural insecticide and later as potential chemical warfare agents (Taylor, 1996). In the late 1990 and 2000, with the advent of increased awareness of terrorism, nerve agents had gained prominence as weapons of mass destruction. In these compounds, the OPs are a group of both synthetic and biogenic OP compounds, characterized by the presence of the binding covalent, carbon to phosphorus (C-P) bond. In OPs, this carbon to phosphorus bond replaces one of the four carbon-to-oxygen-to-phosphorus bonds of the more common phosphate ester (Wanner and Metcalf, 1992). While the vast majority of phosphorus-containing organic compounds contained the phosphate ester bond, both synthetic and naturally occurring phosphonates were still of importance (Blackburn, 1981). The direct C-P linkage is chemically and thermally inert, with the result that most of organophosphonate compounds are resistant to chemical hydrolysis, thermal decomposition and photolysis compared to analogous compounds containing the more reactive N-P, S-P or O-P linkages (Figure 1). The letter R represents either ethyl or methyl. Phosphonate contains an alkyl(R-) in place of one alkoxy group (RO-). X is called leaving group and is the principal metabolite for a specific identification (Kazemi *et al*., 2012).

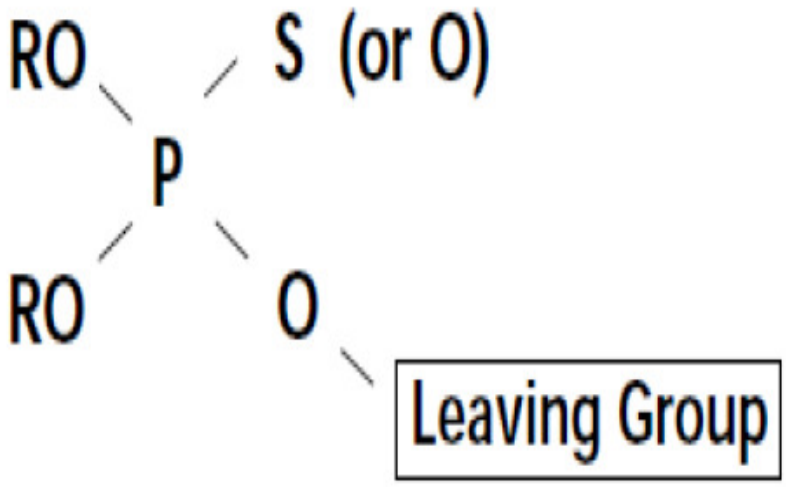

Figure 1: General chemical composition of organophosphate pesticides

Source: Kazemi *et al*., (2012).

1.2.4 Pesticide metabolism

Presently, there are more of 900 pesticides and more of 600 active pesticide ingredients in the market (Hall *et al*., 2001). Millions of tons of pesticides are applied annually; however, less than 5% of these products are estimated to reach the target organisms, with the remainder being deposited on the soil and non-target organisms, as well as escaping into the atmosphere and water (Pimental and Levitan, 1986). The metabolic fate of pesticides is dependent on abiotic factors (temperature, moisture, soil pH, etc.), microbial community or plant species (or both), pesticide characteristics (hydrophilicity, pKa/b etc.), and biological and chemical reactions. Abiotic degradation is due to chemical and physical transformations of the pesticide by processes such as photolysis, hydrolysis, oxidation, reduction, and rearrangements. Further, pesticides may be biologically unavailable because of compartmentalization, which occurs as result of pesticide adsorption to soil and soil colloids without altering the chemical structure of the original molecule. However, enzymatic transformation, which is mainly the result of biotic processes mediated by plants and microorganisms, is by far the major route of detoxification. Metabolism of pesticides may involve a three-phase process (Shimabukuro, 1985; Hatzios, 1991) (Figure 2). In the first phase involving metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to produce a more water-soluble and less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar, amino acid, or glutathione, which increases further, the water solubility and reduces toxicity compared to the parent pesticide. Phase II metabolites were reported to have little or no phytotoxicity and may be stored in cellular organelles. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also nontoxic (Hatzios, 1991). In leafy spurge (*Euphorbia esula L.*), examples of Phase III metabolism are the

conjugation of the N-glycoside metabolite of picloram with malonate and the formation of a gentibioside from the picloram glucose ester metabolite (Frear *et al*., 1989). McKellar *et al*. (1976) fed chlorpyrifos to dairy cattle for 2 weeks. The parent compound and two (oxidized and hydroxylated) metabolites were found at low levels in milk and cream (fat) and the concentrations of all three compounds decreased rapidly after cessation of administration. Hsu *et al*. (1995) recovered a maximum of 0.14% of intake via the eggs and depletion of residues from the body was rapid. Researchers had shown that pesticides have serious deleterious effects on the rumen fluid (Cook, 1969). Cook (1957) was perhaps the first to suggest that rumen liquor played an active role in hydrolyzing OPs, particularly parathion. Additional evidence by Cook (1957) indicated that metabolism of parathion by rumen microorganisms accounted for its apparent lack of toxicity to cattle. Certain OP pesticides were shown by Williams *et al*. (1963) to stimulate gas production *In vitro* by rumen holotrich protozoa, whereas these compounds had no appreciable effect when rumen bacteria served as the inoculums source.

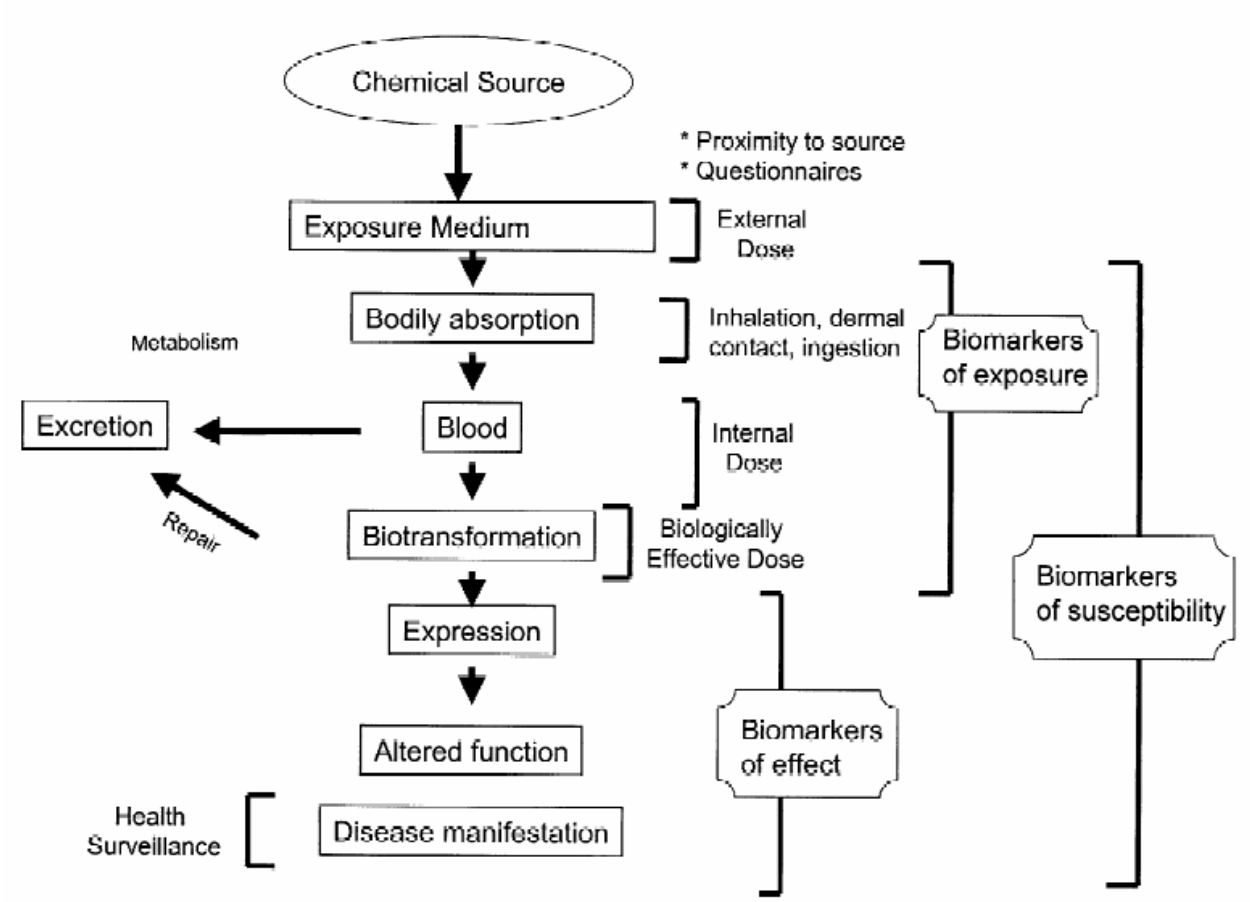

Figure 2: Schematic metabolism of pesticides in body

Source: Barr and Needham (2002).

1.2.5 Mechanism of action of OP pesticides

OP pesticides avidly bind to acetyl cholinesterase (AChE) molecules and share a similar chemical structure (Figure 3). This leads to accumulation of acetylcholine and subsequent over-activation of cholinergic receptors at the neuromuscular junctions and in the autonomic and central nervous systems (Paudyal, 2008). The rate and degree of AChE inhibition differs according to the structure of the OP compounds and the nature of their metabolites. After the initial formation of AChE-OP complex, two reactions take place; at the first reaction, spontaneous reactivation of the enzyme may occur at a slow pace, much slower than the enzyme inhibition and requiring hours to days to occur. The rate of this regenerative process solely depends on the type of OP compound: spontaneous reactivation half life that lasts 0.7 h for dimethyl and 31 h for diethyl compounds. In general, AChE-dimethyl OP complex spontaneously reactivate in less than one day whereas AChE-diethyl OP complex may take several days and reinhibition of the newly activated enzyme can occur significantly in such situation. The spontaneous reactivation can be hastened by adding nucleophilic reagents like oximes, liberating more active enzymes. These agents thereby act as an antidote in OP poisoning (Eddleston *et al.*, 2002). At the second step, with time, the AChE enzyme-OP complex loses one alkyl group making it no longer responsive to reactivating agents. This progressive time dependent process is known as ageing. The rate of ageing depends on various factors like pH, temperature, and type of OP compound; dimethyls OPs have ageing half life of 3.7 h whereas it is 33 h for diethyl OP (Worek *et al.*, 1999). The slower the spontaneous reactivation, the greater the quantity of inactive AChE available for ageing. Oximes, by catalyzing the regeneration of active AChE from enzyme-OP complex, reduce the quantity of inactive AChE available for ageing. Since ageing occurs more rapidly with dimethyl OPs, oximes are hypothetically useful

before 12 h in such poisoning. However, in diethyl OP intoxication, they may be useful for many days (Worek *et al*., 1999).

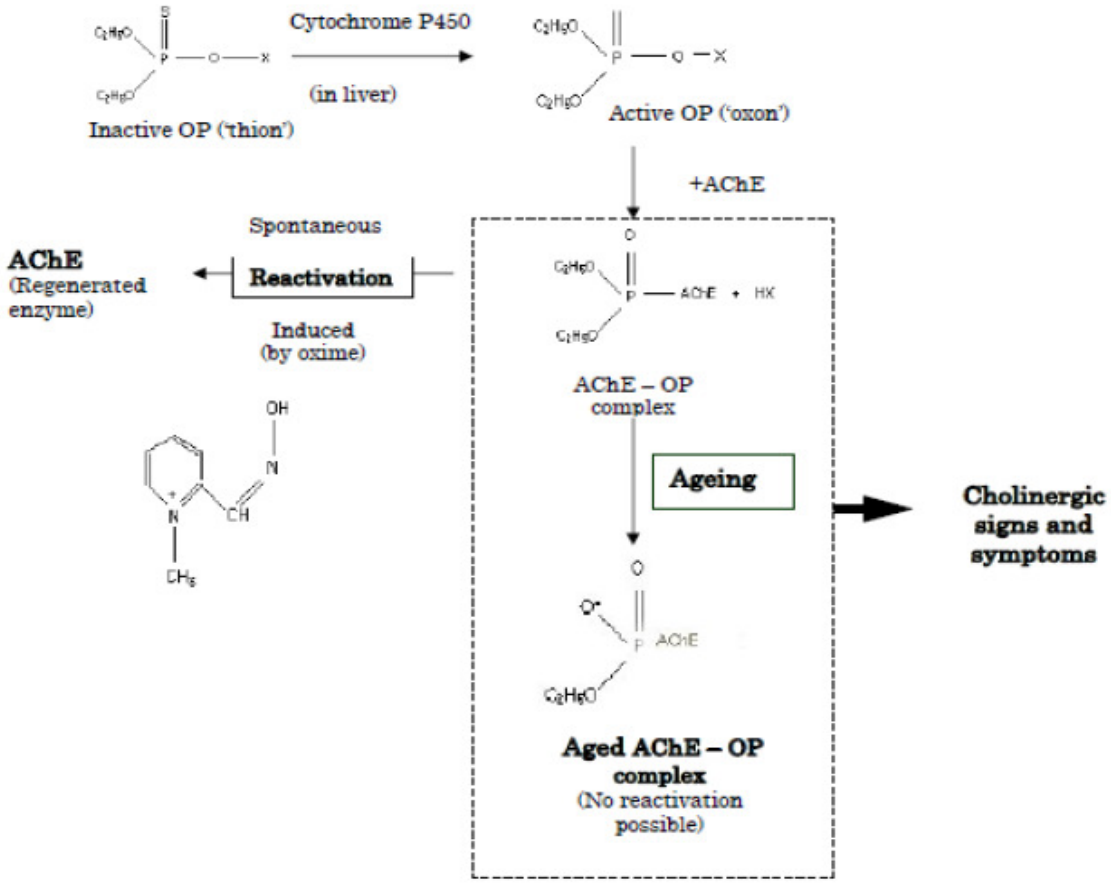

Figure 3: Schematic inhibitory of AChE by organophosphate pesticides

Source: Paudyal (2008)

1.2.6 Oxidative stress induced by pesticides

Reactive oxygen species (ROS), which include all highly reactive, oxygen-containing molecules, are an important part of the defense mechanism against infection, but excessive generation of ROS may damage tissues. ROS formed under both physiological and pathological conditions in mammalian tissues are capable of reacting with membrane lipids, nucleic acids, proteins, and enzymes and other small molecules, resulting in cellular damage. When balance between ROS and antioxidant system is lost "oxidative stress" results. ROS were found to be involved in infertility as evidenced by defective sperm function and in cryptorchidism upon exposure to toxic chemicals. It was shown that the cytochrome P450 enzymes of the steroidogenic pathway use molecular oxygen and electrons transfer from nicotinamide adenine dinucleotide phosphate (NADPH) to hydroxylate the substrate. In this process, superoxide anion or other oxygen free radicals were produced as a result of electron leakage in normal reactions or due to interaction of steroid products or other pseudosubstrates with enzymes. The antioxidant system plays an effective role in protecting testes and other biological tissues below a critical threshold of ROS thus, preventing testicular dysfunction. Antioxidant enzymes constitute a mutually supportive team of defense against ROS (Oschsendorf, 1999).

In living organisms, various ROS are generated in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes, and macrophages and per- . oxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Barlow *et al*., 2005). Pesticides have also been shown to initiate peroxidation of lipids in biological membranes (Koryagin *et al*., 2002; John *et al*., 2001). In erythrocytes, OP was found to produce morphological changes that are associated

with increased LPO (John *et al*., 2001; Thapar *et al*., 2002). The effect of lipid peroxidation (LPO) on membrane lipids, membrane receptors, and membrane-bound enzymes disturbs function, structure, and fluidity of membranes and may result in altered ion flux (Halliwell and Chirico 1993). Generation of oxidative stress and consequent LPO by pesticides was reported in rat and human brain (Ranjbar *et al*., 2002).

Pesticides may irritate lung macrophages resulting in the generation of superoxide radical. Pesticides may be more reactive with an oxygen free radical that re-oxidizes to form superoxide. The pesticide may itself be a free radical, or may deplete antioxidant defenses. The overall effect is the production of more free radicals. The activities of superoxide dismutase (SOD), glutathione (GSH) peroxides, and glutathione reductase (GSH) are decreased owing to consumption of enzymes to neutralize free radicals generated by pesticides (Amer *et al*., 2002).

Earlier studies in animals also showed that pesticides such as paraquat 2,4-D, and endosulfan inhibit the activity of erythrocyte-SOD and induce oxidative stress in hepatocytes as well as in the central nervous system (Julka *et al*., 1993). Moreover, current research indicates that many widely used agricultural chemicals induce oxidative damage in various systems of the body, such as in dopaminergic cells of the brain by modulating the antioxidant defense system (Barlow *et al*., 2005). Increased lipid peroxide levels owing to exposure of OP and carbamate pesticides were reported in several studies (Dave, 1998; Prakasam and Sethupathy, 2001; Patil *et al*., 2003).

1.2.7 Organophosphate pesticdes, oxidative stress, and antioxidant status

OP insecticides, apart from inhibition of AChE and presence of cholinergic effects (Ren~o'n-von Osten *et al*., 2005; Bajgar *et al*., 2009), hyperglycemia (Joshi and Rajini, 2009), and oxidative stress were reported as adverse effects in poisoning by OP in both humans and

animals. Oxidative stress induced by OP leads to disturbances in the function of different organs and tissues. In subchronic or chronic OP, exposure induction of oxidative stress was shown by various investigators as the predominant mechanism of toxicity (Giordano *et al*., 2007; Bűyűkokuroglu *et al*., 2008).

A case control study was conducted to evaluate the existence of oxidative stress, balance between total antioxidant capacity and oxygen free radicals in patients following acute OP exposure. The results showed significant LPO accompanied with decreased levels of total antioxidant capacity, total thiols, and AChE activity. A significant correlation existed between AChE suppression and reduced total antioxidant capacity (Ranjbar *et al*., 2002).

1.2.8 Fenthion

Fenthion (*O,O*-Dimethyl *O*-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate) (Figure 4) is a contact and stomach insecticide used against many sucking and biting pests. It is particularly effective against fruit flies, leaf hoppers, cereal bugs, stem borers, mosquitoes, animal parasites, mites, aphids, codling moths, and weaver birds. It has been widely used in sugar cane, rice, field corn, beets, pome and stone fruit, citrus fruits, cotton, olives, coffee, cocoa, vegetables, and vines (Extension Toxicity Network, 2003). Based on its high toxicity on birds, fenthion has been used to control weaver birds and other pest-birds in many parts of the world. Fenthion is also used in cattle, swine, and dogs to control lice, fleas, ticks, flies, and other external parasites (United States Environmental Protection Agency, 2001; EXTOXNET, 2003; Australian Pesticides and Veterinary Medicines Authority, 2005).

Amid concerns of harmful effects on environment, especially birds, Food and Drug Administration no longer approves uses of fenthion. However, fenthion has been extensively used in Florida to control adult mosquitoes. After preliminary risk assessments on human health

and environment in 1998 and its revision in 1999, USEPA issued an Interim Reregistration Eligibility Decision (IRED) for fenthion in January 2001. The EPA has classified fenthion as Restricted Use Pesticide (RUP), and warrants special handling because of its toxicity (Agency for Toxic Substances and Disease Registry, 2005).

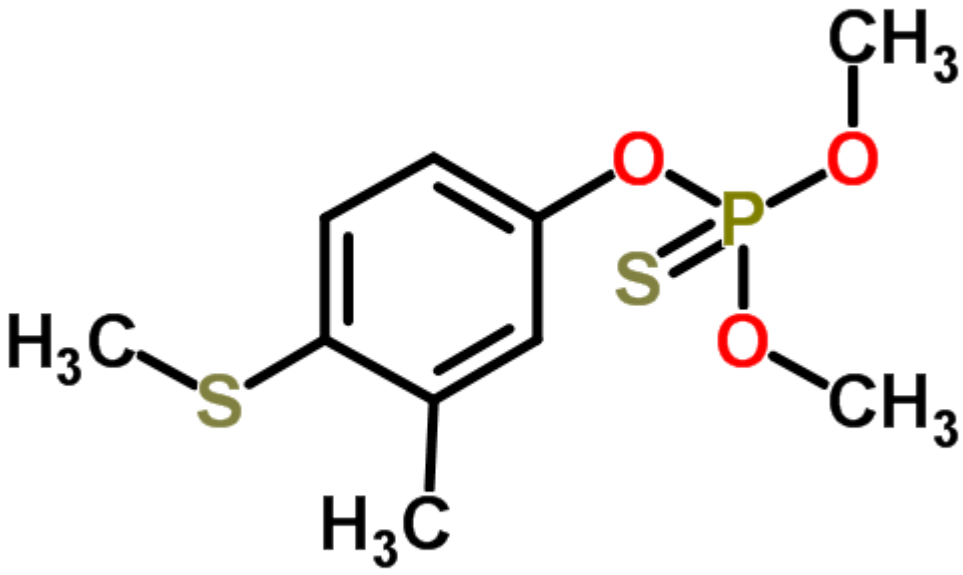

Figure 4: Structure of fenthion

Source: Royal Society of Chemistry (2015).

Fenthion exposure to general population is quite limited based on its bioavailability. Common form of fenthion exposure is occupation related, and occurs through dermal contact or inhalation of dust and sprays (ATSDR, 2005). Another likely means of contamination is through ingestion of food, especially, if it has been applied quite recently with fenthion. So far, ingestion is the most likely severe poisoning case on humans and animals. To avoid this, crops applied with fenthion should be allowed enough degradation time before harvesting. Normally, 2 - 4 weeks time is enough depending upon the type of crop. It is a moderately toxic compound in EPA toxicity class II (probable human carcinogen). It is classified by the U.S Environmental Protection Agency (EPA) as a restricted use pesticide due to the special handling warranted by its toxicity. The half life of fenthion in water under field conditions has been reported to range from 2.9 to 21.1 days for various ocean, river, swamp, lake waters (Vlastos and Ganidi, 2004). Very few studies have demonstrated the effect of fenthion on fish (Thomas and Murthy, 1976).

1.2.9 Description of Clariidae

The family Clariidae belongs to the order Siluriformes and contributes significantly to annual freshwater fish production in South and South-East Asia and Africa (Na Nakorn, 1999). This family is naturally distributed all over Africa, South and South-East Asia with the highest genetic diversity found in Africa (Ilaboya, 2011). Nearly one fifth of all known catfish species occur in Africa and South-East Asia, however, the highest diversity is found in Africa with 14 genera and 92 species (Teugel, 1986), while only two genera with 17 species are presently known from Asia (Teugels, 1996).

Generally, Clariidae catfishes are elongated, have long dorsal and anal fins, and four pairs of barbels. A remarkable characteristic of this family is the possession of suprabranchial organ, formed by folds of the second and fourth branchial arches. With this organ, they are able to

practice aerial respiration, implying that they can survive out of water for a long time. They are also known for walking on land over distances of several hundred meters, breathing atmospheric air and using their pectoral spines as a support (Teugels and Gourène, 1997).

The genus *Clarias* is the most common and popular of the Clariidae containing 32 species in Africa (Teugels, 1986), one of which is *Clarias gariepinus* (Burchell, 1822). It is of great economic importance as it is the most cultured catfish in Africa and the third most cultured catfish species in the world (Garibaldi, 1996). In Nigeria, it is the most abundant culturable fish apart from *Tilapia* (Elliot, 1985). *Clarias gariepinus* was selected for this study because it is of commercial importance as well as an aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries (Nwani *et al*., 2013a). The species serve as an attractive model for toxicity testing due to its availability throughout the season, wide distribution in the environment being open swimmers, food source for human (Amin and Hashem, 2012) and ability to acclimate easily to laboratory conditions (Nwani *et al*., 2013a).

Research has shown that *Cyprinus carpio* exposed to sub lethal concentrations (0.38, 0.19, 0.96 mg/l) of fenthion suffered alterations in blood parameters. There was decrease in RBC count, hemoglobin concentration, ESR, and clothing time whereas WBCs count and platelet count were found to be increased (Muralidharan, 2012). The author concluded that fenthion is moderately toxic to *C. carpio* based on the 96 h LC_{50} recorded. Exposure to chronic sublethal concentrations of fenthion suggests that treated fish are faced with severe metabolic stress as a result of blood alterations thereby indicating that the use of this pesticide in the fields may be a threat to fishes as well as humans. Another study on the same species revealed that its exposure to fenthion destroyed melanophores of the exposed fish and the destructive changes observed is directly related to the strength of the dose induced (Muralidharan and Pillai, 2012). The result

showed that melanophores changes noted is a significant abnormality providing a symptomatic index of toxicity.

Previous studies showed that fenthion caused significant disturbances in glutathione (GSH) redox status in the liver and brain of *Cyprinus carpio* (Sevgiler *et al*., 2007; Uner *et al*., 2009) and in the brain of *Oreochromis niloticus*. Research has also shown that fenthion affected the GSH redox cycle in a tissue-specific manner (Sevgiler and Uner, 2010). According to the authors, while GSH content decreased, GST activity increased in the liver and decreased in the kidney but the same concentration of fenthion did not affect the brain GSH contents and GST activity of the same fish. The studies above showed that fenthion altered the haematological parameters as well as antioxidant enzymes in humans, frogs, *Cyprinus carpio* but its effects on *Clarias gariepinus* is unknown hence, the need for the present study.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Experimental Fish and Chemicals

A total of 360 apparent healthy fish specimens of *Clarias gariepinus* (Burchell, 1822) (Family: Clariidae, Order: Siluriformes) of mean standard length 27.36 $\pm$ 0.23 cm and weight of 197.39 $\pm$ 2.34 g were procured from Freedom Fisheries Ltd at No.4 University Market Road Nsukka, Enugu, Nigeria. They were transported to the Fisheries wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria Nsukka. The fish were treated with 0.05% potassium permanganate ($KMnO_4$) for two minutes to avoid any dermal infections and were later acclimated for three weeks in plastic tanks of 300 litre capacity. They were fed *ad libitum* daily with available food (Coppens commercial feed) containing 35% crude protein. To maintain hygienic condition and prevent pollution caused by food and faeces, fecal matter and other waste materials were siphoned off daily. Dead fish were also removed with plastic forceps to avoid possible deterioration of the water quality. During the period of acclimation, the water in the tanks was renewed daily with well aerated tap water. The feeding was terminated 24 h prior to the range finding and acute toxicity tests to avoid interference of faeces. The acclimated fish were used for the experiments.

Commercial formulation of fenthion (600 g/ L) manufactured by Yufull Industry Co, Ltd., China was purchased from the local market and used for the study.

2.2 Determination of Median Lethal Concentration (acute toxicity), NOEC and LOEC

2.2.1 Determination of median lethal concentration (LC_{50})

Determination of the $LC_{24\text{-}96\ h}$ of fenthion was conducted by using a total of 210 fish specimens. Briefly, triplicate sets of 10 fish were randomly exposed to fenthion at

concentrations of 12.0, 24.0, 36.0, 48.0, 60.0, and 72.0 mg/ L derived from a range finding test using plastic tanks of 25 litre capacity each. Ten litres of water was poured into each of the tanks. Another set of 10 fish was simultaneously maintained with equal amount of tap water but without the test insecticide and considered as the control. Fish were not fed throughout the experiment and lethality was the toxicity end point. Fish were visually examined daily and considered dead when no respiratory movements or sudden swimming in response to gentle touch were observed. Dead fish were removed and the mortality was recorded at intervals of 24, 48, 72 and 96 h. The LC_{50-96} values of the test insecticide for the species at 24, 48, 72 and 96 h was determined by probit analysis (Finney, 1971).

2.2.2 Determination of no observed effect concentration (NOEC)

During the acute toxicity test, the NOEC was determined by the mortality data generated as the highest concentration in which no mortality was observed.

2.2.3 Determination of lowest observed effect concentration (LOEC)

LOEC was also determined using acute toxicity results. It is the highest concentration that low mortality was observed.

2.3 Determination of Safe Levels

The safe levels of the test pesticide was estimated by multiplying the 96 h LC_{50} with different application factors (AF) and were based on the methods of Hart *et al*. (1948), Sprague (1971), Committee on Water Quality Criteria (1972), National Academy of Sciences/National Academy of Engineering (1973), International Joint Commission (1977), and Canadian Council of Resources Environmental Ministry (1991).

2.4 Observation of Behavioral Responses

The behavioral responses of *C. gariepinus* upon exposure to different concentrations of fenthion were observed from 24 h to 96 h of the exposure.

2.5 Experimental Design for Chronic Exposure

The experiment consisted of four treatments of 2.0 mg/L, 4.0 mg/L, 8.0 mg/L and the control, each with three replicates. Each tank that was used contained 10 litre dechlorinated tap water with 10 fish in each of the tanks. Fish maintained in dechlorinated tap water served as the control treatment while the three other treatments were exposed to water containing 2.0, 4.0 and 8.0 mg/L of fenthion corresponding to 1/20, 1/10 and 1/5 of the 96 hour LC_{50} value that was determined after the acute toxicity assay. The exposure lasted a period of 21 days during which the fish were fed small quantity of feed approximately 1% of total body weight about an hour before the test solution was renewed daily. The feeding was to avoid catabolism and subsequent mortality. On each sampling day, (1, 7, 14, and 21), one fish from each of the treatment groups including the control was sacrificed after anesthetizing with tricaine methsnessulfonate (MS 222) to minimize stress. The blood samples were collected by piercing the caudal peduncle using 5ml sterile disposable syringe with a 22 gauge needle (Molnar, 1960). EDTA was used as anti coagulant. The blood was transferred to EDTA tubes and haematological parameters were analyzed while the liver and gill tissues were dissected and weighed separately. The tissues were quickly rinsed in cold 0.9% sodium chloride solution. Tissues from each triplicate were homogenized immediately in pre-chilled potassium phosphate buffer (1: 10 w/v, 0.1M, pH 7.0). One part of the homogenate was used for the estimation of lipid peroxidation while the other part was centrifuged for 20 minutes at 10, 500 rpm under 4^{o}C to obtain the supernatant which was stored at 4 ^{o}C for enzyme assay.

After the duration of exposure, the fish in each of the concentrations were withdrawn from the exposure of the insecticide and placed in chemical-free water. They were then observed for 7 days after which the tissues were collected and assayed.

2.6 Lipid Peroxidation (LPO)

LPO was assayed by measuring malondialdehyde (MDA) formation as described by Sharma and Krishna Murti (1986) as follows:

Procedure:

1.0ml of homogenate prepared in KCL solution was incubated at 37^0C for 30 minutes. Proteins were precipitated by adding 1ml of 10% trichloroacetic acid (TCA) and then centrifuged at 2,000 rpm for 15 minutes. 1ml of supernatant was taken as aliquot in a separate tube to which 1ml of thiobarbituric acid (TBA) reacting substance solution was added. The tubes were kept in boiling water bath for 10 minutes. After cooling the tubes, the optical density was read at 535nm. MDA activity was expressed as nmol/protein.

Calculation:

$$\text{LPO} = \frac{OD \ x \ 1000}{156 \ x \ mg \ \ protein}$$

2.7 Changes in Activities of Antioxidant Enzymes.

2.7.1. Catalase activity (CAT)

Catalase activity was assayed as described by Aebi (1984) as follows:

Procedure:

10% homogenate was prepared in 0.9% NaCl and centrifuged at 2500 rpm for 15 minutes. The supernatant was used for assay. A typical reaction mixture containing 1 ml 50nM potassium phosphate buffer (pH 7.4) and 25µl sample was added to the mixture. The reaction was initiated by the addition of dichromate acetic acid and 1 ml of H_2O_2 . The amount of H_2O_2 that was consumed was determined by recording absorbance of solution at λ 240nm. CAT activity was expressed as U/mg protein.

Calculation:

$$CAT = \frac{\Delta\ OD\ of\ Test\ x\ total\ volume\ x1000}{43.6\ x\ 0.05ml\ sample\ x\ mg\ protein}$$

2.7.2 Superoxide dismutase (SOD)

SOD activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 at 30^0C as described by Misra and Fridovich (1972).

Procedure:

Assay mixture contained 0.5 ml bicarbonate buffer, 0.5 ml EDTA, 50µ1 sample, 1ml dH_20 and was incubated for 5 minutes at room temperature. The reaction was started by the addition of 0.3 ml Epinephrine and absorbance was recorded at λ480nm for 3 minutes. SOD activity was expressed in U/mg protein.

Calculation:

$$SOD = \frac{\Delta\ OD\ of\ control\ -\Delta\ OD\ of\ test}{\Delta\ OD\ of\ control\ x mg\ protein}\ x\ 100$$

2.7.3 Reduced glutathione (GSH):

Activity of GSH was measured according to Brehe and Burch (1976).

Procedure:

0.1 ml of homogenate was put in a tube to which 0.9 ml of distilled water and 1.0 ml sulphosalicylic acid were added. The contents were mixed thoroughly and then centrifuged at 5,000 rpm for 10 minutes. Now, 0.5ml supernatant was put in a tube and similarly, blank and standards were prepared by adding 0.5 ml of distilled water and 0.5 ml of GSH standard respectively. To all the tubes, 4.5 ml of tris buffer and 0.5 ml of DTNB solution were added. After 6 minutes OD was read at λ 412 nm.

Calculation:

$$\text{Re}\,duced\ glutathion\,e = \frac{OD\ of\ unknown\ x\ 20}{OD\ of\ known}$$

2.7.4 Glutathione reductase (GR)

GR was estimated by measuring the rate of conversion of NADPH using the method of Tayarani *et al*. (1989).

Procedure:

Glutathione reductase was estimated by measuring the rate of conversion of NADPH to $NADP^+$. 10% homogenate was prepared in 1.15% KCl and centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for the assay. A typical reaction mixture containing 0.7 ml phosphate buffer, 0.1 ml oxidized glutathione (GSSG) and 0.1 ml suitably diluted homogenate was incubated at room temperature for 10 minutes. The reaction was initiated by the addition of 0.1 ml NADPH and absorbance change was recorded for 5 minutes at λ 340 nm. Specific activity was expressed as μmol NADPH oxidized $min^{-1}mg^{-1}$ protein, taking motor extinction coefficient of NADPH as 6300 m^{-1} cm^{-1}.

Calculation:

GR=

$$\frac{\Delta\ OD\ \ change\ /\min\ \ x6.3\,x1000}{mg\ of\ \ \ protein}$$

2.7.5 Glutathione peroxidase (GPx)

The activity of GPx was determined by monitoring the rate of NADPH oxidation at 340nm by the coupled reaction with glutathione reductase. The specific activity was determined using the extinction coefficient 6.22mMcm^{-1} (Lawrence and Burk, 1976).

2.8 Haematological Parameters.

2.8.1 Determination of packed cell volume (PCV)

PCV was determined using the microhematocrit method of Nelson and Morris (1989) as follows;

Procedure:

The capillary tubes were filled with blood and centrifuged for 5 minutes at 14 000 rpm using a microhematocrit centrifuge (Hawkesley & Sons, Ltd, Lancing, UK) at room temperature. Soon after centrifuging, the hematocrit was read using the microhematocrit reader. The result was expressed as the percentage of whole blood.

2.8.2 Determination of haemoglobin (Hb)

Hb determination was done using the cyanmethemoglobin method (Blaxhall and Daisley, 1973).

Procedure:

0.02mL of blood was mixed with 4.0 mL of Drabkin's solution. This was allowed to stand for 10 minutes for full colour development to occur. Absorbance was read at 540 nm with a unican spectrophotometer against the blank

2.8.3 Determination of red blood cell count (RBC)

RBC counts were estimated using a Neubauer hemocytometer as described by Rusia and Sood (1992).

Procedure:

0.02 mL of blood was pipetted from the blood sample and added to 4mL of the RBC diluting fluid (Toisson's solution) in a clean test tube to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded onto a Neubauer counting chamber, and all RBCs in the five groups of 16 small squares in the central area of the Neubauer chamber was counted using a light microscope at 40×objective. The number of cells counted for each sample was multiplied by 10 000 to obtain the RBC count per microliter of blood.

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2.8.4 Determination of white blood cell count (WBC)

For determination of leucocytes, 0.02mL of blood was pipetted into a small test tube containing 0.38mL of WBC diluting fluid (Turk's solution).to make a 1:20 dilution of the blood sample. The diluted sample was loaded on to the Neubauer counting chamber, and all cells on the four corner squares were counted using a light microscope at 10× objective. The total number of WBCs was calculated in $mm^3 \times 10^4$.

While counting, the numbers of different types of leucocytes (neutrophils, monocytes, lymphocytes, eosinophils and basophils) in the blood smears were identified as described by Hibiya (1982) and Chinabut *et al*. (1991). The number of each type of leukocytes was calculated as a percentage.

Erythrocyte indices, such as the mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), were calculated from the results of RBC count, WBC count, Hb and PCV (Dacie and Lewis, 2001) according to standard formulae:

$$\text{MCHC (g/dl)} = \frac{Hb\ (g/dl)}{PCV\ (\%)} \times 100$$

$$\text{MCH (pg/cell)} = \frac{Hb\ (g/dl) \times 10}{RBC\ count\ in\ million\ /mm3}$$

$$\text{MCV (fl/cell)} = \frac{Hb\ (g/dl) \times 10}{RBC\ count\ in\ million\ /mm3}$$

2.9 Condition Factor (CF)

On each sampling day (1, 7, 14, 21 and 7 days recovery), the condition factors of the treated fish as well as the control were calculated using the method of Anderson *et al*. (1988) after weighing the fish and measuring the standard length.

Calculation:

$$K = \frac{W \times 100}{L^3}$$

Where K= condition factor, W= weight of the fish (g), L= standard length of the fish (cm).

2.10 Hepatosomatic index (HSI)

The weight of a fish from control and each treatment (triplicate) was sampled on day 1, 7, 14, 21 and 7 days recovery test of the experiment and recorded. After collecting blood for haematological parameters, the abdominal cavity was incised from the anus to the isthmus and the liver was dissected out using a sharp safety razor. This was weighed and hepatosomatic index (HSI) was calculated (Khallaf and Authman, 1991) as follows:

$$\text{HSI} = \text{Liver weight (g)} \div \text{Body weight (g)} \times 100$$

2.11 Physico-Chemical Parameters of the Test Water

On each sampling day, water samples were collected in triplicate from each of the test water as well as the control using oxygen, and Biological Oxygen Demand (BOD) bottles and small plastic bottles of 50ml. The sampling bottles were labelled with dates and treatment groups. Until analysis, the collected water samples were kept in a cool container.

2.11.1 Measurement of water temperature

Water temperature was measured at the laboratory using a mercury-in-glass thermometer graduated in degree Celsius (0-100^{0}C).

2.11.2 Determination of dissolved oxygen

Dissolved Oxygen (DO) was determined by Winkler's method (Welch, 1948) as follows;

Procedure:

The samples were collected in BOD bottles to which 2 ml of manganous sulphate and 2 ml of potassium iodide were added and sealed. It was then mixed well and the precipitate was allowed to settle down. After settling down, 2 ml of concentrated sulphuric acid was added and mixed well until all the precipitate dissolved. 203 ml of the sample was then measured into a

conical flask and titrated against 0.025N sodium thiosulphate using starch as indicator. The end point was the change of colour from blue to colourless.

Calculation:

203 ml because (200) (300)/ (200-4) = 203 ml

1 ml of 0.025N $Na_2S_2O_3$ = 0.2 mg of oxygen

D.O. as mg/L = (0.2) x 1000 ml of thiosulphate/200

2.11.3 Determination of pH

Hydrogen ion concentration (pH) was measured using pH meter according to APHA (1992) as follows;

Procedure:

The probe and pH meter were calibrated according to manufacturer's specifications. The test water of each of the concentrations in plastic containers was deep enough to cover the tip of the probe. Care was taken to ensure that the water do not splash to avoid inaccuracy of the reading. The probe was rinsed with water from the sample before placing it in the sample container. After rinsing the probe, it was put into the sample. The meter was adjusted for temperature according to manufacturer's specifications. The meter was allowed to be in equilibrium when the measurement was steady. The pH measurement of the sample was read from the probe according to manufacturer's specification.

2.11.4 Determination of turbidity

Turbidity of samples was analyzed using a Hach ratio Turbidimeter as in APHA (1992).

Principle:

Nephelometric measurement is based on comparison of the intensity of scattered light of the sample with the intensity of light scattered by a standard reference suspension (Formazin polymer) under similar conditions.

Procedure:

1.0 g of Hydrazine sulphate was dissolved in 100 ml of distilled water. 10.0 g of Hexamethylenetetramine was dissolved in distilled water and made up to 100 ml in a volumetric flask. 5 ml of the Hydrazine sulphate solution and Hexamethylenetetramine were mixed in a volumetric flask and allowed to stand for 24 hours at about 25^0C and diluted to 1000 ml with distilled water to give 400 NTU. 10 ml of the stock solution was diluted to 100 ml with distilled water to give a standard solution of 40NTU. The nephelometer was calibrated using distilled water (zero NTU) and a standard turbidity suspension of 40 NTU. The sample was thoroughly shaken and was put in the nephelometric tube and the value was recorded.

Calculation:

Turbidity (NTU) = Nephelometer reading× Dilution factor

2.11.5 Ammonia

Ammonia concentration was determined using the method of APHA (1992).

Procedure:

10 ml of the sample was pipetted into 100 ml of conical flask. 1 cm^3 of $ZnSO_4$ and 0.5 cm^3 of NaOH were added into the conical flask. The mixture was diluted to volume with distilled water and allowed to stand for 5 minutes. It was then centrifuged for 10 minutes. The supernatant

was collected into 125 cm^3 of conical flask. 2 drops of Rochelle salt solution was added into the conical flask. 1cm^3 of Nesslers reagent was added. It was then thoroughly mixed and allowed to stand for 15 minutes. The absorbance was measured at 425nm.

2.11.6 Nitrate

The concentration of nitrate in the test water was determined using the method of APHA (1992).

Procedure:

0.722 g of AR potassium nitrate was dissolved in distilled water and made up 100 ml of the stock solution. The standard nitrate solution was prepared by evaporating 50 ml of the stock solution to dryness in the water bath. The obtained residue was dissolved in 2 ml of phenol disulfonic acid and diluted to 500 ml to give 1 ml = 10µg. The solution of various strengths ranging from 0.0 (blank) to 1.0 mg/L was prepared by diluting stock solution with water. A known volume of the sample (50 ml) was pipetted into a porcelain dish and evaporated to dryness on a hot water bath. 2 ml of phenol disulphuric was added to dissolve the residue by constant stirring with a glass rod. Concentrated solution of NaOH and distilled water was added as it was being stirred to make it alkaline. This was filtered into Nessler's tube and made up to 50 ml with distilled water. The absorbance was read at 410 nm using a spectrophotometer after the development of colour.

Calculation:

$$\text{Nitrate (mg/L)} = \frac{Absorbance\ of\ sample \times conc.of\ Std. \times 1000}{Absorbance\ \ of\ Std. \times sample\ taken}$$

2.11.7 Phosphates

The amount of phosphate was determined using the method of APHA (1992) as follows;

Procedure:

25 g ammonium molybdate was dissolved in 175 ml of distilled water. 280 ml concentrated sulphuric acid was added to 400 ml distilled water and cooled. Molybdate solution was added and the mixture was diluted to 1000 ml. 2.5 g fresh stannous chloride was dissolved in 100 ml glycerol, heated on water bath and stirred with a glass rod to hasten dissolution. 219.5 mg of dried AR potassium hydrogen phosphate was dissolved in distilled water and made up to 1000 ml, where 1 ml = 50.0 µg of phosphate. 10 ml of the stock solution was made up to give 1 ml = 0.05 mg. Standards of strength ranging from 0.0 (blank) to 0.005 mg/L at intervals of 0.01mg was prepared by diluting the stock with distilled water.

To 50 ml of the filtered sample, 4mg of ammonium molybdate reagent and about 4-5 drops of stannous chloride reagent was added. After about 10 minute but before 12 minutes, the colour that was developed was measured photometrically at 690 nm and calibration curve was prepared. A reagent blank was always run with same treatment with distilled water as sample. The value of phosphate was obtained by comparing absorbance of samples with the standard curve and expressed as mg/L.

Calculation:

$$\text{Phosphates (mg/L)} = \frac{Absorbance\ of\ sample \times conc.of\ Std. \times 1000}{Absorbance\ \ of\ Std. \times sample\ taken}$$

2.12 Histopathological Examination

On day 1, 7, 14, 21 and 7 days recovery of the research, parts of liver and gill tissues from control and each treatment were removed and preserved in 10 % phosphate buffered formalin for 24 hours, and then dehydrated by a series of upgraded ethanol solution, embedded

in paraffin, and sectioned at 5 μm thick. Tissue sections were routinely processed and stained with Hematoxylin and Eosin (H & E) and examined by light microscopy according to Roberts (2001).

Histological Procedures:

Samples of fish from both control and experimental groups were excised, rinsed in physiological saline and fixed in aqueous Boulin's fluid for 6, 12 and 8 hours respectively. The gills and liver tissues were subjected to micro techniques using the method of Bucke (1989). The tissues were dehydrated in an ethyl alcohol series of ascending concentrations, embedded in paraffin and sectioned at 5μm thickness. The tissues sectioned were then stained with haematoxilin-eosin (HE). Stained slides were cleared in xylene and mounted in synthentic resin medium. The slides were examined under low power (x4) objective and high power (x10) objective with Tension Binocular microscope. Under extreme low power, the condenser was moved into a very low position to prevent the image of the lamp bulb from obscuring the image of the micrographs. Photomicrographs were then taken at (x100) objective with Samsung ES25 4X Zoom lens digital camera. Photomicrographs of control groups were compared with those of exposed groups under the guidance of a pathologist.

2.13 Statistical Analysis

The data obtained were statistically analyzed by using SPSS 21.0 software (SPSS, Inc., Chicago, IL). Data were subjected to three-way analysis of variance and Duncan's multiple range tests to determine the significant difference at the 5% probability level. Results were expressed as means ± standard error.

CHAPTER THREE

RESULTS

3.1 Median Lethal Concentration (LC_{50}), NOEC and LOEC

3.1.1 Median lethal concentration (LC_{50})

The number of dead *C. gariepinus* exposed to different concentrations of fenthion is given in Table 1. Fish mortality increased as the concentration of fenthion increased. At 96 h exposure duration, the control group had 0% mortality while the group that had the highest concentration (72.0 mg/L) had 100% mortality. However, the experimental group exposed to 12.0 mg/L of fenthion had 0% mortalities as the control. The highest and most rapid fish mortality was observed in fish exposed to the highest concentration of fenthion. During acute toxicity assay, no mortality was recorded in the control group.

Table 1: Cumulative mortality of *Clarias gariepinus* exposed to various concentrations of fenthion.

Concentration (mg l^{-1})	Number of fish exposed	Number of deaths				Mortality (%)	Survival (%)
		24h	48h	72h	96h		
Control	30	0	0	0	0	0	100
12	30	0	0	0	0	0	100
24	30	2	2	2	4	13	87
36	30	2	6	10	12	40	60
48	30	4	10	12	18	53	47
60	30	12	16	22	24	80	20
72	30	24	30	30	30	100	0

3.1.2 Statistical endpoints of acute toxicity test

The statistical end points results of acute toxicity test are shown in Figure 5. NOEC is the highest concentration that no mortality was observed while LOEC is the highest concentration that has the lowest observed effect. NOEC was not significantly different during acute toxicity test ($p > 0.05$). LOEC of the exposure duration was not significant ($p > 0.05$) except at day 24 of the exposure where it was significant ($p < 0.05$). The LC_{50} values (with 95% confidence limits) of different concentrations (Table 2) of fenthion for *C. gariepinus* were found to be 61.99mg/L, 50.32mg/L, 45.06mg/L, and 39.97mg/L for 24 hours, 48 hours, 72 hours and 96 hours respectively following Finney's (1971) method and using statistical package for social sciences (SPSS) version 21. The LC_{50} was found to be significant throughout the duration of acute toxicity ($p < 0.05$). A dose-dependent increase and time dependent decrease were observed in mortality rate (LC_{50}) such that as the exposure time increases from 24 to 96 hours, the median lethal concentration required to kill the fish was reduced.

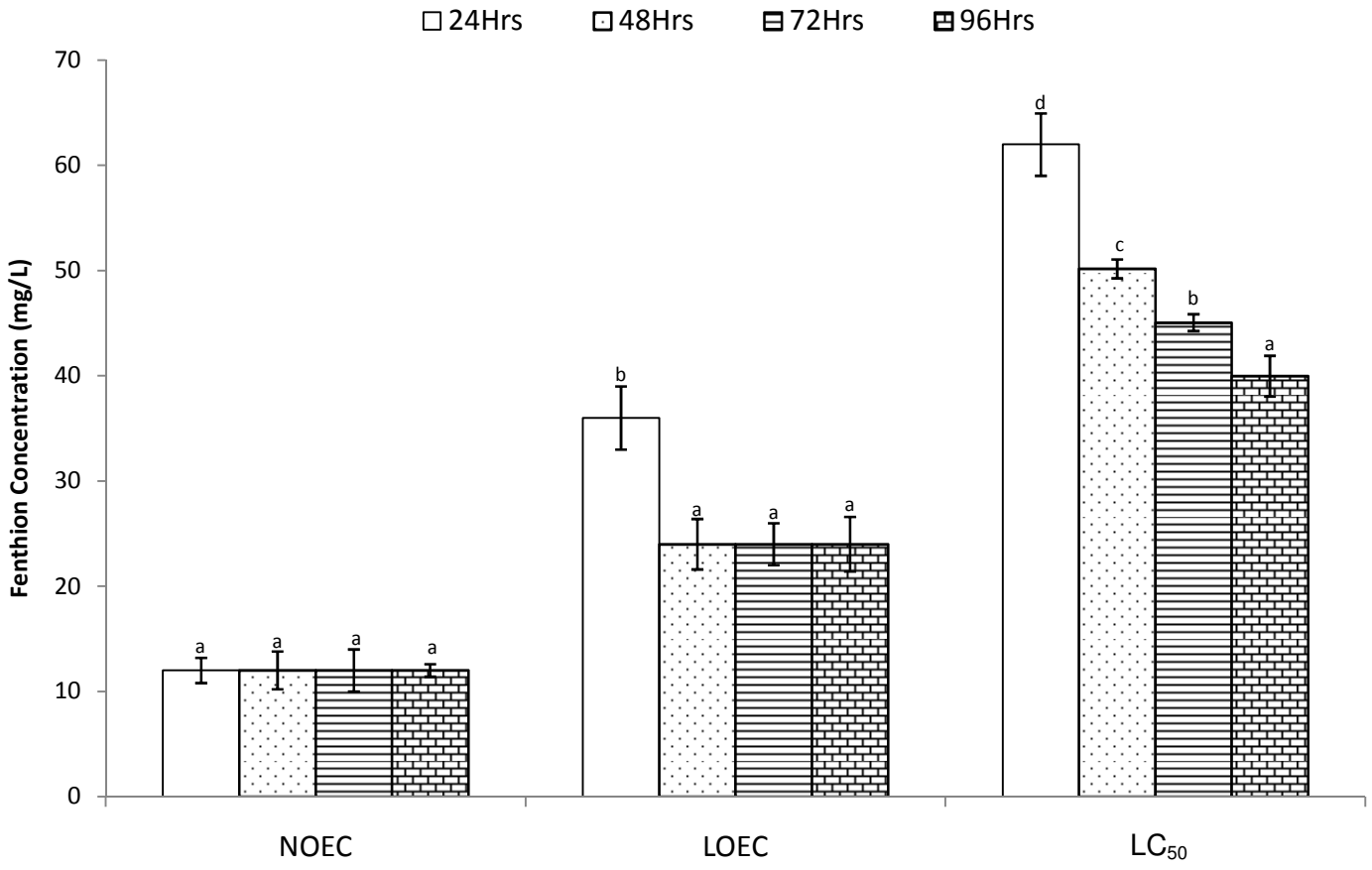

Figure 5: Statistical end points of acute toxicity testing in *Clarias gariepinus* exposed to fenthion for different durations (24, 48, 72 and 96 hours)

Table 2: Lethal concentration of fenthion (mg/L) (95% confidence interval) depending on exposure time for *Clarias gariepinus* (n=10 in three replicates).

Lethal conc. (mg/L)	Exposure time (h)			
	24	48	72	96
LC_{10}	36.14 (0.592-48.163)	30.90 (8.026-40.364)	28.04 (14.078-35.389)	23.97 (18.840-27.852)
LC_{20}	43.49 (4.730-58.259)	36.53 (14.623-46.073)	33.00 (19.957-40.116)	28.57 (23.734-32.273)
LC_{30}	49.70 (17.789-79.514)	41.22 (21.941-52.063)	37.11 (25.372-44-425)	32.42 (27.919-36.037)
LC_{40}	55.71 (35.336-161-943)	45.69 (29.749-60.291)	41.02 (30.675-49.219)	36.12 (31.920-39.793)
LC_{50}	61.99 (45.761-461.744)	50.32 (37.152-73.600)	45.06 (35.869-55.316)	39.97 (35.949-43.932)
LC_{60}	68.96 (52.311-1491.500)	55.41 (43.432-95.981)	49.49 (40.865-63.422)	44.22 (40.163-48.892)
LC_{70}	77.30 (57.828-5458.200)	61.43 (48-884-133.893)	54.71 (45.753-76.337)	49.27 (44.801-55.334)
LC_{80}	88.35 (63.651-25453.083)	69.31 (54.389-204-.045)	61.52 (51.024-96.370)	55.91 (50.413-64.594)
LC_{90}	106.33 (71.562-218778.349)	81.94 (61.549-374.994)	72.40 (58.104-136-001)	66.64 (58.744-80.917)

3.2 Estimation of Safe Levels

The results of estimated safe levels of fenthion as calculated by multiplying the 96 hour LC_{50} with different application factors are given in Table 3. The estimated safe levels of fenthion in *C. gariepinus* varied from 3.997 to 3.99 x 10^{-4}

Table 3: Estimate of safe levels of fenthion at 96 hour exposure time in *Clarias gariepinus.*

Pesticide	96h LC_{50} (mgl^{-1})	Method	AF	Safe level (mgl^{-1})
Fenthion	39.968	Hart *et al.,* (1948)*	-	9.96×10^{-1}
		Sprague (1971)	0.1	3.9968
		CWQC (1972)	0.01	3.99×10^{-1}
		NAS/NAE (1973)	0.01-0.00001	$3.99 \times 10^{-1} – 3.99 \times 10^{-4}$
		CCREM (1991)	0.05	1.9984
		IJC (1977)	5% of 96h LC_{50}	1.9984

*$C = 48\text{ h } LC_{50} \times 0.03/S^2$, where C = presumable harmless concentration and $S = 24\text{ h } LC_{50}/48\text{ h } LC_{50}$

3.3 Behavioural Responses of *Clarias gariepinus* Exposed to Fenthion

Behavioural characteristics results of *C. gariepinus* exposed to fenthion at different exposure durations are presented in Table 4. Fenthion affected the behavioural characteristics of the fish. The behavioural responses of the test fish were observed from 24 h of the exposure to 96 h (4 days). In the control group, no mortality was observed throughout the experimental period and normal behavioural responses such as non hyperactivity, normal swimming patterns and fin movements were observed. Fish exposed to different concentrations of fenthion displayed behavioural abnormalities in response to the test chemical. At the initial exposure, fish that were alert stopped swimming and remained static in position in response to the sudden changes in the surrounding environment. After a few seconds, fish in the experimental group tried to avoid the test water by swimming rapidly and trying to jump out of the water. Faster opercula movement, gulping of air, repeated closing and opening of the mouth and secretion of mucus were also observed. The mucus secreted were deposited on the buccal cavity and gills. With increasing fenthion concentrations and exposure durations, hyperactivity and jerky movement increased but swimming rate, fin movement and equilibrium status were slowed down. Fish in tanks with higher concentrations of the pesticide showed abnormal behaviour such as jumping and displaying erratic with vigorous jerky movements and gulping of air. Skin discoloration was mostly observed in fish that were exposed to the highest concentration of fenthion while it was least in the lowest concentration. After sometime, the fish lost their balance and became exhausted owing to respiratory difficulty. They settled down passively at the bottom of the tanks and died.

Table 4: Behavioural characteristics of *Clarias gariepinus* exposed to fenthion at different exposure durations

Concentration (mg/L).	Equilibrium Status	Fin movement	Hyperactivity	Jerky movement	Swimming rate
24 h					
Control	+++	+++	-	-	+++
12	+++	+++	-	-	+++
24	+++	+++	-	-	+++
36	++	++	+	++	++
48	++	++	+	+ ++	++
60	+	+	+	+++	+
72	+	+	+	+++	+
48 h					
Control	+++	+++	-	-	+++
12	+++	+++	-	-	+++
24	+++	+++	-	-	+++
36	++	++	+	+	++
48	+	++	+	++	++
60	+	+	++	+++	+
72	+	+	++	+++	+
72 h					
Control	+++	+++	-	-	+++
12	++	++	-	+	+++
24	+	+	+	++	++
36	+	+	+	++	+
48	+	+	++	++	+
60	-	-	+++	+++	-
72	-	-	+++	+++	-
96 h					
Control	+++	+++	-	-	+++
12	++	++	-	+	++
24	++	++	+	++	++
36	+	+	+	++	+
48	+	+	++	+++	+
60	-	-	+++	-	+
72	-	-	+++	-	-

None -, mild+, moderate ++, strong +++

3.4 Effects of Fenthion on Lipid Peroxidation (MDA) in the Liver and Gill Tissues of *Clarias gariepinus*

The results of changes in lipid peroxidation (MDA) in the liver and gills of *C. gariepinus* exposed to three sub-lethal concentrations of fenthion are presented in Table 5. The induction of lipid peroxidation (MDA) was both time and concentration dependent in both tissues with the lowest MDA formation observed on day 1 of the exposure. The values gradually increased as the experiment progressed with the highest formation recorded on 7 days recovery. At the 8mg/L concentration of fenthion, MDA formation in the exposed fish increased from 24.68% and 27.94% on day 1 to 75.32% and 71.17% on day 28 in the liver and gill tissues of *Clarias gariepinus*, respectively. Induction of LPO was significantly increased in fish exposed to the highest concentration throughout the duration of exposure ($p < 0.05$). No significant difference was observed in MDA of the liver and gills of fish exposed to 2.0 mg/L of fenthion throughout the experiment ($p > 0.05$).

Table 5: Effects of fenthion on lipid peroxidation (MDA, nmol/protein) in the liver and gill tissues of *Clarias gariepinus*

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	14.65 ± 3.97^{a2A}	26.81 ± 5.16^{c3B}	19.33 ± 1.72^{b1A}	33.16 ± 1.00^{c2B}	46.57 ± 0.80^{d2B}
	2.0	7.88 ± 0.91^{a1A}	12.45 ± 1.60^{b1A}	16.68 ± 1.44^{b1A}	24.31 ± 2.16^{c1A}	25.52 ± 2.30^{c1A}
	4.0	11.15 ± 0.92^{a1A}	15.97 ± 1.88^{a1A}	19.70 ± 0.94^{a1A}	32.78 ± 0.88^{b2B}	41.44 ± 5.57^{c2B}
	8.0	17.81 ± 0.95^{a2B}	24.57 ± 1.38^{b2B}	30.63 ± 1.20^{c2B}	34.47 ± 1.26^{c2B}	54.35 ± 1.43^{d3B}
Gill	control	14.43 ± 0.50^{a3A}	11.46 ± 1.20^{a1A}	17.77 ± 1.62^{b1A}	26.23 ± 0.62^{c2A}	27.56 ± 1.42^{c1A}
	2.0	6.32 ± 0.70^{a1A}	11.30 ± 0.56^{b1A}	16.57 ± 0.55^{c1A}	24.96 ± 0.72^{d1A}	31.67 ± 0.80^{e1A}
	4.0	10.35 ± 0.45^{a2A}	13.61 ± 1.33^{a1A}	17.61 ± 1.55^{b1A}	28.52 ± 1.15^{c2A}	28.58 ± 0.45^{c1A}
	8.0	11.92 ± 1.98^{a2A}	15.37 ± 0.43^{a2A}	25.70 ± 1.25^{b2A}	25.37 ± 1.06^{b1A}	30.75 ± 1.67^{c1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.5 Effects of Different Concentrations of Fenthion on Antioxidant Enzymes in *Clarias gariepinus*

3.5.1 Effects of fenthion on catalase (CAT) activity in the liver and gill tissues of *Clarias gariepinus*

Results of changes in CAT activity are presented in Table 6. The increase in CAT activity was both time and concentration dependent in all the tissues. The activity of CAT in the liver of the exposed fish increased significantly ($p < 0.05$) to the highest concentration of fenthion compared to the gills throughout the exposure except on day 7 where there was no significant difference ($p > 0.05$).

Table 6: Activity of catalase (CAT, unit/mg protein) in the liver and gill tissues of *Clarias gariepinus*

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	1.80 ± 0.39^{a1A}	1.07 ± 0.18^{a1A}	2.71 ± 1.89^{a1A}	1.30 ± 0.23^{a1A}	0.97 ± 0.33^{a1A}
	2.0	3.77 ± 0.74^{b1A}	1.20 ± 0.30^{a1A}	2.34 ± 0.65^{ab1A}	1.30 ± 0.41^{a1A}	1.69 ± 0.40^{a1A}
	4.0	4.18 ± 0.24^{b1B}	4.55 ± 0.55^{b2B}	7.07 ± 0.89^{c2B}	1.76 ± 0.12^{a1B}	0.68 ± 0.34^{a1A}
	8.0	7.39 ± 1.41^{b2B}	7.72 ± 1.00^{b3A}	7.13 ± 1.09^{b2B}	2.50 ± 0.60^{a1B}	2.64 ± 0.15^{a2A}
Gill	control	2.68 ± 0.25^{a1A}	2.47 ± 0.29^{a2A}	3.03 ± 0.32^{b2A}	2.12 ± 0.43^{a2B}	4.73 ± 0.15^{c2B}
	2.0	2.50 ± 0.14^{b1A}	5.67 ± 0.18^{d2B}	4.21 ± 1.12^{c2A}	0.68 ± 0.10^{a1A}	3.25 ± 0.10^{b1B}
	4.0	3.53 ± 0.07^{b1A}	0.87 ± 0.38^{a1A}	0.92 ± 0.27^{a1A}	1.66 ± 0.06^{a2A}	4.77 ± 0.34^{c2B}
	8.0	3.93 ± 0.77^{b1A}	7.97 ± 0.16^{c3A}	4.78 ± 1.09^{b2A}	1.33 ± 0.18^{a1A}	4.72 ± 0.08^{b2B}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.5.2 Effects of fenthion on superoxide dismutase (SOD) activity in the liver and gill tissues of *Clarias gariepinus*

Results of SOD activity are presented in Table 7. SOD activity in the liver decreased from day 1 of the exposure to day 7 in all the concentrations compared to the control but the decrease was not significant ($p > 0.05$). Activity of SOD slightly increased from day 14 of the exposure to post exposure (7 days recovery). Fluctuations were observed in SOD activity in gill tissues as both increase and decrease were observed. The increase was not significant between the gills and the liver tissues ($p > 0.05$) except on day 21 where there was significant increase in the liver of fish exposed to 8.0 mg/L of fenthion compared to the gill tissues ($p < 0.05$). Fish exposed to 2.0 mg/L and 4.0 mg/L recorded significant difference at day 14 of the exposure compared to the remaining days ($p < 0.05$).

Table 7: Activity of superoxide dismutase (SOD, unit/mg protein) in the liver and gill tissues of *Clarias gariepinus*

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	1.01 ± 0.02^{a1A}	1.01 ± 0.02^{a1A}	0.94 ± 0.01^{a1A}	1.12 ± 0.00^{a1A}	0.69 ± 0.34^{a1A}
	2.0	1.00 ± 0.01^{b1A}	0.94 ± 0.01^{a1A}	1.02 ± 0.01^{b2A}	1.10 ± 0.02^{c1A}	0.95 ± 0.02^{a1A}
	4.0	0.95 ± 0.03^{a1A}	0.88 ± 0.04^{a1A}	1.05 ± 0.01^{b2A}	1.14 ± 0.00^{c1A}	0.98 ± 0.03^{a1A}
	8.0	0.98 ± 0.01^{ab1A}	0.63 ± 0.26^{a1A}	1.05 ± 0.02^{ab2A}	1.41 ± 0.31^{b1B}	0.98 ± 0.02^{ab1A}
Gill	control	0.85 ± 0.15^{a1A}	0.96 ± 0.05^{a2A}	0.96 ± 0.01^{a1A}	1.13 ± 0.01^{b2A}	0.91 ± 0.01^{a1A}
	2.0	0.99 ± 0.01^{a1A}	0.94 ± 0.01^{a2A}	1.04 ± 0.10^{a1A}	0.90 ± 0.04^{a1A}	0.98 ± 0.05^{a1A}
	4.0	0.98 ± 0.01^{b1A}	0.83 ± 0.01^{a1A}	1.00 ± 0.03^{b1A}	1.14 ± 0.00^{c2A}	0.95 ± 0.01^{b1A}
	8.0	0.95 ± 0.03^{a1A}	0.91 ± 0.01^{a2A}	0.93 ± 0.09^{a1A}	0.89 ± 0.00^{a1A}	0.95 ± 0.04^{a1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.5.3 Effects of fenthion on reducedglutathione (GSH) activity in *Clarias gariepinus*

Table 8 shows the results of the effects of different concentrations of fenthion on the activity of GSH. There was decrease in GSH activity throughout the experiment in the liver and gill tissues compared to the control. Significant difference was observed in GSH activity in the liver of fish exposed to different concentrations of fenthion compared to the control on day 1 of the exposure ($p < 0.05$). In the gill tissues, fish exposed to 8.0 mg/L showed significant difference in GSH activity compared to the control on day one of the exposure ($p < 0.05$). In the findings, the difference in GSH activity was not significant ($p > 0.05$) between the tissues except on day 1 of the experiment where there was significant increase in GSH activity in the liver compared to the gills ($p < 0.05$).

Table 8: Effects of fenthion on reduced glutathione (GSH, mg/protein) activity in the liver and gill tissues of *Clarias gariepinus*

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	9.89 ± 0.42^{c2A}	7.52 ± 0.64^{b1A}	4.77 ± 0.43^{a1A}	5.27 ± 0.50^{a1A}	5.77 ± 0.51^{a2B}
	2.0	7.96 ± 1.33^{b3A}	8.33 ± 0.30^{b1A}	4.59 ± 0.30^{a1A}	4.93 ± 0.43^{a1A}	4.99 ± 0.15^{a1B}
	4.0	7.78 ± 0.16^{b1A}	7.80 ± 0.37^{b1A}	4.54 ± 0.27^{a1A}	4.34 ± 0.25^{a1A}	4.48 ± 0.10^{a1A}
	8.0	7.43 ± 0.11^{b1B}	7.75 ± 0.66^{b1A}	4.22 ± 0.19^{a1A}	4.22 ± 0.38^{a1A}	4.20 ± 0.15^{a1A}
Gill	control	7.29 ± 0.10^{b2A}	7.97 ± 0.16^{c1A}	4.30 ± 0.31^{a1A}	5.47 ± 0.35^{b1A}	4.60 ± 0.29^{a1A}
	2.0	7.70 ± 0.05^{b2A}	8.13 ± 0.26^{d1A}	4.09 ± 0.23^{a1A}	4.80 ± 0.19^{b1A}	3.63 ± 0.60^{a1A}
	4.0	7.28 ± 0.12^{c2A}	8.07 ± 0.03^{b1A}	4.01 ± 0.01^{a1A}	4.60 ± 0.15^{b1A}	4.02 ± 0.11^{a1A}
	8.0	6.51 ± 0.14^{b1A}	7.71 ± 0.18^{c1A}	4.00 ± 0.12^{a2A}	4.46 ± 0.54^{a1A}	4.58 ± 0.10^{a1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.5.4 Effects of fenthion on glutathione reductase (GR) activity in the liver and gill tissues of *Clarias gariepinus*

The results of the effects of fenthion on GR activity in the liver and gill tissues of exposed fish are presented in Table 9. Slight decrease in GR activity was observed throughout the experiment in the liver and gill tissues compared to the control. Significant difference was observed between the liver and the gill tissues ($p < 0.05$). There was increase in GR activity in the post-exposure (7 days recovery) compared to the exposure days.

Table 9: Effects of fenthion on glutathione reductase (GR, nmol/mg protein) activity in the liver and gill tissues of *Clarias gariepinus*

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	15.14 ± 0.60^{b3A}	11.39 ± 0.88^{a1A}	12.01 ± 1.16^{ab2A}	11.08 ± 0.33^{a1A}	25.23 ± 1.67^{c3B}
	2.0	10.02 ± 0.01^{a1A}	11.43 ± 0.90^{a1B}	13.38 ± 0.68^{b2A}	11.65 ± 0.99^{a1A}	16.03 ± 1.12^{c2A}
	4.0	12.48 ± 0.88^{b2A}	12.40 ± 1.18^{b1A}	10.35 ± 0.89^{a1A}	12.92 ± 1.40^{b1B}	16.94 ± 0.69^{c2A}
	8.0	9.04 ± 0.60^{ab1A}	9.13 ± 1.17^{ab1A}	8.35 ± 0.67^{a3A}	10.13 ± 0.72^{b1A}	20.60 ± 1.10^{c2B}
Gill	control	14.48 ± 1.82^{a1B}	12.58 ± 1.30^{a2B}	14.34 ± 0.34^{a2B}	14.46 ± 0.64^{a2B}	14.99 ± 0.85^{a1A}
	2.0	10.02 ± 0.58^{b1A}	6.40 ± 0.33^{a1A}	10.02 ± 0.59^{b1A}	12.12 ± 0.93^{b2A}	15.13 ± 0.87^{c1A}
	4.0	12.13 ± 1.57^{ab1A}	11.79 ± 0.89^{ab2A}	14.35 ± 0.34^{b2B}	9.62 ± 1.16^{a1A}	14.67 ± 0.49^{b1A}
	8.0	10.07 ± 1.56^{a1A}	12.14 ± 1.02^{a2B}	9.01 ± 0.00^{a1A}	10.93 ± 0.53^{a1A}	16.86 ± 1.46^{b1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.5.5 Effects of fenthion on glutathione peroxidase (GPx) activity in the liver and gill tissues of *Clarias gariepinus*

The results of changes in GPx activity are shown in Table 10. There was a general reduction in GPx activity throughout the duration of the experiment but no significant difference ($p > 0.05$) was observed except on days 1 and 7 where liver tissues exposed to the highest concentration differed significantly compared to the gill tissues ($p < 0.05$). Post exposure test (7 days recovery) showed no significant difference in GPx activity between the liver and gill tissues ($p > 0.05$).

Table 10: Activity of glutathione peroxidase (GPx, nmol /mg protein) in the liver and gill tissues of *Clarias gariepinus* exposed to sub-lethal concentrations of fenthion.

Tissue	Conc.	Exposure duration (days)				
	(mg/L)	1	7	14	21	7 days recovery
Liver	control	0.28 ± 0.07^{a2A}	0.24 ± 0.06^{a2B}	0.39 ± 0.34^{a1A}	0.06 ± 0.03^{a1A}	0.04 ± 0.04^{a1A}
	2.0	0.20 ± 0.09^{b2A}	0.13 ± 0.05^{ab2A}	0.09 ± 0.03^{ab1A}	0.02 ± 0.01^{a1A}	0.02 ± 0.00^{a1A}
	4.0	0.16 ± 0.03^{b2A}	0.02 ± 0.00^{a1A}	0.08 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.00^{a1A}
	8.0	0.02 ± 0.00^{a1A}	0.01 ± 0.07^{a1A}	0.07 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.10^{a1A}
Gill	control	0.27 ± 0.06^{a1A}	0.16 ± 0.05^{a1A}	0.33 ± 0.30^{a1A}	0.03 ± 0.02^{a1A}	0.01 ± 0.01^{a1A}
	2.0	0.20 ± 0.05^{b1A}	0.16 ± 0.03^{b1A}	0.06 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.00^{a1A}
	4.0	0.16 ± 0.00^{b1A}	0.20 ± 0.05^{b1B}	0.03 ± 0.00^{a1A}	0.02 ± 0.01^{a1A}	0.05 ± 0.03^{a1A}
	8.0	0.14 ± 0.05^{b1B}	0.17 ± 0.02^{b1B}	0.05 ± 0.01^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.01^{a1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

3.6 Haematological Parameters.

3.6.1 Effects of exposure to sub-lethal concentrations of fenthion on Packed cell volume (PCV) in *Clarias gariepinus*.

The results of changes in PCV are presented in Table 11. PCV of the treated fish were generally lower between the treated groups and the control throughout the experiment but the difference was not significant ($p > 0.05$). However, fish exposed to 8.0 mg/L of the concentration differed significantly in PCV compared to the control on day 1 of the exposure ($p < 0.05$). There was a progressive increase in PCV in fish exposed to the highest concentration across the duration of the experiment and the increase was significant ($p < 0.05$). Significant increase was also observed in other concentrations across the duration ($p < 0.05$).

Table 11: Effects of exposure to sub-lethal fenthion concentrations on packed cell volume (PCV, %) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	28.33 ± 1.76^{b2}	21.00 ± 2.31^{a1}	30.67 ± 3.71^{a2}	34.00 ± 1.73^{a2}	40.67 ± 4.06^{a3}
2.0	27.33 ± 2.91^{b2}	20.33 ± 0.88^{a1}	26.00 ± 4.93^{a2}	33.67 ± 2.96^{a2}	42.67 ± 4.37^{a3}
4.0	27.33 ± 2.85^{b2}	20.00 ± 2.08^{a1}	26.33 ± 0.88^{a2}	29.00 ± 2.08^{a2}	42.33 ± 2.19^{a3}
8.0	15.67 ± 2.40^{a1}	17.00 ± 3.61^{a1}	22.00 ± 4.16^{a2}	26.33 ± 1.86^{a2}	36.00 ± 2.65^{a3}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.2 Haemoglobin (Hb) changes in *Clarias gariepinus* exposed to sub-lethal concentrations of fenthion.

The results of the effects of exposure to sub-lethal concentrations of fenthion on haemoglobin are shown in Table 12. Compared to the control, haemoglobin was lower in the treated fish throughout the experiment. Significant decrease was observed in fish exposed to the highest concentration on day 1 and 21 of the exposure as well as in fish exposed to 4.0 mg/L at the post exposure ($p < 0.05$).

Table 12: Effect of exposure to sub-lethal fenthion concentrations on haemoglobin (Hb, g/dL) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	9.43 ± 0.59^{b1}	7.00 ± 0.75^{a1}	10.23 ± 1.25^{a2}	11.30 ± 0.78^{a2}	13.53 ± 1.36^{a3}
2.0	9.10 ± 0.99^{b2}	6.77 ± 0.29^{a1}	8.67 ± 1.66^{a1}	11.23 ± 1.01^{a2}	14.20 ± 1.46^{a3}
4.0	9.10 ± 0.99^{b2}	6.67 ± 0.71^{a1}	8.77 ± 0.29^{a2}	9.67 ± 0.71^{a2}	14.13 ± 0.72^{a3}
8.0	5.23 ± 0.79^{a1}	5.67 ± 1.20^{a1}	7.33 ± 1.37^{a2}	5.77 ± 0.62^{b1}	8.03 ± 0.88^{b2}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.3 Effects of exposure to sub-lethal fenthion concentrations on red blood cells in *Clarias gariepinus*.

The results of changes in red blood cell counts are presented in Table 13. Within the duration of exposure, a concentration dependent decrease was observed on day 1, 7 and 21 of the experiment. However, across durations within the same concentrations, there was slight increase in RBCs counts in all the concentrations from day 1 of the exposure to day 7, a drop in day 14 of the experiment and an increase from day 21 of the exposure to recovery but none of increase was significant ($p > 0.05$).

Table 13: Effect of exposure to sub-lethal fenthion concentrations on red blood cells (RBC, $\times 10^6$ cells/mm^3) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	146.67 ± 17.64^{b1}	130.00 ± 11.55^{b1}	108.33 ± 4.41^{c1}	126.67 ± 21.86^{b1}	121.67 ± 1.67^{b1}
2.0	123.33 ± 33.33^{b1}	126.67 ± 20.28^{b1}	88.33 ± 22.05^{b1}	129.00 ± 15.28^{b1}	98.33 ± 6.01^{a1}
4.0	108.33 ± 50.87^{a1}	110.00 ± 15.28^{a1}	88.33 ± 25.87^{b1}	130.00 ± 15.28^{b1}	106.67 ± 12.02^{a1}
8.0	98.33 ± 24.55^{a1}	100.00 ± 11.55^{a1}	66.67 ± 8.82^{a1}	100.00 ± 7.64^{a1}	100.00 ± 35.12^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.4 Effects of exposure to sub-lethal concentrations of fenthion on white blood cells in *Clarias gariepinus*

Results of white blood cells counts are presented in Table 14. The WBCs in the treated fish were generally higher than that of control in all the days except at day 7 where they were lower. During recovery, the WBCs of exposed fish were higher than the control.

Table 14: Effect of exposure to sub-lethal fenthion concentrations on white blood cells (WBC, $\times 10^3$ cells/mm^3) in *Clarias gariepinus*

Conc.	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
Control	2766.67±290.59^{a1}	2633.33±272.85^{31}	2600.00 ± 115.47^{a1}	2753.33 ± 120.19^{a1}	2666.67±66.67^{a1}
2.0	4166.67±578.31^{b3}	2766.667±240.37^{a1}	2750.00 ± 251.66^{a1}	2866.67±66.67^{ab2}	2803.33±347.87^{ab2}
4.0	4200.00±721.11^{b3}	2953.33±371.18^{b2}	2833.33 ± 266.67^{ab1}	2873.33±185.59^{ab1}	2933.33±284.80^{b1}
8.0	4800.00±702.38^{c3}	3566.67±240.37^{c2}	2966.67 ± 176.38^{b1}	3066.67±266.67^{b1}	3400±115.47^{c2}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.5 Effect of exposure to sub-lethal fenthion concentrations on MCHC (%) in *Clarias gariepinus*

Results of haematological index (MCHC) are presented in Table 15. There was no significant difference in MCHC ($p > 0.05$) between the treated fish and the control throughout the duration of exposure.

Table 15: Effect of exposure to sub-lethal fenthion concentrations on mean corpuscular haemoglobin concentration (MCHC, %) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	33.29 ± 0.08^{a1}	33.35 ± 0.10^{a1}	33.36 ± 0.08^{a1}	33.24 ± 0.00^{a1}	33.27 ± 0.03^{a1}
2.0	33.28 ± 0.08^{a1}	33.28 ± 0.11^{a1}	33.32 ± 0.07^{a1}	33.35 ± 0.07^{a1}	33.28 ± 0.03^{a1}
4.0	33.29 ± 0.04^{a1}	33.31 ± 0.10^{a1}	33.29 ± 0.09^{a1}	33.32 ± 0.07^{a1}	33.39 ± 0.03^{a1}
8.0	33.44 ± 0.15^{a1}	33.33 ± 0.00^{a1}	33.37 ± 0.10^{a1}	33.29 ± 0.04^{a1}	32.77 ± 0.65^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.6 Effect of exposure to sub-lethal fenthion concentrations on MCH (pg/cell) in *Clarias gariepinus*

The results of the effects of fenthion on MCH in *C. gariepinus* are shown in Table 16. There was no significant difference in MCH ($p > 0.05$) between the control and the treated groups throughout the experiment.

Table 16: Effect of exposure to sub-lethal fenthion concentrations on mean corpuscular haemoglobin (MCH, pg/cell) in *Clarias gariepinus*

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
Control	0.65 ± 0.04^{a1}	0.54 ± 0.01^{a1}	0.96 ± 0.16^{a2}	0.94 ± 0.16^{a2}	1.11 ± 0.12^{a3}
2.0	0.80 ± 0.14^{a1}	0.56 ± 0.10^{a1}	1.01 ± 0.06^{a1}	0.78 ± 0.15^{a1}	1.47 ± 0.22^{a2}
4.0	1.12 ± 0.30^{a1}	0.65 ± 0.16^{a1}	1.13 ± 0.23^{a1}	0.77 ± 0.13^{a1}	1.34 ± 0.17^{a1}
8.0	0.58 ± 0.09^{a1}	0.57 ± 0.08^{a1}	0.74 ± 0.32^{a1}	0.89 ± 0.12^{a1}	1.42 ± 0.31^{a2}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.7 Effect of exposure to sub-lethal fenthion concentrations on MCV (fL/cell) in *Clarias gariepinus*

The fish exposed to different concentrations of fenthion were not significantly different ($p > 0.05$) in mean corpuscular volume (MCV) results compared to control throughout the duration of the experiment (Table 17) ($p > 0.05$).

Table 17: Effect of exposure to sub-lethal fenthion concentrations on mean corpuscular volume (MCV, fL/cell) in *Clarias gariepinus*

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
Control	1.96 ± 0.12^{a1}	1.61 ± 0.03^{a1}	2.87 ± 0.47^{a2}	2.84 ± 0.49^{a2}	3.34 ± 0.35^{a3}
2.0	2.41 ± 0.41^{a1}	1.70 ± 0.30^{a1}	3.03 ± 0.17^{a1}	2.34 ± 0.44^{a1}	4.42 ± 0.66^{a2}
4.0	3.37 ± 0.90^{a1}	1.95 ± 0.49^{a1}	3.39 ± 0.70^{a1}	2.32 ± 0.40^{a1}	3.93 ± 0.54^{a1}
8.0	1.72 ± 0.26^{a1}	1.61 ± 0.21^{a1}	3.24 ± 0.22^{a2}	2.68 ± 0.36^{a2}	4.25 ± 0.92^{a3}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.8 Effect of exposure to sub-lethal fenthion concentrations on neutrophils in *Clarias gariepinus*

The results of effect of exposure to sub-lethal concentration of fenthion on neutrophil counts in *C. gariepinus* are presented in Table 18. Compared to the control, there was significant difference in fish exposed to all the concentrations on day 1 of the exposure ($p < 0.05$). On day 21 of the exposure, significant difference was observed in fish exposed to the highest concentration compared to the control ($p < 0.05$). During the 7 days recovery, neutrophil values in initially exposed fish decreased in all the concentrations of fenthion compared to the control.

Table 18: Effect of exposure to sub-lethal fenthion concentrations on neutrophils (%) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	70.00 ± 0.00^{b2}	70.00 ± 1.15^{b2}	64.67 ± 4.37^{a1}	64.00 ± 5.03^{a1}	70.67 ± 0.67^{b2}
2.0	64.67 ± 3.33^{a1}	66.33 ± 4.81^{a1}	65.67 ± 1.76^{a1}	64.00 ± 2.31^{a1}	65.33 ± 3.71^{a1}
4.0	67.33 ± 2.40^{a1}	72.00 ± 1.16^{b2}	66.00 ± 3.06^{a1}	65.33 ± 5.70^{a1}	67.33 ± 2.40^{b1}
8.0	62.67 ± 3.71^{a1}	73.33 ± 4.81^{b2}	68.67 ± 2.40^{a2}	70.00 ± 1.16^{b2}	68.67 ± 3.33^{a2}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.9 Effects of exposure to sub-lethal fenthion concentrations on lymphocytes in *Clarias gariepinus*

The results of changes in lymphocytes are presented in Table 19. The lymphocytes values of the treated fish were higher compared to the control throughout the duration of exposure but the increases were not significantly different ($p > 0.05$).

Table 19: Effect of exposure to sub-lethal fenthion concentrations on lymphocytes (%) in *Clarias gariepinus*

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
Control	27.33 ± 0.67^{a1}	26.33 ± 2.10^{a1}	32.00 ± 5.13^{a1}	32.67 ± 4.67^{a1}	27.33 ± 0.67^{a1}
2.0	34.00 ± 3.06^{a2}	35.67 ± 4.63^{a2}	24.00 ± 1.73^{a1}	34.00 ± 2.08^{a2}	$33.67 \pm 3.67^{a,2}$
4.0	31.33 ± 1.76^{a1}	26.33 ± 1.33^{a1}	32.00 ± 3.00^{a1}	36.00 ± 9.08^{a1}	31.33 ± 1.76^{a1}
8.0	33.67 ± 3.67^{a1}	35.67 ± 4.63^{a1}	34.00 ± 1.53^{a1}	28.33 ± 0.88^{a1}	34.00 ± 3.06^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.10 Effects of exposure to sub-lethal fenthion concentrations on monocytes in *Clarias gariepinus*

The results of the effects of fenthion on monocytes in *C. gariepinus* are presented in Table 20. There was no overall significant difference ($p > 0.05$) in monocytes of the treated fish compared to the control during the course of the research but lower counts was observed in monocytes of the fish exposed to all the concentrations. No significant difference was observed in monocyte values in 7 days recovery test ($p > 0.05$).

Table 20: Effect of exposure to sub-lethal fenthion concentrations on monocytes (%) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	2.00 ± 0.00^{a2}	2.00 ± 0.00^{a2}	1.67 ± 0.33^{b2}	1.33 ± 0.33^{a2}	0.50 ± 0.00^{a1}
2.0	0.67 ± 0.33^{a1}	1.67 ± 0.33^{a1}	1.33 ± 0.33^{b1}	1.33 ± 0.67^{a1}	0.33 ± 0.33^{a1}
4.0	1.33 ± 0.33^{a1}	1.33 ± 0.88^{a1}	0.30 ± 0.00^{a1}	0.33 ± 0.33^{a1}	0.67 ± 0.33^{a1}
8.0	0.67 ± 0.67^{a1}	1.67 ± 0.33^{a1}	0.33 ± 0.33^{a1}	1.00 ± 0.58^{a1}	0.33 ± 0.33^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.11 Effect of exposure to sub-lethal fenthion concentrations on eosinophils in *Clarias gariepinus*

The results of analysis of eosinophils are shown in Table 21. There was no significant difference in eosinophil values between the treated fish and the control throughout the experiment ($p > 0.05$).

Table 21: Effect of exposure to sub-lethal fenthion concentrations on eosinophils (%) in *Clarias gariepinus*

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
Control	0.40 ± 0.00^{a1}	1.33 ± 0.33^{a1}	1.33 ± 0.33^{a1}	0.33 ± 0.33^{a1}	1.33 ± 0.67^{a1}
2.0	0.33 ± 0.33^{a1}	2.00 ± 0.00^{a2}	2.00 ± 0.00^{a2}	0.33 ± 0.33^{a1}	0.67 ± 0.67^{a1}
4.0	0.33 ± 0.33^{a1}	1.33 ± 0.88^{a1}	2.00 ± 0.58^{a2}	0.00 ± 0.00^{a1}	0.67 ± 0.33^{a1}
8.0	0.00 ± 0.00^{a1}	2.00 ± 0.00^{a2}	1.00 ± 0.58^{a1}	0.00 ± 0.00^{a1}	0.67 ± 0.33^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.6.12 Effect of exposure to sub-lethal fenthion concentrations on basophils in *Clarias gariepinus*

The results of changes in basophils are presented in Table 22. There was no significant difference in basophil values between fenthion exposed fish and the control throughout the duration of the experiment ($p > 0.05$).

Table 22: Effect of exposure to sub-lethal fenthion concentrations on basophils (%) in *Clarias gariepinus*

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
Control	0.00 ± 0.00^{a1}	0.00 ± 0.00^{1}	0.33 ± 0.33^{a1}	1.00 ± 1.00^{a1}	0.00 ± 0.00^{a1}
2.0	0.33 ± 0.33^{a1}	0.00 ± 0.00^{1}	0.00 ± 0.00^{a1}	0.33 ± 0.33^{a1}	0.00 ± 0.00^{a1}
4.0	0.67 ± 0.33^{a1}	0.00 ± 0.00^{1}	0.00 ± 0.00^{a1}	1.00 ± 0.58^{a1}	0.00 ± 0.00^{a1}
8.0	0.33 ± 0.33^{a1}	0.00 ± 0.00^{1}	0.00 ± 0.00^{a1}	0.67 ± 0.67^{a1}	0.33 ± 0.33^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.7 Effects of Exposure to Sub-lethal Fenthion Concentrations on Condition Factor in *Clarias gariepinus*

The results of the effects of exposure to sub-lethal fenthion concentrations on condition factor (CF) in *C. gariepinus* during the experimental period are presented in Figure 6. There was no significant difference ($p > 0.05$) in the condition factor between the treated groups and the control during the experiment though the condition factor of the treatment groups were lower than the control. However, increases in the condition factor of the treatment groups were observed at the post exposure (7 days recovery) compared to the control but the increase was not significant ($p > 0.05$). A significant increase was observed in fish exposed to 4mg/L on the 7^{th} day of post exposure ($p < 0.05$).

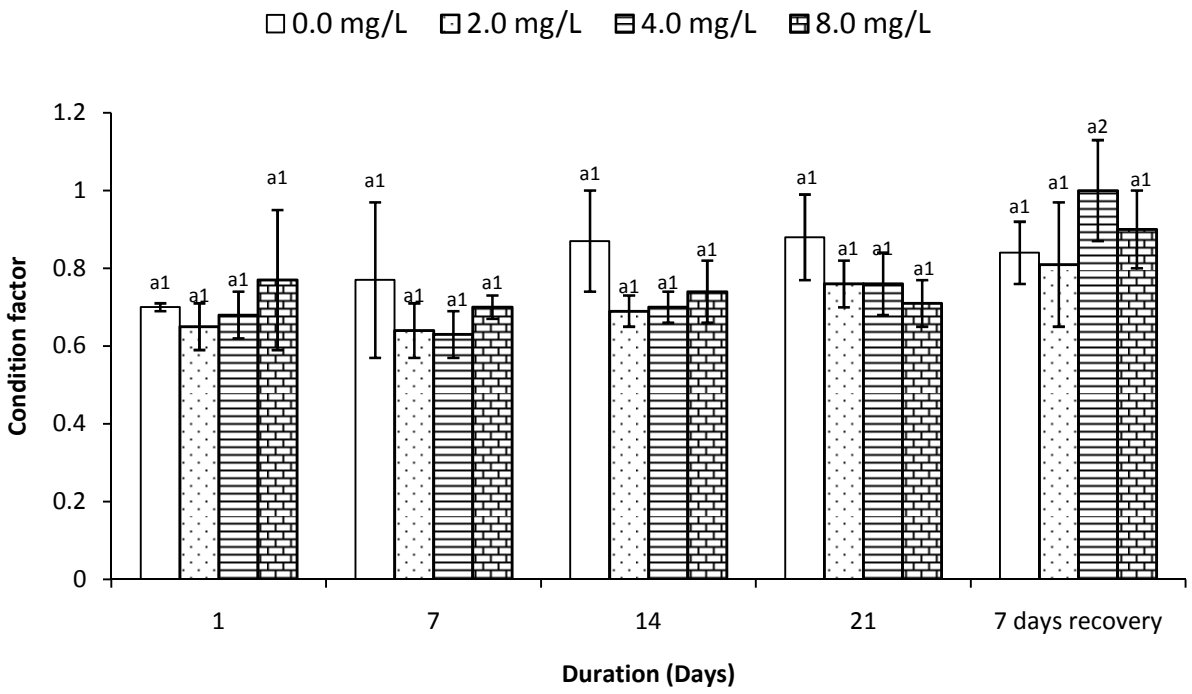

Figure 6: Effect of exposure to sublethal fenthion concentrations on the condition factor in *Clarias gariepinus*

3.8 Effects of Exposure to Sub-lethal Fenthion Concentrations on Hepatosomatic Index (HSI) in *Clarias gariepinus*

The hepatosomatic index results of *C. gariepinus* exposed to fenthion are presented in Figure 7. There was no significant difference in hepatosomatic index of the treated fish compared to the control ($p > 0.05$). However, significant increase was observed in fish exposed to all the concentrations compared to the control on day 21 of the exposure ($p < 0.05$). There was no significant difference in HSI between the control and the initially treated fish during the 7 days recovery ($p > 0.05$).

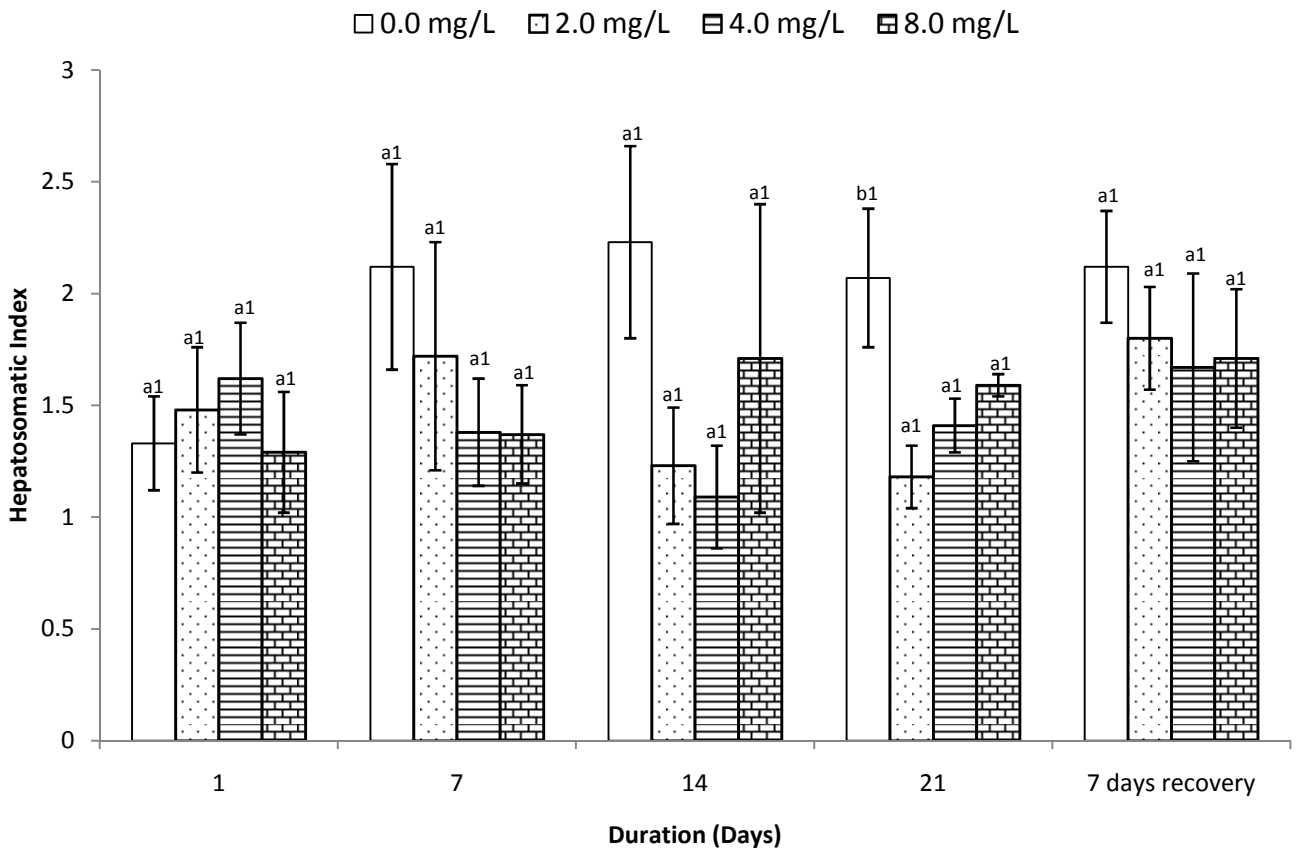

Figure 7: Effect of exposure to sub-lethal fenthion concentrations on the hepatosomatic index in *Clarias gariepinus*

3.9 Physico-chemical Parameters of the Test Water

3.9.1 Effect of exposure to sub-lethal fenthion concentrations on temperature of the test water

The results of the effect of exposure to sub-lethal concentrations of fenthion on temperature of the test water are presented in Table 23. The temperature of the control and that of the exposed fish were not significantly different in all the concentrations during the exposure period ($p > 0.05$). No significant difference in temperature was observed in the recovery test ($p > 0.05$).

Table 23: Mean values of temperature (^{o}C) for the different concentrations of fenthion

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
control	25.50 ± 0.50^{a1}	23.50 ± 0.50^{a1}	24.50 ± 0.50^{a1}	24.95 ± 1.55^{a1}	26.95 ± 0.05^{a1}
2.0	25.50 ± 0.50^{a1}	23.40 ± 0.40^{a1}	24.50 ± 0.60^{a1}	25.00 ± 0.70^{a1}	26.75 ± 0.75^{a1}
4.0	25.60 ± 0.80^{a1}	23.51 ± 0.51^{a1}	24.50 ± 0.70^{a1}	25.00 ± 0.50^{a1}	26.60 ± 0.10^{a1}
8.0	25.50 ± 0.50^{a1}	23.50 ± 0.60^{a1}	24.50 ± 0.80^{a1}	25.50 ± 0.50^{a1}	26.70 ± 1.00^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.9.2 Effect of exposure to sub-lethal concentrations of fenthion on dissolved oxygen of the test water

Results of the effects of exposure of fenthion concentrations on dissolved oxygen are presented in Table 24. There was no significant difference in dissolved oxygen concentration between the treated groups and the control throughout the duration of the exposure ($p > 0.05$). No significant difference in dissolved oxygen was observed in the recovery test ($p > 0.05$).

Table 24: Mean values of dissolved oxygen (mg/L) for the different concentrations of fenthion

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
control	5.70 ± 0.30^{a1}	7.60 ± 0.60^{a1}	6.90 ± 0.90^{a1}	5.50 ± 0.50^{a1}	6.35 ± 0.55^{a1}
2.0	5.30 ± 0.70^{a1}	8.20 ± 0.20^{a2}	6.40 ± 0.50^{a1}	$5.51 \pm 0.20^{a\,1}$	6.20 ± 0.80^{a1}
4.0	5.30 ± 0.30^{a1}	8.00 ± 0.60^{a2}	6.40 ± 0.70^{a1}	6.40 ± 0.70^{a1}	6.20 ± 0.10^{a1}
8.0	5.25 ± 0.20^{a1}	7.10 ± 0.20^{a2}	5.90 ± 0.30^{a1}	6.50 ± 0.70^{a1}	5.80 ± 0.30^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.9.3 Effect of exposure to sub-lethal fenthion concentrations on pH of the test water

Results of the changes in pH of the test water after fish exposure are shown in Table 25. No significant difference was observed in all the treated groups compared to the control throughout the duration of the exposure ($p > 0.05$). Significant difference was not observed in pH of the initially exposed fish in the recovery test ($p > 0.05$).

Table 25: Mean values of pH for the different concentrations of fenthion

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
control	7.40 ± 0.10^{a1}	7.40 ± 0.40^{a1}	7.40 ± 0.40^{a1}	7.70 ± 070^{a1}	7.04 ± 0.86^{a1}
2.0	7.60 ± 0.70^{a1}	7.20 ± 0.57^{a1}	7.10 ± 0.57^{a1}	7.50 ± 0.60^{a1}	7.00 ± 0.80^{a1}
4.0	7.50 ± 0.40^{a1}	6.70 ± 0.70^{a1}	6.80 ± 0.70^{a1}	7.50 ± 0.60^{a1}	7.45 ± 0.45^{a1}
8.0	7.70 ± 0.70^{a1}	6.60 ± 0.60^{a1}	6.60 ± 0.60^{a1}	7.70 ± 0.80^{a1}	7.75 ± 0.75^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.9.4 Effect of exposure to sub-lethal concentrations of fenthion on turbidity of the test water

Table 26 shows the results of mean values of turbidity for the different concentrations of fenthion. Turbidity was significantly low in the control compared to the treated groups throughout the experiment ($p < 0.05$). On post exposure (7 days recovery) however, there was significant difference in turbidity between the control and the exposed fish ($p < 0.05$). Turbidity was observed to be concentration dependent on day 7 of the exposure.

Table 26: Mean values of turbidity (mg/L) for the different concentrations of fenthion

Concentration	Exposure duration (days)				
(mg/L)	1	7	14	21	7 days recovery
control	23.40 ± 0.20^{a1}	19.01 ± 0.01^{a2}	21.50 ± 0.10^{a1}	10.10 ± 0.20^{a1}	30.50 ± 4.50^{a3}
2.0	30.50 ± 3.50^{b2}	23.50 ± 0.50^{b2}	28.60 ± 5.36^{b2}	12.78 ± 0.82^{b1}	21.25 ± 0.25^{a2}
4.0	35.50 ± 0.60^{b3}	23.50 ± 0.60^{b2}	31.13 ± 3.63^{b3}	12.57 ± 0.50^{b1}	22.00 ± 2.00^{a2}
8.0	35.72 ± 0.90^{b3}	25.00 ± 0.10^{b2}	37.50 ± 3.50^{b3}	18.50 ± 0.50^{c1}	23.90 ± 0.90^{a2}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration

3.9.5 Effect of exposure to sub-lethal concentrations of fenthion on ammonia concentration of the test water

The effects of exposure to sub-lethal concentration of fenthion on ammonia are shown in Table 27. Ammonia was significantly lower in fish exposed to 4mg/L of the toxicant compared to control on day 14 of the exposure period ($p < 0.05$). No significant difference was observed in ammonia concentration of the initially treated fish in the recovery test ($p > 0.05$).

Table 27: Mean values of Ammonia (mg/L) for the different concentrations of fenthion

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
control	1.03 ± 0.03^{a1}	2.12 ± 0.12^{a1}	2.01 ± 0.20^{b1}	2.15 ± 0.15^{a1}	1.84 ± 0.84^{a1}
2.0	2.16 ± 0.49^{a1}	2.03 ± 0.05^{a1}	2.20 ± 0.10^{b1}	2.17 ± 0.10^{a1}	2.34 ± 0.45^{a1}
4.0	2.08 ± 0.60^{a2}	2.65 ± 0.60^{a2}	0.29 ± 0.19^{a1}	2.02 ± 0.50^{a2}	1.95 ± 0.60^{a2}
8.0	2.48 ± 0.60^{a1}	2.58 ± 0.60^{a1}	2.23 ± 0.35^{b1}	1.67 ± 0.10^{a1}	2.41 ± 0.09^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.9.6 Effect of exposure to sub-lethal fenthion concentrations in nitrate of the test water

Table 28 shows the results of changes in nitrate of the test water. There was no significant difference in nitrate concentration in fish treated with fenthion compared to the control throughout the duration of the exposure and the recovery test ($p > 0.05$).

Table 28: Mean values of nitrate (mg/L) for the different concentrations of fenthion

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
control	0.33 ± 0.00^{a1}	0.35 ± 0.20^{a1}	0.29 ± 0.10^{a1}	0.21 ± 0.10^{a1}	0.65 ± 0.48^{a1}
2.0	0.35 ± 0.05^{a1}	0.36 ± 0.20^{a1}	0.21 ± 0.01^{a1}	0.28 ± 0.10^{a1}	0.20 ± 0.07^{a1}
4.0	0.27 ± 0.10^{a1}	0.43 ± 0.10^{a1}	0.26 ± 0.16^{a1}	0.25 ± 0.10^{a1}	0.24 ± 0.06^{a1}
8.0	0.33 ± 0.20^{a1}	0.33 ± 0.20^{a1}	0.28 ± 0.10^{a1}	0.28 ± 0.18^{a1}	0.21 ± 0.80^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.9.7 Effect of exposure to sub-lethal fenthion concentrations in phosphate of the test water

The results of exposure to sub-lethal concentrations of fenthion on phosphate of the test water are presented in Table 29. No significant difference was observed in phosphate of the treated groups in all the concentrations compared to the control ($p > 0.05$). However, there was significant difference in all the concentrations compared to the control on day 21 of the exposure ($p < 0.05$). During the recovery, no significant difference was observed ($p > 0.05$).

Table 29: Mean values of Phosphate (mg/L) for the different concentrations of fenthion

Concentration (mg/L)	Exposure duration (days)				
	1	7	14	21	7 days recovery
control	5.00 ± 0.01^{a2}	5.43 ± 0.50^{a2}	2.13 ± 0.10^{a3}	2.26 ± 0.30^{a1}	6.02 ± 0.11^{a3}
2.0	4.10 ± 0.20^{a1}	5.50 ± 0.50^{a1}	5.28 ± 0.20^{a1}	5.27 ± 0.50^{b1}	4.75 ± 1.25^{a1}
4.0	4.70 ± 0.70^{a1}	4.96 ± 0.60^{a1}	5.56 ± 0.50^{a1}	5.58 ± 0.60^{b1}	5.20 ± 0.20^{a1}
8.0	5.43 ± 0.60^{a1}	5.01 ± 0.30^{a1}	5.58 ± 0.50^{a1}	5.56 ± 0.90^{b1}	4.30 ± 1.20^{a1}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between exposure periods within the same concentration.

3.10 Histopathological Results

3.10.1 Histopathological observations in gills of *Clarias gariepinus* exposed to fenthion

Histopathological changes in *C. gariepinus* were observed during the experiment. No alterations were observed in the gills of the control (Plate 1). The severity and frequency of gill lesions were found to be more pronounced in gills of fish exposed to the highest concentration of fenthion (plates 2 to 10). Histopathological alterations were concentration and duration dependent. The most common pathological changes in the gills of the exposed fish species were fusion of the tip of lamella, deformed lamella, congestion of blood spaces, epithelial lifting, intercellular space variation and hypertrophy of lamella.

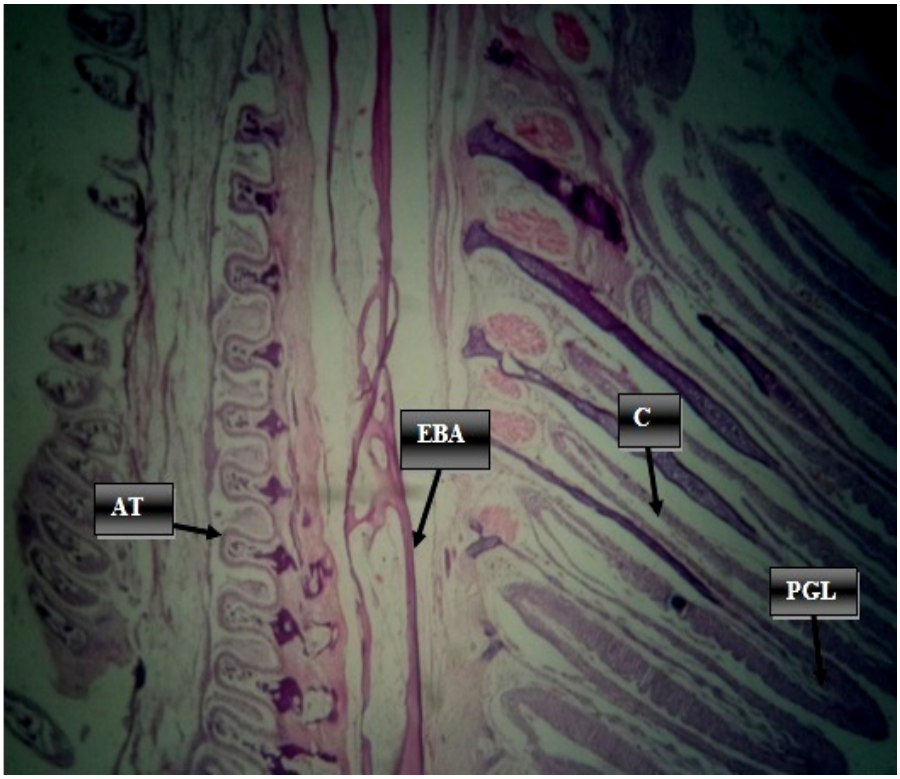

Plate 1: Photomicrograph of a section of the gill from a control *Clarias gariepinus* showing normal adipose tissue (AT), efferent branchial artery (EBA), cartilage (C), primary gill lamella (PGL). H&E mag x100

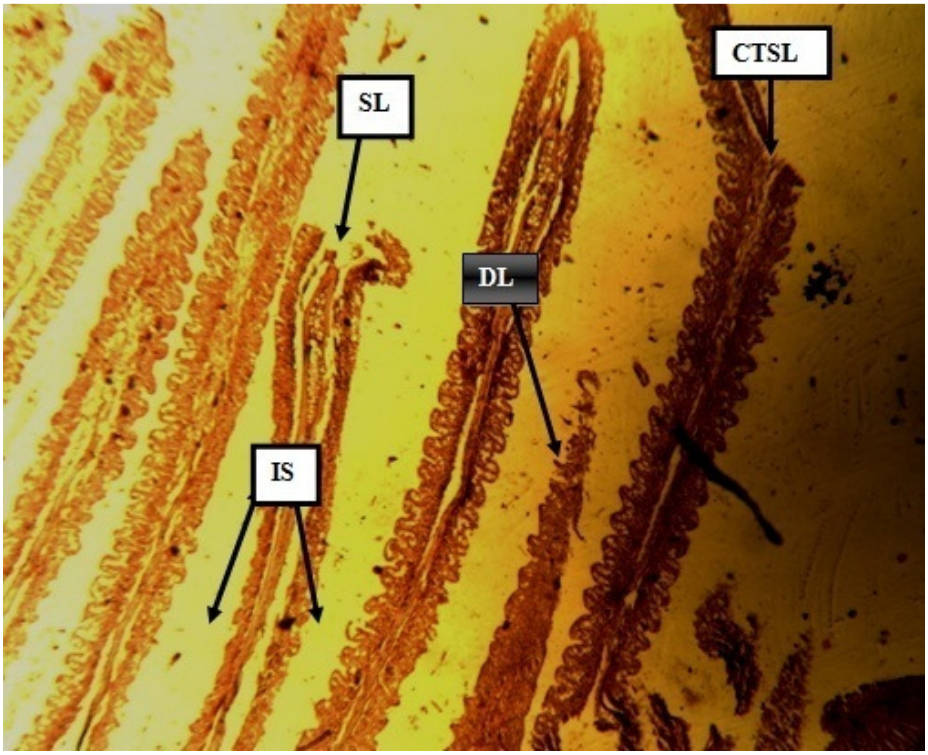

Plate 2: Photomicrograph of sections of the gills of *Clarias gariepinus* exposed to 2.0mg/L of fenthion showing variation in intercellular spaces (IS), fusion of the tip of secondary lamella (SL), deformed lamella (DL) and destruction of the curved tip of secondary lamella (CTSL) at day 1 of the exposure. H&E mag x100

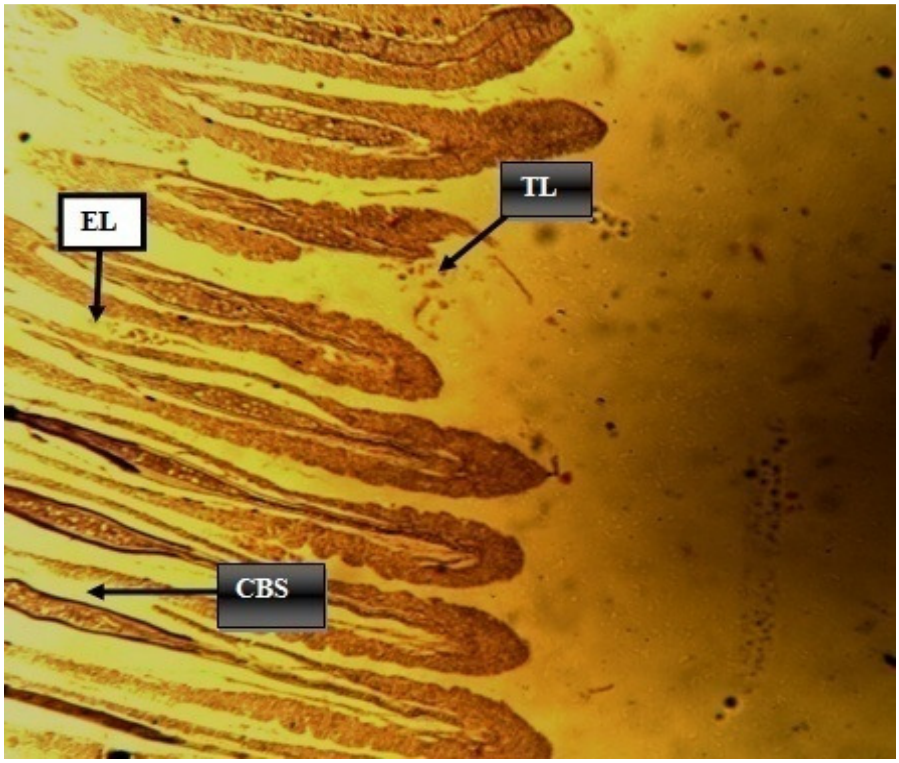

Plate 3: Photomicrograph of sections of the gills of *Clarias gariepinus* exposed to 2.0mg/L of fenthion showing epithelial lifting (EL), destruction of the tips of lamella (TL) and congestion of blood spaces (CBS) at day 14 of the experiment. H&E mag x100

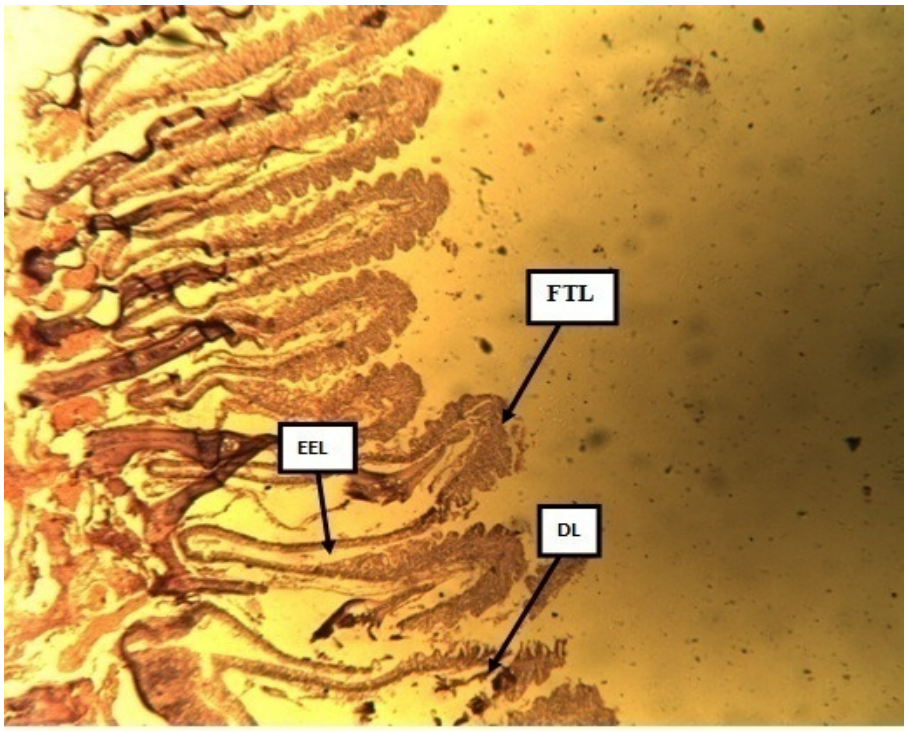

Plate 4: Photomicrograph of sections of the gills of *Clarias gariepinus* exposed to 2.0mg/L of fenthion showing extensive epithelial lifting (EEL), fusion of the tips of lamella (FTL) and deformed lamella (DL) at post exposure. H&E mag x100

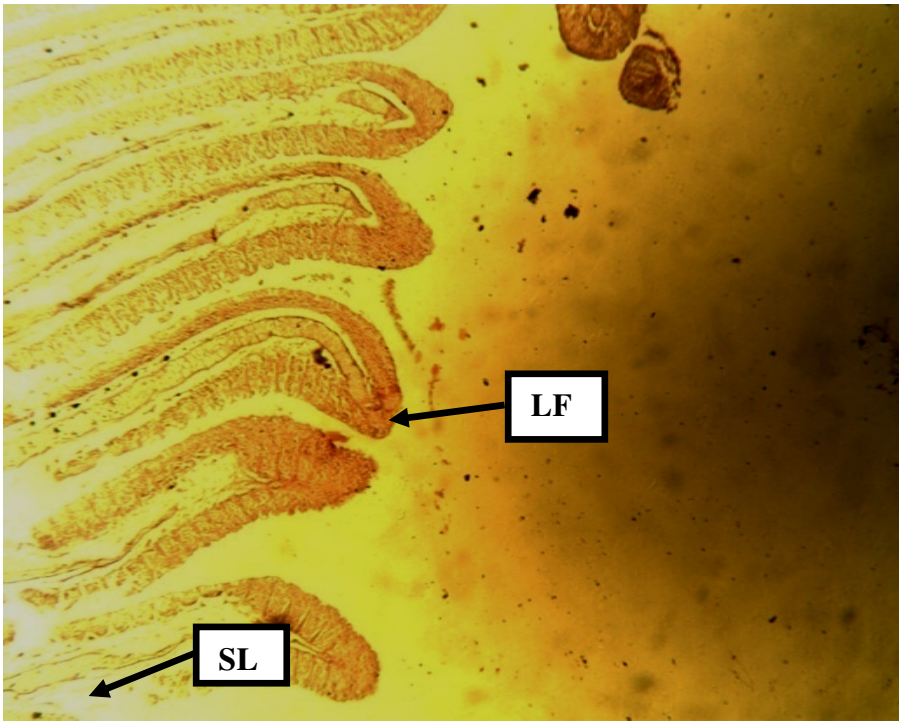

Plate 5: Photomicrographs of sections of gills of *Clarias gariepinus* exposed to 4.0mg/L of fenthion showing severe lamella fusion (LF) and severe destruction of secondary lamella (SL) at day 1 exposure.

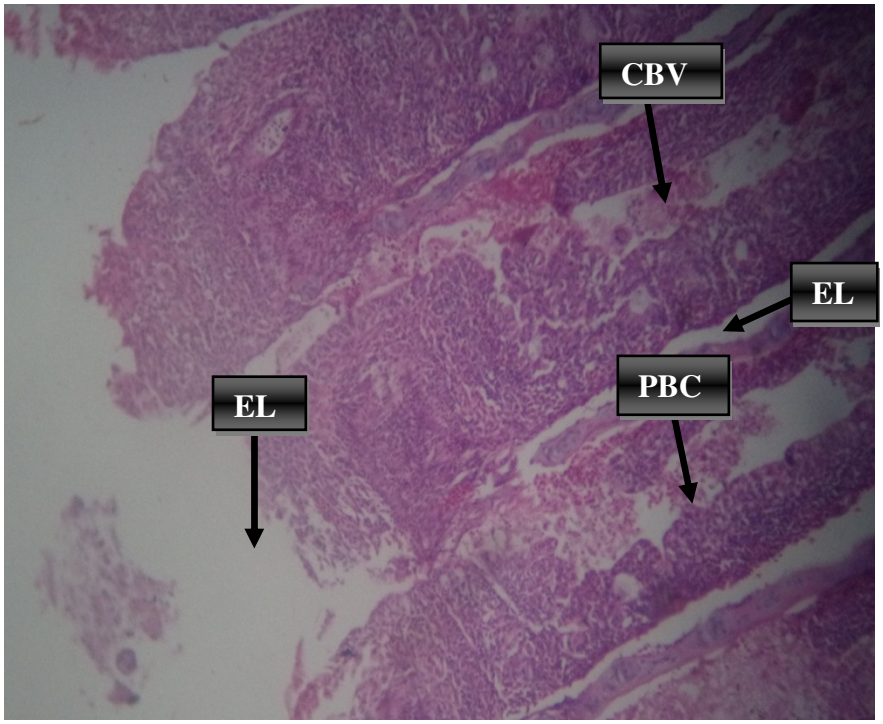

Plate 6: Photomicrograph of sections of the gills in *Clarias gariepinus* exposed to 4.0mg/L of fenthion showing .proliferation of blood cells (PBCs), epithelial lifting (EL) and congestion of blood vessels (CBV) at day 14 of the exposure.

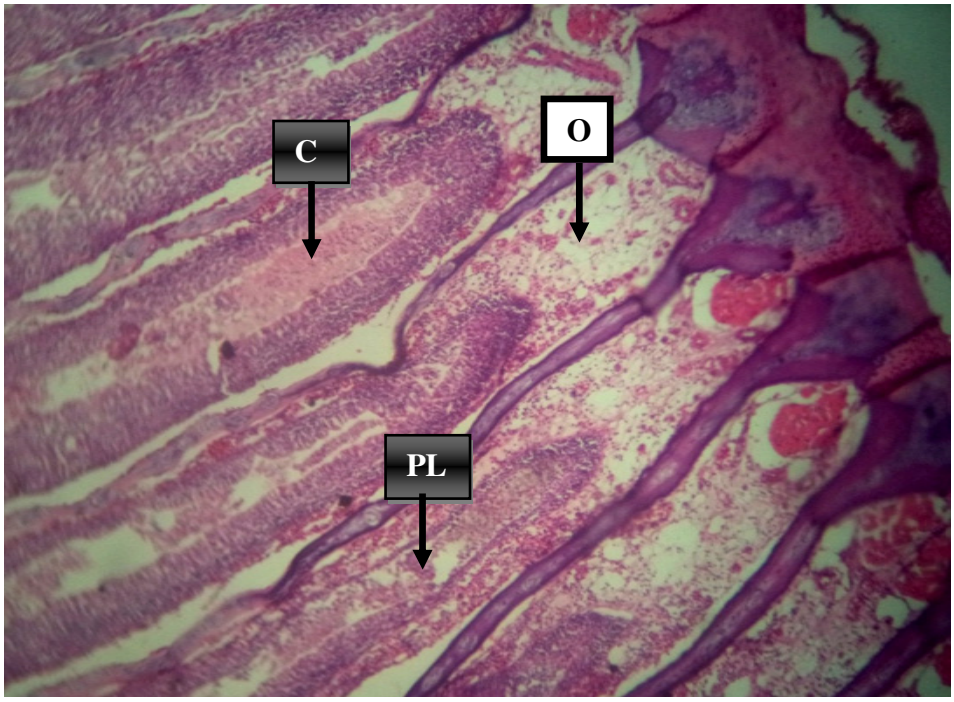

Plate 7: Photomicrograph of sections of the gills of *Clarias gariepinus* exposed to 4.0mg/L of fenthion showing congestion (C), oedema (O) and destruction of primary lamella (PL) at post exposure.

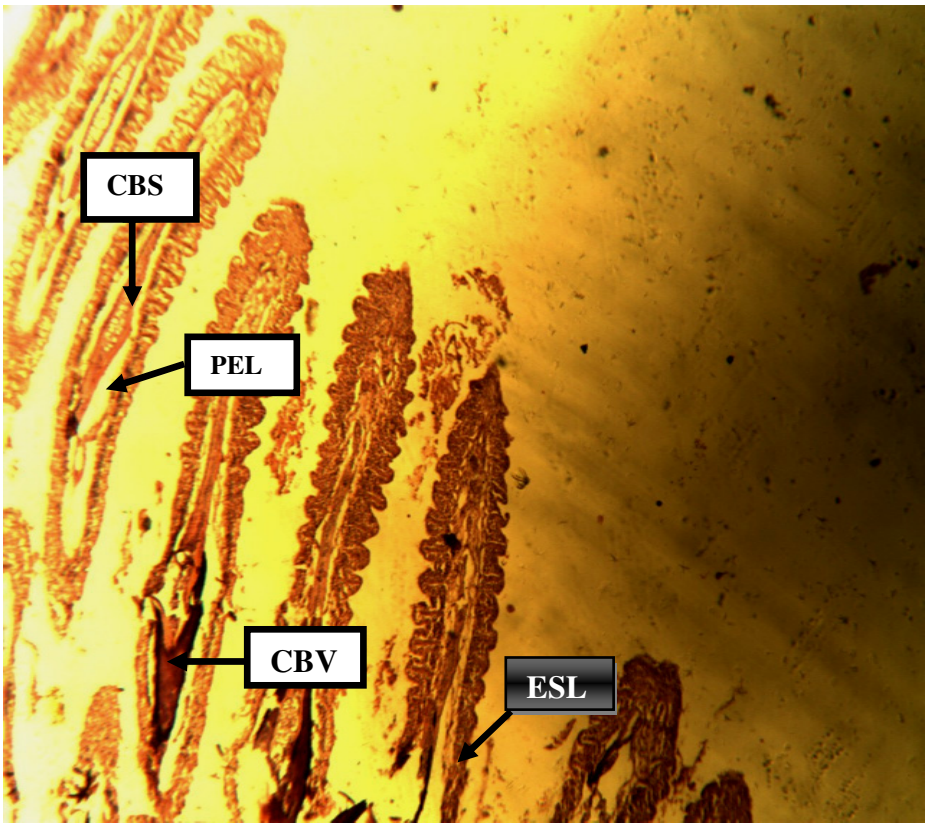

Plate 8: Photomicrograph of sections of gills in *Clarias gariepinus* exposed to 8.0mg/L showing congestion of blood spaces (CBS), partial epithelial lifting (PEL), clogged blood vessel (CBV) and erosion of part of the secondary lamella (ESL)at day 1 of the exposure. H&E. mag x100

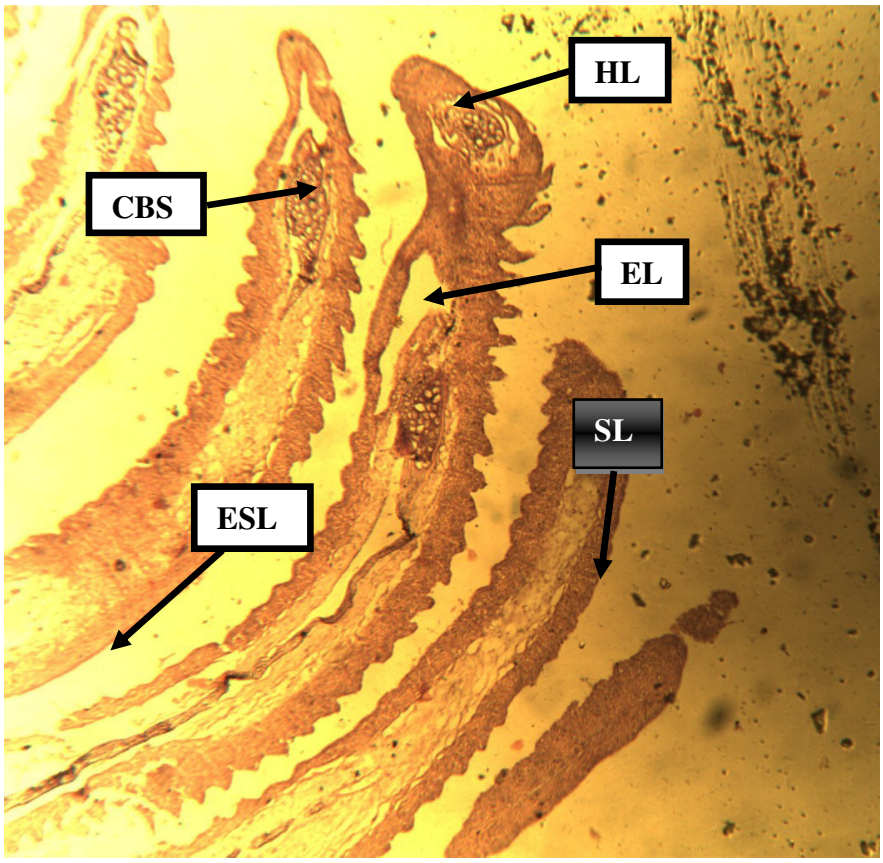

Plate 9: Photomicrograph of the gill sections of *Clarias gariepinus* exposed to 8.0mg/L of fenthion showing hypertrophy of the tip of lamella (HL), epithelial lifting (EL), erosion of the secondary lamella (ESL) and congestion of blood spaces (CBS) at day 14 of the exposure. H& E mag x100.

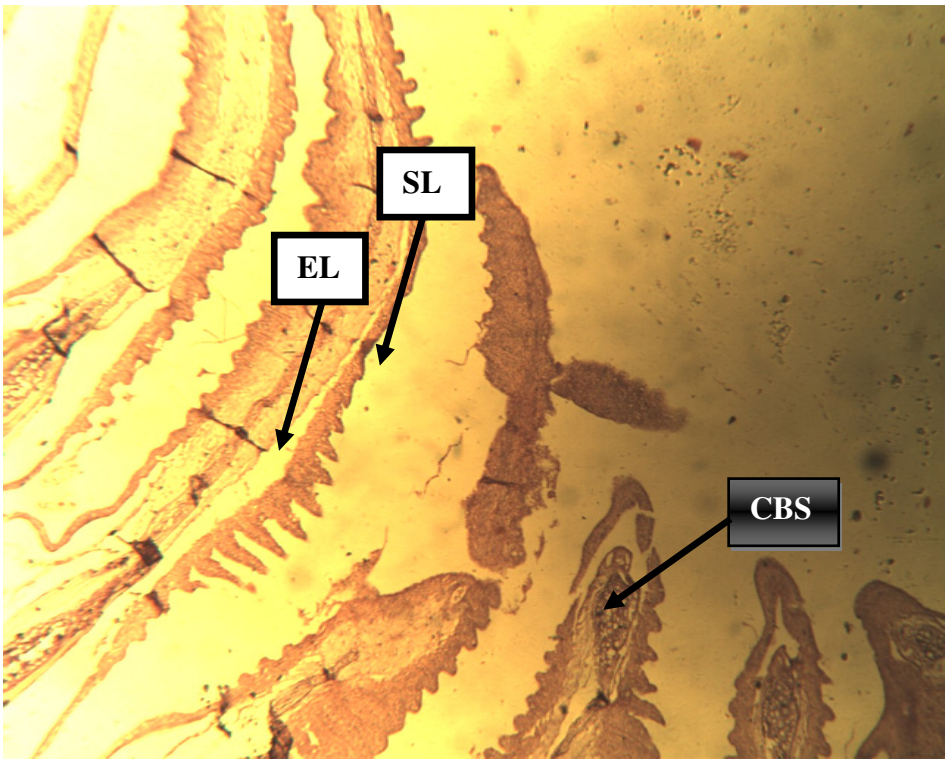

Plate 10: Photomicrograph of sections of the gills of *Clarias gariepinus* exposed to 8.0mg/L of fenthion showing destroyed secondary lamella (SL), congestion of blood spaces (CBS), and epithelial lifting (EL) at post exposure. H&E. mag x100.

3.10.2 Histopathological observations of liver in *Clarias gariepinus* exposed to fenthion

Histopathological changes observed in the liver of *C. gariepinus* exposed to fenthion are presented in Plates 11 to 19. No alterations were observed in the liver of the control (Plate 11) as cellular architecture with distinct hepatic cells and sinusoid spaces were observed to be intact. The sinusoids have irregular boundaries composed of only signal layer of fenestrated endothelial cells and large irregular phagocyte cells which are known as kuffer cells and a central vein. During the period of exposure as well as post- exposure, the microscopical examination of liver cells revealed loss of their normal morphology. Fenthion treated groups showed several signs of toxicity as necrotic cells. The severity and frequency of liver lesions were found to be more pronounced in fish exposed to the highest concentration of fenthion. The liver alterations were concentration and duration dependent. The most common pathological changes observed in the liver of the exposed fish species were cytoplasmic vacuolation in the cytoplasm of the liver cells, congestion of blood, necrosis, disarray of hepatic chords and sinusoids enlargement.

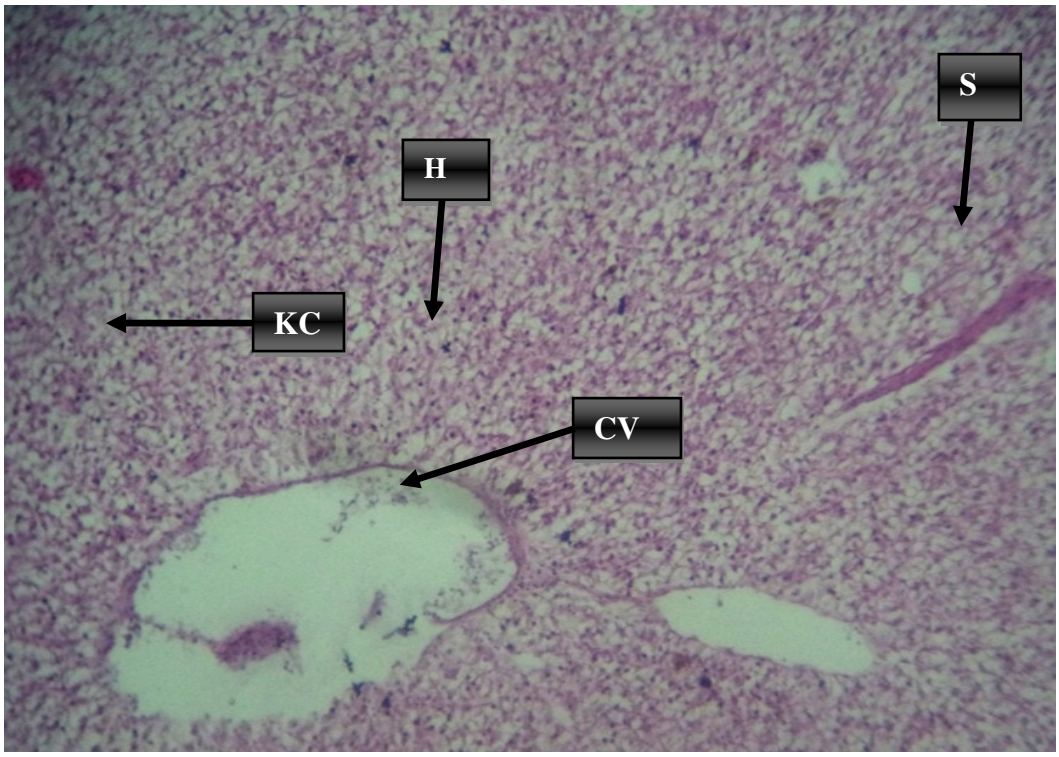

Plate 11: Microphotograph of sections of liver (control) showing kuffer cells (KC), central vein (CV), hepatocytes (H) and sinusoids (S). H&E. mag. X100.

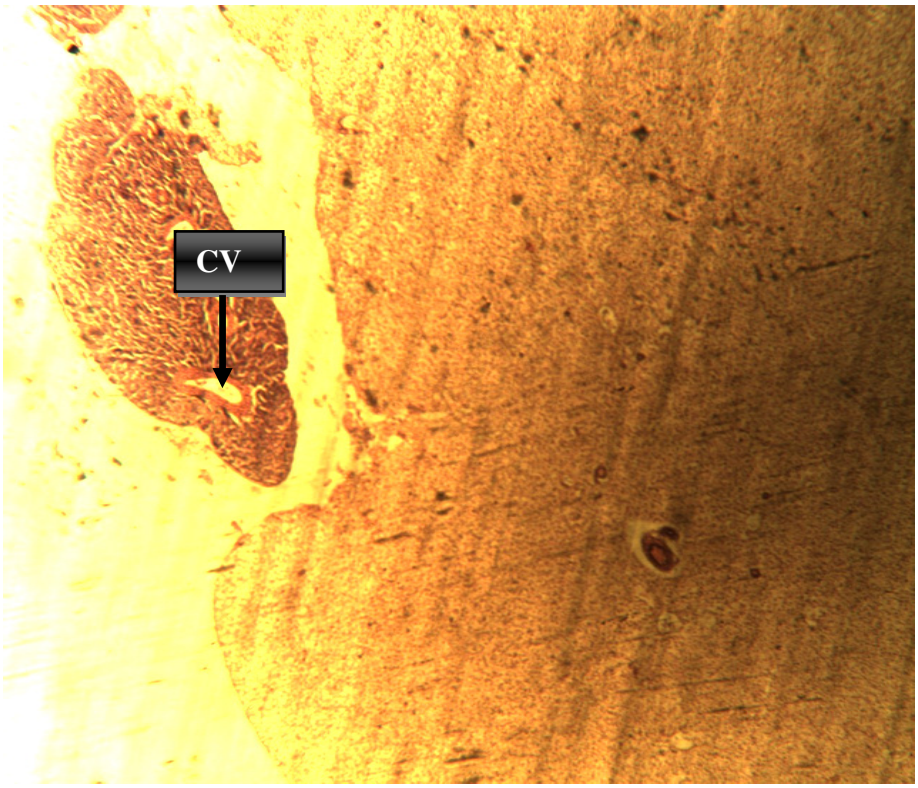

Plate 12: Photomicrograph of liver section of *Clarias gariepinus* exposed to 2.0mg/L of fenthion showing central vein (VC) at day 1 of the exposure. H&E mag x100

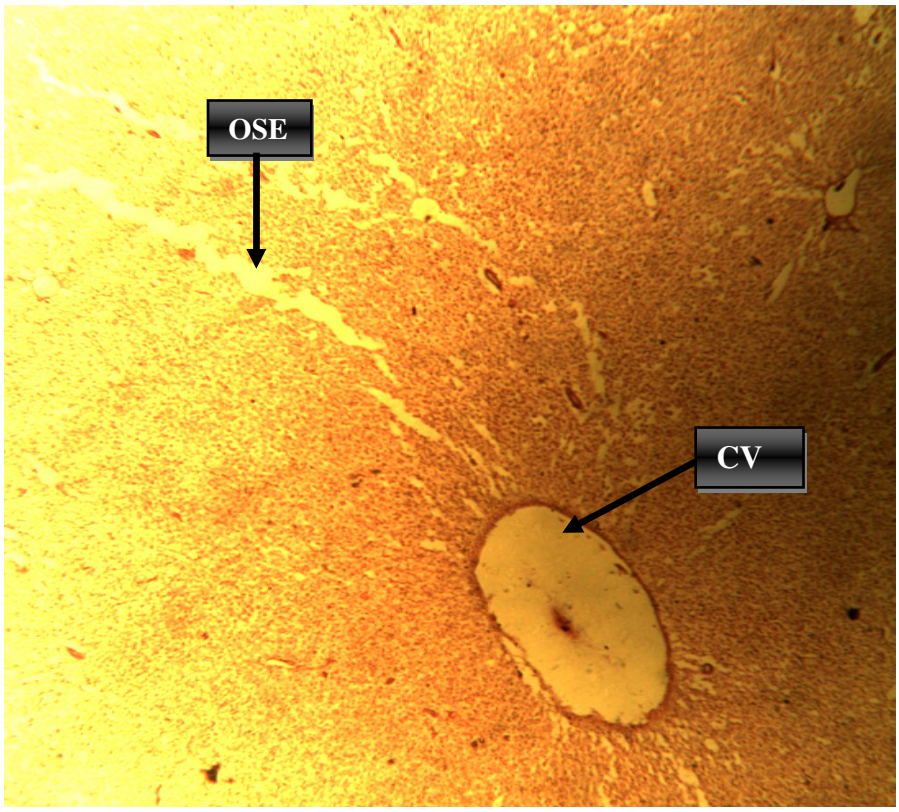

Plate 13: Microphotograph of section of the liver exposed to 2.0mg/L of fenthion in *Clarias gariepinus* showing the onset of sinusoid enlargement (OSE) at day 14 of exposure. H&E mag. X100.

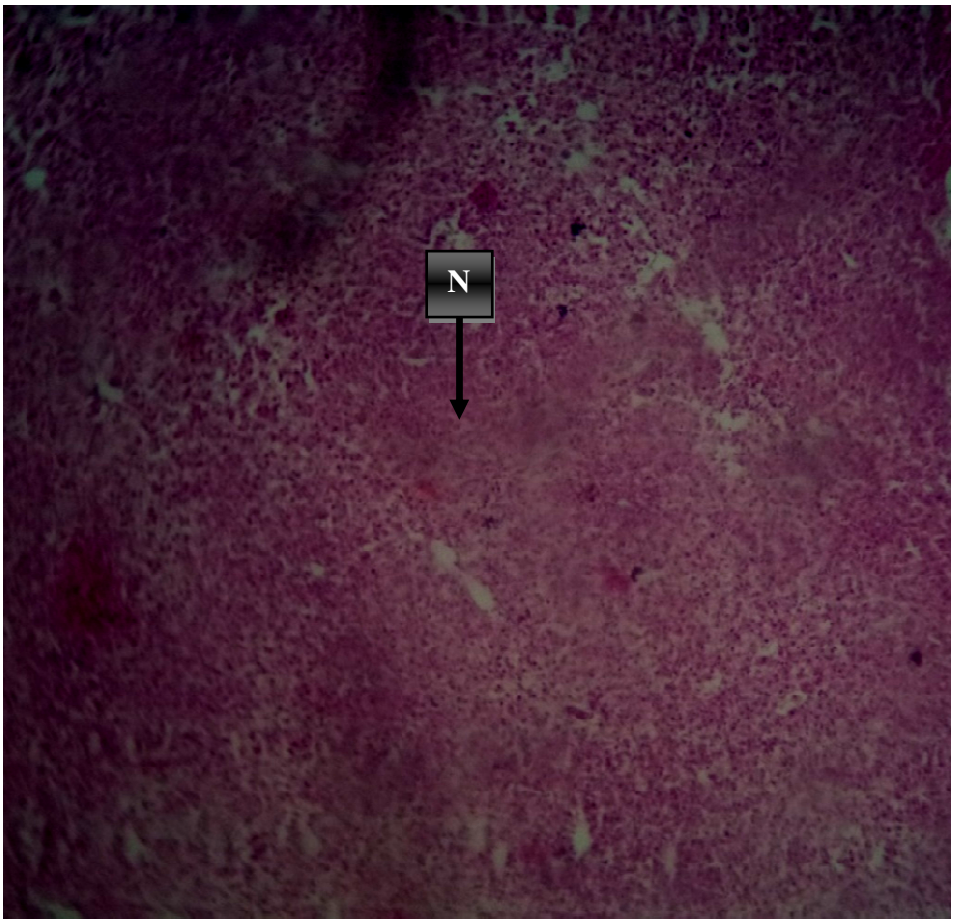

Plate 14: Microphotograph of section of the liver exposed to 2.0mg/L of fenthion at post exposure showing necrosis (N). H&E mag. X100.

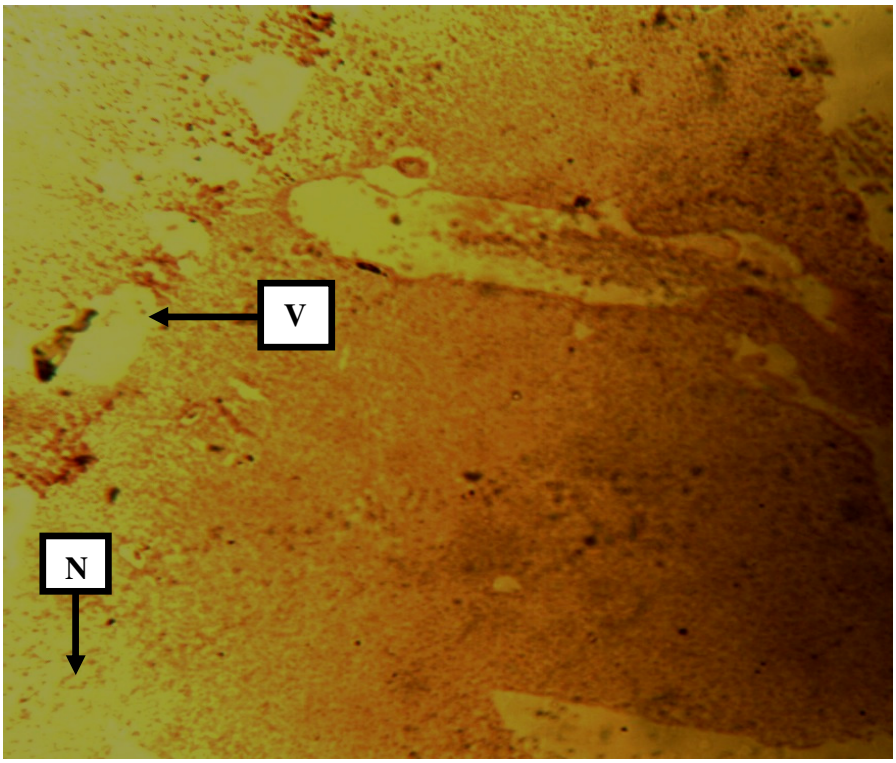

Plate 15: Microphotograph of section of the liver exposed to 4.0mg/L of fenthion at day 1 of the exposure showing necrosis (N) and vacuolation (V). H&E mag. X100.

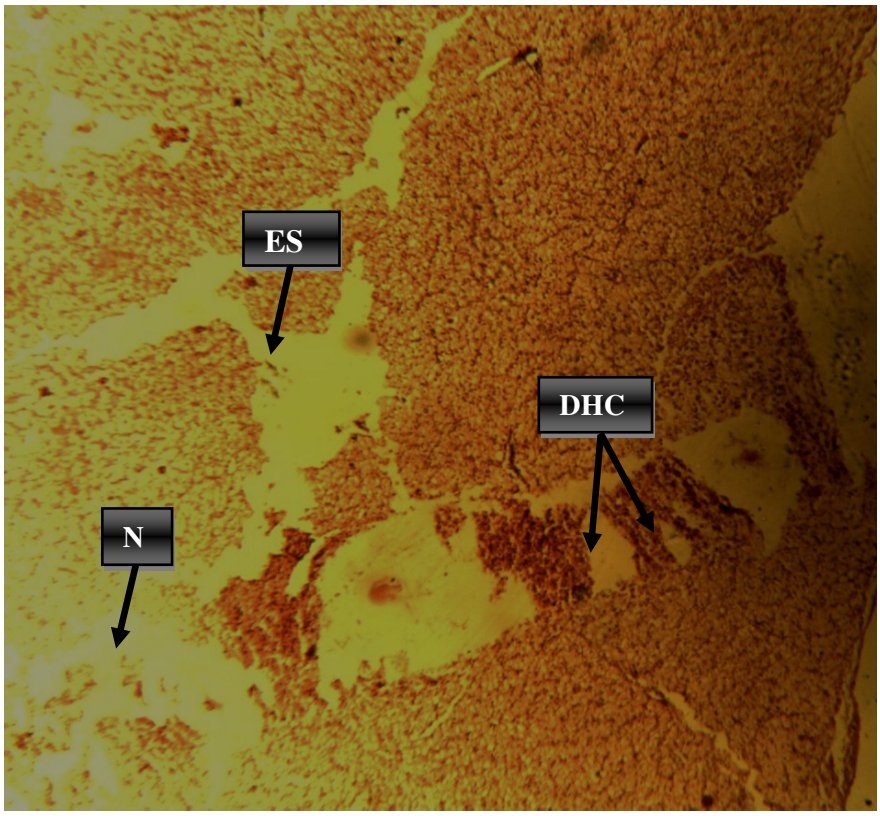

Plate 16: Microphotograph of section of the liver exposed to 4.0mg/L of fenthion at day 14 of the exposure showing necrosis (N), extravasated sinusoid (ES) and disarrangement of hepatic chords (DHC). H&E mag. X100.

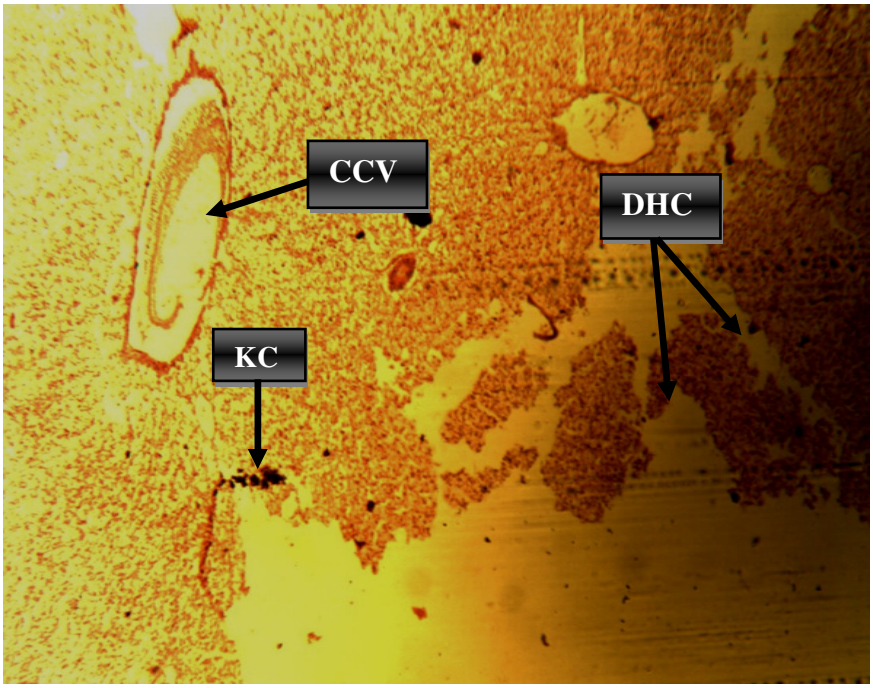

Plate 17: Microphotograph of section of the liver exposed to 4.0mg/L of fenthion at the post exposure showing onset of congestion of central vein (CCV), accumulation of kuffer cells (KC) and disarray of hepatic chords (DHC). H&E mag. X100.

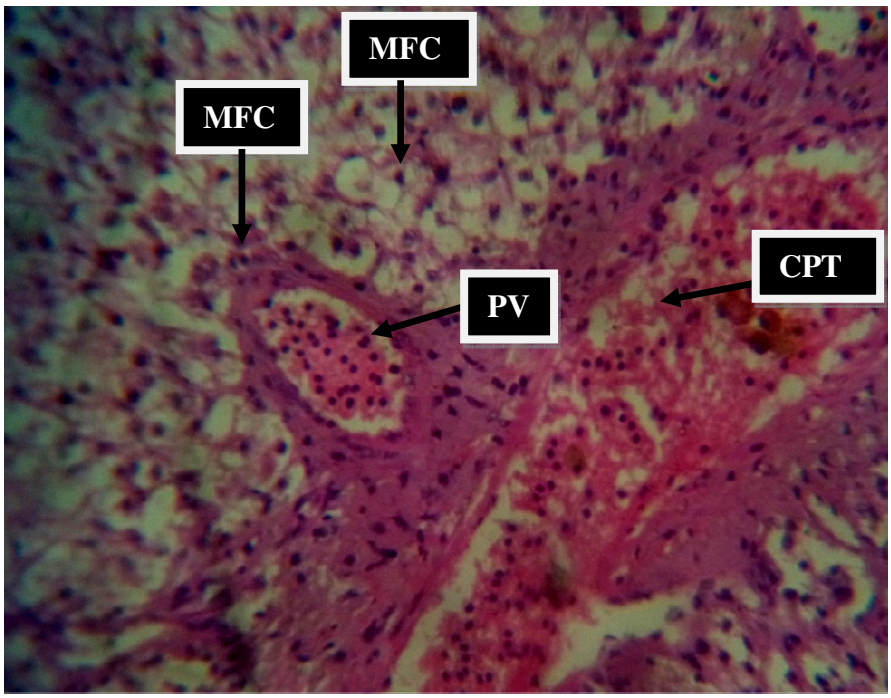

Plate 18: Microphotograph of section of the liver exposed to 8.0mg/L of fenthion during the experiment showing the onset of micro and macro fatty change (MFC), congestion of the portal triad (CPT) and the portal vein (PV). H&E mag. X100.

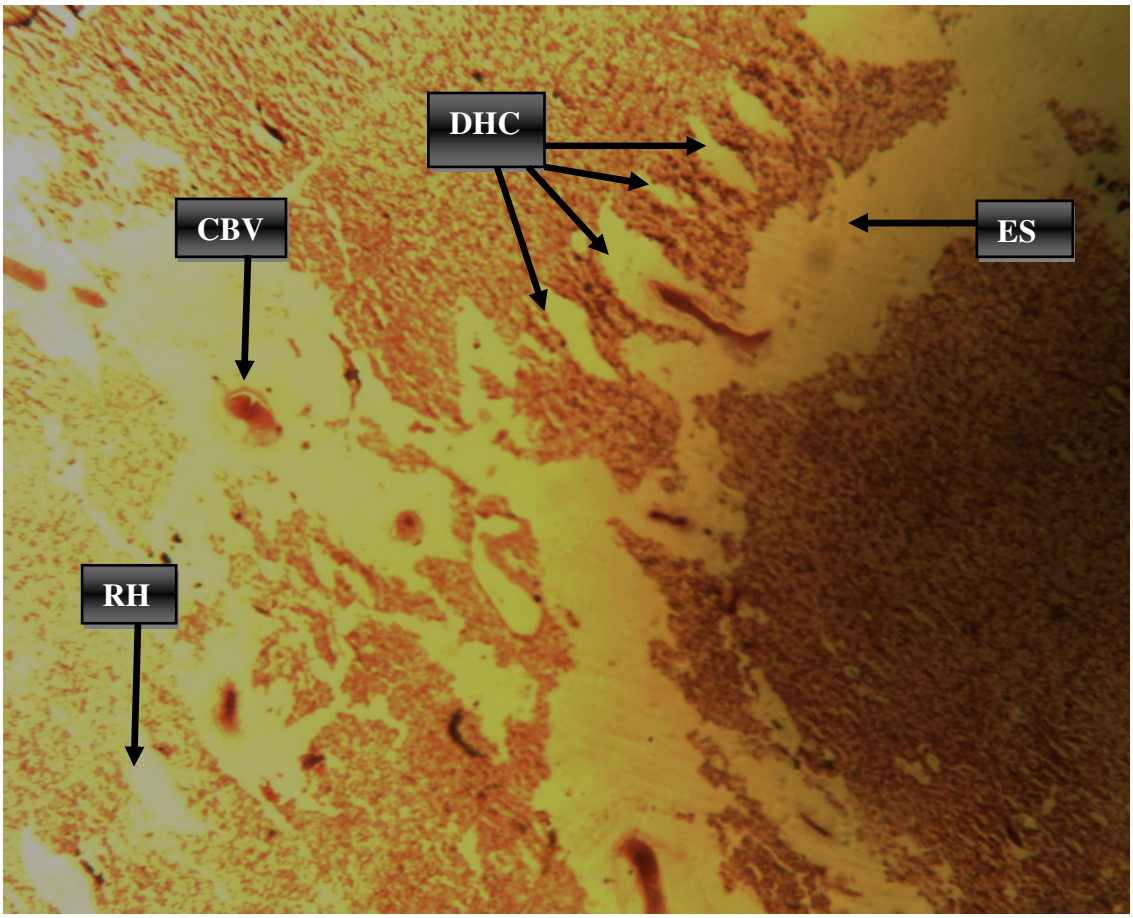

Plate 19: Microphotograph of section of the liver exposed to 8.0mg/L of fenthion at day 14 of the exposure showing clogged blood vessel (CBV), extenstive extravasated sinusoids (ES) and ruptured hepatocytes (RH) around necrotic region. H&E mag. X100.

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

Organic insecticide poisoning remains one of the major health issues in both developing and developed communities (Peter and Cherian, 2000). A great proportion of acute poisoning cases are caused by exposure to pesticides, especially organophosphate (OP) compounds. The primary mechanism of action of OP pesticides is based on inhibition of the acetylcholinesterase (AChE) enzyme (Eto, 1979). Like all organophosphate insecticides, fenthion acts on the nervous system as inhibitor of acetylcholinesterase (AChE), an enzyme that hydrolyzes acetylcholine (ACh). Ach is a molecule that is involved in the transmission of nervous signals from nerves to muscles and between neurons in the brain (Junquera, 2014).

Acute and chronic toxicity tests are mostly used to assess the toxicity of chemicals on non-target organisms (Santos *et al*., 2010). The 96 hour LC_{50} is one of the most important parameters for evaluating the toxic effects of pollutants (Nwani *et al*., 2015a). In the present study, the 96 hour LC_{50} values of fenthion for the African catfish, *C. gariepinus* was found to be 39.97mg/L. This suggests that fenthion is toxic to fish. There was dose-dependent increase and time-dependent decrease in mortality such that as the exposure time increased from 24 hour to 96 hour, the median lethal concentration required to kill the fish was reduced. This agreed with Muralidharan (2014a) who reported that acute toxicity of fenthion does not increase with time. In the present study, there was 100% mortality in *C. gariepinus* exposed to 72.0 mg/L at the end of 96 h.

The 96 hour LC_{50} value of the present investigation was higher than the 96 hour LC_{50} value of fenthion previously reported in *Cyprinus carpio* (1.5 mg/L) by Muralidharan (2012).

The disparity in the toxic potential of the pesticide may be attributed to differences in hardiness of the test species and water quality parameters. According to Johnson and Toledo (1993), the difference in the toxic potential of fenthion can be related to its accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in varied patterns of biotransformation leading to more or less toxic metabolites. In general, toxicity of chemicals to aquatic organisms has been reported to be affected by temperature, pH, dissolved oxygen, size and age, strain of species, water quality, concentration and formulation of test chemicals (Pandey *et al*., 2005; Nwani *et al*., 2010; Rauf and Arain, 2013). The magnitude of toxic effects of pesticides also depends on the length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murty, 1986). Varied inhibition of acetylcholinesterase, detoxification and absorption are factors causing the selective toxicity of pesticides for various species of fish (Oh *et al*., 1991).

The safe levels obtained for fenthion in the present study varied from 3.99×10^{-1} to 1.9984. However, the large variation in safe levels determined by various methods has resulted in controversy over its acceptability (Buikema *et al*., 1982; Pandey *et al*., 2005). According to Kennega (1979), the major weakness in calculation of application factor (AF) is its dependence on LC_{50} values. Pandey *et al*., (2005) observed that the extrapolation of laboratory data to field data was not always meaningful hence, the difficulty in deciding the acceptable concentration that may be considered 'safe' based on laboratory experiments.

Behavioural changes in fish are the most sensitive indicators of potential toxic effects of pesticide exposure (Banaee *et al*., 2011; Rauf and Arain, 2013). Erratic swimming indicates loss of equilibrium which shows that the region of the brain which is associated with the maintenance of equilibrium must have been affected. The abnormal behaviour exhibited by the experimental

groups such as abnormal swimming behaviour are due to inhibition of AChE activity leading to accumulation of acetylcholine in choligenic synapses with ensuing hyper stimulation. Fenthion may inhibit AChE activity due to its accumulation in the brain tissues or due to intake of fenthion with ChE group. This may lead to impairment of the normal functioning of nerve impulse causing physiological and behavioural modifications that could lead to reduction in survival ability (Muralidharan, 2014a).

It has been documented that under stress condition, fish become hyperactive, perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy demand (Alkahem *et al*., 2011). According to Muralidharan (2012), hyperactivity of fish as a result of exposure to pollutants could also be attributed to impaired gill function. Also, fish secrete increased amount of mucus to coat the body especially the gills to get relief from the irritating pollutants (Muralidharan, 2012). The secretion of copious mucus by fish could be a defense mechanism to neutralize the effect of pesticides which gradually covers the body, gills, and buccal cavity. Repeated opening and closing of the mouth and opercula covering accompanied by partially extended fin (coughing) as was observed in the present study could be due to clearance of the accumulated mucus debris in the gill region for proper breathing. The behavioural alterations observed during exposure of *C. gariepinus* to fenthion in this study agreed with to observations by other researchers who studied the responses of other fishes exposed to organophosphate pesticides. Nwani *et al*. (2013a) reported similar behavioural changes in *C. gariepinus* exposed to termifos. Rauf and Arain (2013) also reported similar behavioural changes in *Cirrhinus mrigala* exposed to diazinon. The observed behavioural alterations of fish in this study are also consistent with the findings of Nwani *et al*. (2010; 2013b)

who observed similar behavioural changes in *Channa punctatus* and *Clarias gariepinus* exposed to atrazine and chlorpyrifos respectively.

LPO is one of the main manifestations of oxidative damage induced by various compounds including pesticides hence; it has been used as an indication of pollution. MDA are produced by lipid peroxidation and considered as indicators of oxidative stress, which results from the free radical damage to membrane components of cells. Even in the absence of any pollutants, generation of free radicals occurs in the living system since they have been identified as by-products of a subset of enzymes that engage in electron transfer. The results of the present study revealed the presence of free radical activity because of the significant increase in the level of malondialdehyde (MDA). Increase in MDA level is usually associated with loss of membrane integrity, as well as disintegration of polyunsaturated fatty acids in the membrane bilayer, which exerts unfavourable effects on the susceptible organ structure and function. Exposure to sub-lethal fenthion concentrations significantly increased in a time and concentration dependent manner in LPO in both tissues thus reflecting increased oxidative stress and lipoperoxidation. Exposure of *C. gariepinus* to the highest concentration of fenthion showed a marked increase in MDA in the liver and gill. This marked increase could be attributed to the ability of fenthion to produce free radicals. High level of LPO may differ among species (Dar *et al*., 2014). According to Marigoudar *et al*. (2009), differences observed in MDA could reflect variation in the antioxidant mechanisms of fish, duration of exposure and the pesticide tested. The elevated values of LPO obtained in this study are in agreement with previous reports. Altuntas and Delibas, (2002) reported that fenthion caused a significant increase in LPO in wister rats weighing between 200 and 230g. Nwani *et al*. (2014c) reported elevated level of LPO in *Clarias gariepinus* exposed to premextra herbicide. Dar *et al*. (2014) also reported that the LPO in blood

of *Carassius carassius* increased significantly upon chronic exposure to endosulfan and it was also found to be time and concentration dependent. The authors also stated that the level of LPO may differ among species. Exposure to sublethal concentrations of deltamethrin in tissues of *Clarias gariepinus* also resulted to elevated levels of MDA in liver, gill and kidney tissues (Amin and Hashem, 2012).

Antioxidant levels, enzymological activity, biochemical parameters and haematological profiles are widely used as indicators to assess toxic stress, functional status and homeostasis in animals (Saravanan *et al*., 2012; Gecit *et al*., 2014). The activities of enzymes have been reported to be an indicator of the ability of tissues to cope with oxidative stress. However, when there is a high increase in oxidative stress, the defense mechanisms against ROS collapse (Nwani *et al*., 2015b). ROS then affects the antioxidant defense mechanisms thereby reducing intercellular GSH and decreasing SOD and CAT activities. Pesticides have been reported to induce oxidative stress and suppress cellular activities in animals (Dhasarathan *et al*., 2010). The gills are important for respiration, osmoregulation, acid-base balance and excretion of nitrogenous wastes in fish (Heath, 1995) and represents the greatest surface area of the fish in contact with external environment (Nahed, 2011). The liver in fish is an organ that performs numerous functions associated with metabolism of xenobiotics. Liver cells are dependent on antioxidant enzymes for protection against ROS produced during biotransformation of xenobiotics (Londis and Yu, 1995). Liver and gill antioxidant enzymes are used to determine if the tissues are functioning normally or if they have an injury or disease. Among the enzymes involved in defense system are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and glutathione reductase (GR). SOD is responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water (Vander Oost *et al*., 2003). It also metabolizes

superoxide radical (Nahed, 2011). CAT degrades hydrogen peroxides, which results from the degradation of the anion superoxide by SOD (Nwani *et al*., 2014c). GPx is known to be protective antioxidant that acts cooperatively with CAT in scavenging out H_2O_2 and other peroxides to ensure optimum protection against oxidative stress and tissue-specific damage (Gate *et al*., 1999). The major regulator of the intercellular thiol redox status is glutathione GSH, with its active –SH group having reducing necleophilic properties (Sevgiller and Uner, 2010). The most widely recognized function of GSH is its defense against toxic compounds, whether exogenous, such as electrophilic xenobiotics or endogenous, such as ROS generated during oxidative metabolism and/or stress (Estrela *et al*., 2006). It can directly scavenge ROS or act as a substrate for glutathione peroxidase (GPx) (Sevgiller and Uner, 2010).

Toxicants may induce different antioxidant/prooxidant responses depending on their ability to produce reactive oxygen species (ROS) (Barata *et al*., 2005). Under normal physiological status, the antioxidant defense systems can be induced by a slight oxidative stress as a compensatory response and thus, the reactive oxygen species (ROS) can be removed to protect the organisms from oxidative damage (Livingstone, 2001). The activity of the antioxidant enzymes may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as the susceptibility of the exposure species (Nahed, 2011).

Experimental factors such as time of exposure and dose or environmental factors such as water quality should also be taken into account for possible antioxidant system responses to oxidative stress caused by pollutants (Monserat *et al*., 2007; Baysoy *et al*., 2012; Alak *et al*., 2013). The present study shows the usefulness of liver and gill enzymes (SOD, CAT, GPx, GR, and GSH) to evaluate fenthion toxicity on fish in the aquatic environment.

Induction of SOD/CAT system provides a first line of protection against ROS. Increase in CAT activity may be to cope with increase in oxidative stress caused by fenthion. The results of CAT activity in this study also agreed with earlier reports of increased CAT activity in the liver of rainbow trout exposed to carbonsulfan (Capkin and Altinok, 2013).The increase in CAT activity may be in response to H_2O_2 produced by SOD activity since CAT is responsible for the conversion of H_2O_2 to water (Dar *et al*., 2014). The reduction in CAT activity in fish gills exposed to 8.0 mg/L on day 21 of the exposure could be justified by the instability of superoxide radicals which have been reported to inhibit CAT activity. It could also be as a result of decrease in the rate of chemical reaction as a result of excess production of H_2O_2. CAT activity was also reported to be significantly reduced in *C. gariepinus* exposed to deltamethrin (Amin and Hashem, 2012). According to the authors, the significant reduction could be attributed to the influx of superoxide radicals which have been reported to decrease in CAT activity. Decreased CAT activity suggests that fenthion produces hepatic dysfunction.

In the present study, fluctuations were observed in SOD activity during the duration of the experiment as both increase and decrease in SOD activity were observed. Increased SOD activity could be as a result of oxidative stress. The increase in SOD was to remove the superoxide radical but increased SOD activity might not be able to cope with increased ROS. If the ROS generated is more than SOD production, there will be depletion in SOD production so; activity of SOD will decrease via direct oxidative damage. Canada and Calabrese (1989) reported that the activity of SOD in fish can increase or decrease after exposure to various xenobiotics. In *Ictalurus punctatus* exposed to pollutants, CAT activity increased but not SOD or GPx, whereas in trout, the same pollutants increased SOD as well as CAT activity (Marther and

Di, 1991). Chlorpyrifos exposure increased SOD activity in *Oreochromis niloticus* (Oruc, 2010). The present research revealed similar results with the above reports.

GSH form the main non-protein thiol and is one of the main reductants found in the cell (Nwani *et al*., 2015b). GSH depletion leads to oxidative damage and greater LPO levels which in turn promote cell damage (Dabas *et al*., 2012). In the present study, the liver and gills showed similar pattern in the decrease in GSH activity (though not significantly different from the control). Highest decrease in GSH activity was recorded in fish exposed to the highest concentration. The decreased GSH levels in gill and liver tissues of *C. gariepinus* exposed to fenthion might be related to the increased use of GSH to stabilize fenthion in its oxidative stress for preventing the redox cycling and free radical generation. These results are similar to that of Nwani *et al*. (2015b) who recorded decrease in GSH activity in rats exposed to carbosulfan. Oxidative stress is known to rapidly consume glutathione. According to Nwani *et al*. (2010), oxidative stress may also be due to depletion of cellular GSH content below the critical level which prevents the conjugation of xenobiotics like atrazine to GSH and thus enables them to freely combine covalently with cell protein. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. The activity of GPx in the treated fish were generally lower compared to the control and could be as a result of the inability to detoxify H_2O_2 effectively. These results are consistent with the findings of Kanchan *et al*. (2002) who evaluated enzymatic and antioxidant activity in the liver and kidney of pregnant rats. There were fluctuations in GR activities in the present study as both increase and decrease were observed.

Liver tissues of *C. gariepinus* exposed to fenthion showed higher levels of LPO, CAT, SOD, GPx, GR and GSH compared to the gill tissues throughout the duration of the experiment

which suggests that the rate of production of ROS was higher in the liver compared to the gills thereby causing the liver to be at risk of exposure to fenthion compared to the gills. The higher values of these parameters in the liver may also be associated with its high metabolic reactions and free radical generation in the liver that requires the presence of antioxidants for possible protection against oxidative stress induced by ROS (Pereira *et al*., 2013).

Haematological indices are good pointers of physiological changes to evaluate the toxic stress, health functional status and internal environment of an organism (Saravanan *et al*., 2012). In the present study, reduction was observed in PCV, RBCs and Hb compared to the control and might be due to failure in the formation of red blood cells in red blood cell forming tissues of the body caused by fenthion. Significant reduction in PCV in the highest concentration of fenthion on day 1 of the exposure is in accordance with the reports of Ahmed (2012) who studied the effects of malathion (organophosphate) pesticide in *C. gariepinus*. Similar to the present results, decrease in PCV, RBC counts and haemoglobin of diazinon exposed fish was reported by Barnee *et al*. (2011) and related it to the destruction of cells and/or decrease in size of cells due to adverse effects of pesticides. Generally, toxicant exposure exerts an adverse effect on the hemopoietic organs which in turn alters blood parameters.

The results of this study showed that haemoglobin was lower in the treated fish compared to the control. Similar results were reported in *Cyprinus carpio* treated with fenthion (Muralidharan, 2012). The decrease in haemoglobin may be associated with either an increase in the rate at which Hb is destroyed or to a decrease in the rate of Hb synthesis. The decrease in haemoglobin may also be attributed to iron synthesis machinery caused by the inhibition of aerobic glycolysis in fish exposed to fenthion. Haemoglobin decrease after exposure to sub-lethal concentrations of toxicants may impair oxygen supply to various tissues, thus resulting in a slow

metabolic rate and low energy production (Ahmed *et al*., 1995). Toxicants may also cause undesirable effects on the haematopoietic organs which reduce the supply of RBCs either due to less production and/or increased rate of removal from circulation (Khalid, 2012). Poisoning from pesticide residues may also led to the development of anaemia due to interference of the Hb biosynthesis and shortening of the life span of the circulating erythrocytes (Jyostana *et al*., 2003). Nwani *et al*. (2015b) attributed decreased Hb to intravascular hemolysis, anaemia or suppression of hemopoiesis. Reduction in the level of haemoglobin may be the consequence of toxic effects of fenthion on the synthesis of this molecule. The insecticide may disrupt the synthetic pathway by affecting the activity enzymes involved in the synthesis of haemoglobin, consequently reducing the level of Hb of the exposed fish.

Generally, leucocytes (WBCs) alter immunological functions in animals, including fish (Nwani *et al*., 2015). The increase in WBC counts in the treated fish compared to the control in the present study indicates the occurrence of leukocytosis (WBC increase). The observed leukocytosis in the present study was perhaps a normal immune defensive response of the fish against a toxic invasion. Secondly, it also suggested that fenthion stimulated the immune system with a simultaneous release of lymphocytes from the lymphomyeloid tissue as a defense response. These resulted in leukocytosis and/or lymphocytosis which altered body physiology. Leukocytosis was also reported in *Cyprinus carpio* exposed to lindane (Saravanan *et al*., 2011) and in *Oreochromis niloticus* exposed to deltamethrin (El-sayed *et al*., 2007). Increase in WBC counts might be due to leukaemia or blood cancer during which the numbers of WBCs increase. The results of this work are similar to that of Muralidharan (2014a) who also observed increase in WBCs in *Cyprinus carpio* exposed to fenthion. The increase in WBCs was attributed to the interference with development of erythrocytes in the haemopoietic tissues thereby creating

leukomogenic condition which could be an adaptive response towards the stress induced by the pollutant. The results of the present study are consistent with the findings of some other authors studying the response of other fishes exposed to organophosphate pesticides. This regards changes in WBCs after acute exposure to malathion in *Oreochromis niloticus* (Khalid, 2012). The author stated that changes in leukocyte system manifests in the form of leukocytosis with heterophilia and lymphopenia, which are characteristics of leukocytosis in animals exhibiting stress. Significant reductions in RBCs, PCV and WBCs have been reported following the exposure of *Onchorrhynchus mykiss* (Dogan and Can, 2011), *Clarias gariepinus* (Ahmad 2012) to dimethoate and *Rutilus frisil* (Mohammed *et al*., 2012) exposed to different concentrations of diazinon. In another study however, Lohna *et al*. (2001) recorded leucopenia (reduced WBC counts) in sunfish inhabiting selenium laden coal ash effluents and associated it with increasing metal concentrations. This is in agreement with the findings of Sampath *et al*. (1993) after exposing *Oreochromis niloticus* to a toxic environment.

Significant decrease in RBCs observed in the study is in accordance with the results of Muralidharan (2012) who studied the haematological alterations in *C. carpio* exposed to fenthion. The reduction in RBC counts and Hb are often accompanied by a decrease in packed cell volume and demonstrates the physiological dysfunction of the haemopoietic system. The decreased RBC counts may be due to inhibited RBC production and/or due to haemoglobin synthesis. Surrounding toxicants might have caused reduction in RBCs, which in turn have caused reduction in haemoglobin and packed cell volume (Jaya and Ajar, 2014). Blood cell indices like mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) seem to cause changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. In order to

overcome the hypoxic conditions in stressful media, fish usually responds by increasing the MCV and MCH of erythrocytes. In the present studies, slight increase was observed in MCH and MCV in fish exposed to fenthion. Similar results were observed by Muralidharan (2012) in MCH and MCV. MCV and MCH values depend upon RBC counts hence their variation determined the anaemic condition of the exposed fish. Changes in white blood cell differentials have been used as good pointers of stress (Cole *et al*., 2001). Neutrophils and lymphocytes make up the majority of WBC and proliferate in circulation in response to stress (Jain, 1993; Thrall, 2004). The observed significance in neutrophils and lymphocytes might have been provoked by the stress imposed by fenthion in *C. gariepinus*. Stress-induced lymphopenia and neutrophilia have been shown to be linked to elevated glucocorticoids secretion which acts to increase their percentage levels (Davies *et al*., 2008). Similar results have been reported in fishes exposed to different toxicants (Li *et al*., 2011; Nwani *et al*., 2014b). Changes in blood parameters suggest that there were osmotic disturbances and change in oxygen carrying capacity during the exposure to pesticides.

Condition factor and hepatosomatic index have been proposed as indices of environmental contamination (Nwani *et al*., 2015a). According to the authors, condition factor is a measure of fish fitness for a given length. For instance, a heavier fish is a better condition factor (Li *et al*., 2011). The condition factor is also an organism-level response, to factors such as nutritional status, pathogen effects and toxic chemical exposure, causing greater-than normal and less-than-normal weights (Andu and Kangur, 1996; Azmat *et al*., 2007). The condition factors are used as indicator of the well being of individual organism, because it integrates many levels of the organizational processes. For example, a decrease in condition factor is considered a reflection of depletion in energy reserves because these indices are positively related to muscle

and livers energy content (Lizama *et al*., 2002; Hasan and Seces, 2003; Ensibi *et al*., 2013). Recently, organosomatic indices and fish condition factor have been used to determine the sub lethal effects of these pollutants during the clinic diagnosis of fish physiology (Yi *et al.,* 2007; Ozer *et al.,* 2008; Nwani *et al*., 2015a).

The use of various organosomatic indices is based on the assumption that there is proportional relationship between fish size and the particular ratio in assessing fish stress by pollutant (Ronald and Bruce, 1990). In the present study, the non-significant decrease in condition factor of the treated group compared to the control indicates that fenthion had no direct effects on the CF in *C. gariepinus*. Similar decrease in condition factor was reported by other authors studying the effects of other environmental pollutants on the condition factors. Arellano *et al.* (1999) exposed *Solea senegalensis* to cypermethrin pesticide and observed a decrease in condition factor which depicted a reduction in growth rate which may be due to a reduction in oxygen carrying protein levels and red blood cells, while increase occurred in the number of white blood cells. Anderson *et al*. (1988) also observed a decrease in condition factor when Indian shad was exposed to bleached kraft mill effluent. However, an increased condition factor was observed by Memaster *et al*. (1991) in the white sucker (*Astostomes commessomi*) and suggested a disruption in metabolic capability and altered energy allocation. The decrease in condition factor may be due to the impairment of the olfactory systems which might have affected feeding, resulting in alterations of metabolic activities and energy allocation of the fish systems.

Hepatosomatic index refers to the relative liver size and relates to the hepatic enzyme activity for chemical detoxification (Fang *et al*., 2009). Hepatosomatic index (HSI) can also be referred to as the general measurement of the overall condition of fish or the growth status of

liver (West, 1990) and can be an excellent predictor of adverse health in fish (Adams and McLean, 1985). In the present study, decrease in HSI of the treated fish compared to the control (though not significant) except on day 21 of the exposure can be attributed to the damage caused to liver cells in response to stress induced by Fenthion. This is consistent with the findings of Muralidharan (2014c). On the other hand, Abdelmeguid *et al*. (1999) reported that hepatosomatic index of *Oreochromis niloticus* exposed to lead acetate was increased as a result of change in quantity of fat in liver. Munshi and Dutta (1996) stated that the HSI of *Osteichthyes* is normally between 1% and 2%. Although hepatosomatic indices can vary with nutrition, season, fish condition and disease, a relationship between hepatosomatic index and levels of contamination was reported by Sloof *et al.* (1983).

The water used for the cultivation of fish will not give maximum production if the physico-chemical parameters are not optimal for fish and other aquatic organisms (Keremi *et al*., 2014). Water temperature observed in this study corroborated the report of Keremi *et al.* (2014) who analyzed the physic-chemical parameters of fish pond water in freshwater areas of Bayelsa state, Nigeria. Water pH affects metabolism and physiological processes of fish and also exerts considerable influence on toxicity of ammonia (Indian Council of Agricultural Research, 2006). The values of pH observed in the present study range between 6.60 and 7.75. This is within desirable range according to Boyd (1990). Huet (1972) also observed that pH values of 6.5 to 9.0 are good for fish production. Ammonia is introduced into the pond through dead phytoplankton, uneaten feeds, dead and decaying organic matter (Keremi *et al*., 2014). Fish are very sensitive to un-ionized ammonia and needs an optimum range of 0.02-0.05mg/l (ICAR, 2006). Robinette (1976) reported that 0.12mg/l NH_3 caused reduced growth and gill damage in Channel Catfish. All the observed values in this study were higher than desired range and limits and this may

affect the growth of fish. Keremi *et al*. (2014) also reported similar findings to that of this study. Dissolved oxygen is a measure of the amount of gaseous oxygen dissolved in an aqueous solution that plays a vital role in the biology of cultural organisms (Ehiagbonare and Ogundiran, 2010). The mean DO values obtained in this study ranged between 5.25 mg/L and 8.20 mg/L. These values agreed with the minimum DO of 5.0 mg/l reported for tropical fishes by Saloom and Duncan (2005). Turbidities in waters rarely exceed 20,000mg/l (Irwin, 1945). This value is within the range obtained in the present study. The turbidity values obtained in ponds though, are within the desirable range are above the lower limit as reported by Boyd (1990).

Alterations in liver structure can be significant in evaluation of fish health (Kolbasi *et al*., 2009). The liver also plays an important role in fish physiology, both in anabolism and catabolism and it acts as storage centre for many substances, mainly glycogen. The changes observed in the liver could be attributed to direct effects of pollutants on the hepatocytes as found in pesticide toxicity (Mohammed, 2009), because it is the site for detoxification of toxicants. The vacuolation of the hepatocytes could probably be due to metabolic damage related to the exposure with fenthion contaminated water. Alterations observed in liver parenchyma such as necrosis and vacuolation are known to be often associated with degenerative necrotic condition (Myers *et al*., 1987). Gills are among the most sensitive organs which react first in the environment (Devi and Mishra, 2013) as functions such as respiration, osmoregulation and excretion are performed through the gills (Lease *et al*., 2003; Ramah, 2011).

The histological results observed in the liver and gill tissues of *C. gariepinus* in the present study indicate that sub-lethal concentrations of fenthion caused moderate to severe alterations in gill and liver architecture which are important organs performing vital structures as stated above. The exposure of organisms to sub-lethal concentrations of pesticides in their

environment has been reported to result in various biochemical, physiological and histopathological alteration in vital tissues (Anbu and Ramaswamy, 1991). The different concentrations of fenthion used in the present study as well as the different exposure periods showed different degrees of pathological changes.

Histopathological lesions observed in the liver and gill tissues of *C. gariepinus* exposed to fenthion in the present study are similar to reports in *Cyprinus carpio* exposed to fenthion and the extent of damage to the organs tested were also dose dependent (Muralidharan, 2014b). Cengiz and Unlu (2006) documented similar findings in *Puntius gonionotus* exposed to paraquat. Elezaby *et al*. (2001) recorded similar results in *Oreochromis niloticus* exposed to dimethoate. Muralidharan (2014b) attributed the damage done to the liver to the accumulation of fenthion since the liver is an important detoxifying organ.

Changes in gill structure might have been responsible for changes in the activities of enzymes. Epithelial hypertrophy could be as a result of epithelial detachment as stated by Machado and Fanta (2003). Epithelial lifting (chloride cells, pavement cells) increases the distance through which the toxicant reach the blood stream thereby causing impaired oxygen uptake (Kumar *et al*., 2010) and lamella fusion could be a protective mechanism as it reduces the amount of vulnerable gill surface area. The epithelial lifting could result in dysfunction or even non-functional gills and eventually suffocate the fish. Hypertrophy, fusion of the secondary lamella and epithelial lifting observed in the present study were considered to be of the first degree of gill lesions. According to Olurin *et al*. (2006) these pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the pollutants through the gill surface and probably increased capillary permeability. The oedema observed in fish gills exposed to fenthion could be due to acute inflammatory nature of the lesion induced by fenthion.

Oedema with epithelial separation was reported by Eller, (1971) and Van valin *et al*. (1968) as additional change in fish gills exposed to toxicants.

Though the histological changes observed in the present study and that of Muralidharan, (2014b) revealed that the changes were concentration dependent, some authors reported that such structural changes are non toxicant specific (Meyers and Hendricks, 1985), which could be mere stress response of fish to toxicant exposures (Gabriel *et al*., 2007). The structural changes observed in the liver and gill tissues during the duration of exposure were expected to normalize as reported by Myers *et al*. (1992) who stated that it is possible for the gill to regenerate and recover from the alterations but no such generation and recovery were observed at the post exposure of the experiment which shows that the changes observed were permanent.

The histopathological results of this study clearly indicate that in the liver and gill tissues, sub-lethal concentrations of fenthion had destructive effects in *C. gariepinus* and were related to the concentration as well as the duration of exposure. Gills were found to be the most seriously affected tissues as they were in contact with fenthion in water thus, serving as major path of entry of the pollutant in the fish. The liver tissues were also damaged possibly due to its role in detoxifying fenthion as may lead to impairment of metabolic activities.

4.2 Conclusion

Based on the major findings of this study, it can be concluded that fenthion has the potential to damage the physiology of fish leading to observed changes in behavioural pattern with resultant dose dependent mortality. The study shows the significance of behavioural parameters in assessing the risk associated with pesticide exposure to fish. Higher concentrations of fenthion induced oxidative stress in *C. gariepinus* as evidenced by the induction of lipid peroxidation and

subsequent changes in antioxidant enzymes. Sub-lethal exposure to the organophosphate pesticide fenthion, altered the haematological parameters in *C. gariepinus.* Histopathological results clearly indicated that sub-lethal concentrations of fenthion had destructive effects on the liver and gill tissues of *C. gariepinus* and the effects are irreversible as no positive response was observed at the post exposure experiment (7 days recovery). All effects that were observed in the liver and gill tissues reduced the general state of health of *C. gariepinus* at sub-lethal concentrations of the toxicants.

4.3 Recommendations

The following are the recommendations;

1. The activities of antioxidant enzymes and oxidative stress can be used as a biological indicator to monitor unacceptable levels of environmental contamination.
2. Environmental monitoring programs for the pesticide is necessary in view of the hazard and side effects that might be associated with excessive use on non-target organisms especially fish.
3. Further studies to investigate the underlying mechanisms of action of fenthion in freshwater would be a promising research.

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