TOXICITY AND OXIDATIVE EFFECTS OF PESTICIDE MIXTURE ON LIVER AND GONADS OF MALE ALBINO RATS

BY

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CERTIFICATION

This is to certify that this work: "Toxicity and Oxidative Effects of Pesticide Mixture on Liver and Gonads of Male Albino Rats" was carried out by PETER CHUKWUDI (20164997028) in partial fulfilment for the award of the degree of M.Sc. in Biotechnology in the Department of Biotechnology of the Federal University of Technology, Owerri.

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DEDICATION

This work is dedicated to God Almighty, the author and giver of life.

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ABSTRACT

Commercial mixtures of organophosphates and pyrethroids have become very popular in the insecticide markets in developing nations and have resulted in an elevation in the prevalence of mixed toxicity. The present research is aimed at evaluating the toxic effects of commercial preparation of a pesticide mixture, MagicForce, which contains Dimethoate (DM) and Lambda-cyhalothrin (LC) in the ratio of 20:1. For this purpose, forty (40) adult male albino rats were divided into four (4) equal groups. Dimethoate group (DM) received: (10.7mg/kg b.w/orally/daily), Lambda-cyhalothrin group (LC) received: (3.9mg/kg b.w/orally/daily), MagicForce group (MF) received: (1.2mg/kg/b.w/orally/daily) while Control group (C) received only food and water daily. The general health of the rats was monitored daily for toxicity signs and mortality while taking the weekly body weights. At the end of the study (28 days), the rats were sacrificed. The liver and gonads were excised and weighed, the tissue homogenates prepared for biochemical analysis together with the blood samples. The estimation of the cell death marker Lactate dehydrogenase (LDH), Liver function enzymes (AST, ALT and ALP), Total Serum Protein, Total Albumin, Total Bilirubin were carried out. Oxidative markers malondialdehyde (MDA), catalase (CAT), glutathione (GSH), glutathione-s-transferase (GST) and glutathione peroxidase ($GP_{x)}$ were determined. Heamatological parameters (WBC, RBC, HGB and MCHC) were also examined. The reproductive status was determined by analyzing the testosterone levels, sperm motility, sperm count and sperm viability. The results revealed that nephrotic cell death occurred most in the LC treated group (73.83µ/L, 352.53µ/L, 334.53µ/L) than in the DM and MF. Liver function enzymes (AST, ALT and ALP) activities were significantly elevated in LC-treated group (138.13 µ/L, 44.73 µ/L and 29.90 µ/L) respectively, than observed in the other treatment groups. The Total Protein Profile level visibly decreased mostly in the LC exposed group (58.12µ/L, 17.19µ/L) compared to the other treatment groups. The oxidative stress and hematological studies showed that the LC-treated group was most adversely affected than the DM and Magicforce. There was no much significant impact on the reproductive status of the animals by the other pesticides except on the testosterone levels which was affected by the LC. It can be concluded that the LC, singly had more adverse effect on the overall parameters measured than the Dimethoate (DM) and in their combined commercial state (Magicforce). This suggests an antagonistic interaction in the mixture.

Keywords: Antagonistic, Dimethoate, Lambda-Cyhalothrin, MagicForce, Organophosphate, Pyrethroids.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

In the quest for industrialization and modernization, man has contributed immensely to environmental pollution. Increased demand for food and fiber has lead to the chemicalization of agriculture and presently, modern agriculture is inevitably dependent on fertilizers and pesticides (Suseela *et al*., 2017).

Pesticides are classified into compounds that have similar chemical structures and modes of toxic action. The organophosphate insecticides (OPs) seem to be the most popular pesticide. So many biochemical changes have been observed in organs of animals due to the organophosphorus insecticides (Betrosian *et al.*, 1995; and Senanayke, 1998). These organs include central nervous system, (Desi *et al.*, 1998; and Lengyl *et al.*, 2005), liver (Gomes *et al*., 1999), and kidney (Kossmann *et al*., 1997). Dimethoate is a contact and systemic organophosphate pesticide and its major toxicological endpoint in animals and humans is the inhibition of acetylcholinesterase (AchE) activity, the key enzyme that hydrolyzes the neurotransmitter acetylcholine (Carlock *et al*., 1999). Inhibition of AChE results in the accumulation of acetylcholine that over stimulates cholinergic receptors, which in turn stimulates neurological activity in the organism (Gallo & Lawryk, 1991). Many other insecticide families such as pyrethroids exhibit neurological activity and damage at different target sites (Kingsley & Iniobong, 2014). Lambda-cyhalothrin, like those of other pyrethroids, will be mainly neuropathy (effects on the nervous system) (Manal *et al*., 2008). Cyhalothrin may act on ion channels within the nerve cells (neurons) to disrupt proper function of the cells of both the peripheral and central nervous systems. At lower doses, this may

take the form of stable, repetitive firing of the neuron, but high doses may result in depolarization of the nerve cell and blockage of conduction (Ray, 1991). Both Dimethote and Lambda-cyhalothrin are two of the most widely used pesticides in agriculture in Africa (Al-Haj *et al.*, 2005).

The exposure to these insecticides may involve large sectors of human population, which comprises the agricultural workers and their families, besides the others who may be exposed through home application of pesticides or via residues in food (Lu *et al*., 2000).

On the other hand, there is an increased commercial interest in the development of insecticide mixtures for higher efficacy. The combination of two or more active compounds in a mixture may provide rapid action and more residual effect than any of them if applied singly in sequence. As part of the efficacy evaluation, there is a need to consider the reduction of pesticide use by justifying that the dose used is appropriate, and to consider resistance management issues according to Draft Efficacy Guideline 607 (2007). It is equally important to highlight or cross-reference to the efficacy, toxicological, and ecotoxicological submissions for any mixed active product which suggests additive or synergistic effects between active ingredients.

However, pesticides can cause damage through varying mechanisms involving multiple reaction pathways (Khan, 2006). The simultaneous exposure to OPs and pyrethroids causes synergistic toxicity through OP-induced esterase inhibition (Denton *et al*., 2003). The mixed effect of pesticides within the same class can be foretold fairly well based on our experience and understanding of their toxic mode of action.

Contrarily, the combined effects of across-classes of insecticides are more difficult to predict and understand (Lydy *et al*., 2004). Few research works have begun to characterize the toxicological

effects of pesticides mixture exposure (Richardson *et al*., 2001; Schuler *et al*., 2005; Moser *et al*., 2006).

To understand these combined toxicological effects of organophosphorus and pyrethroid pesticides, the present study compares and evaluates the toxicity profiles of dimethoate and lambda-cyhalothrin individual exposures and their mixture.

1.2 PROBLEM STATEMENT

Pesticides are the substances intended to control, repel, mitigate, kill or regulate the growth of undesirable biological organisms. These pests not only compete with humans for food, but also transmit diseases. Today pesticide usage has become an indispensible and integral part of world agriculture (Prakasam *et al*., 2001).

In recent years, environmental contamination with pesticides represents one of the problems of the African region. The presence of these toxic chemicals was recorded in air, water, house dust and in the tissues of nonoccupationally exposed people, especially in the adipose tissue, blood and urine (Gunnell *et al*., 2007).

Pesticide self-poisoning is a major public health problem, according to the World Health Organization, about 3 million cases of pesticide poisonings occur annually killing at least 250–370,000 people (Varol *et al*., 2016).

Pesticide residues in food pose potential risk to public health. Thus strict international regulations are in place for pesticide residues in food. The Codex Alimentarius Commission set up the Maximum Residue Limits (MRLs) for pesticides. MRLs represent the maximum concentrations of a pesticide residue (expressed as mg/kg) legally permitted in different food

commodities and animal feeds (Codex Online Pesticide Index). However, a number of studies have shown that pesticide residues in food exceeded MRLs, especially in developing countries. In vegetable samples from Kumasi, Ghana, levels of pesticides in tomatoes and pepper exceeded the MRLs (Darko & Akoto, 2008). Thus the understanding of the potential toxicity of these pesticides is critical.

Dimethoate is moderately toxic by ingestion, inhalation and dermal absorption. As with all organophosphates, dimethoate is readily absorbed through the skin. Organophosphates are easily absorbed through the lungs. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or with liver malfunction may be at increased risk from exposure to dimethoate (Darko & Akoto, 2008). High environmental temperatures or exposure of dimethoate to visible or UV light may enhance its toxicity. Splashing of dimethoate into the eye may cause swollen eyelids and damage to the cornea (the outer surface of the eye). The organophosphate insecticides are cholinesterase inhibitors. They are highly toxic by all routes of exposure. Begum and Vijavaraghaven (1995) observed that, the exposure of Dimethoate to the fresh water fish *Claries batrachus* reduced the carbohydrates and proteins metabolism, and affect the aminotransferase activity in the liver. An increase in blood glucose in experimental rats was reported after Dimethoate oral administration period of 2 months in dose 21 mg/kg. (Hagar & Fahmy, 2009). An increase in lactate dehydrogenase, serum transaminase, and a decrease in the serum total protein, albumin, and globulin was observed in experimental rats after Dimethoate orally administration in dose 75 mg/kg (Attia & Nasr, 2009). Diazinon is an organophoshorus insecticide with anticholinesterase mode of action (Alahyary *et al.*, 2008). At the last 20 decades in Nigeria, a critical increase in number of people suffering from various

liver and kidney diseases as well as diabetic mellitus and reproductive impairment has been noted.

Pyrethroid insecticides (e.g. Lambda-cyhalothrin) bind to voltage-sensitive sodium channels and modify their gating kinetics, thereby disrupting nerve function and producing acute neurotoxic effects in both insects and non-target organisms (Soderlund *et al*., 2002). Lambda-cyhalothrin can irritate the eyes, skin, and respiratory tract. The substance can cause negative side effects on the peripheral nervous system, resulting in convulsions or ataxia.

Lambda-cyhalothrin can produce an effect described as "subjective facial sensation" in some people working with the compound. Subjective facial sensation is a transient phenomenon, and its symptoms are not associated with objective physical signs and individuals can recover from it completely.

The marketing of mixtures of organophosphate and pyrethroid insecticides has become very common in developing countries and has resulted in an increase in the prevalence of toxicity (El-Halwagy & Zaki, 2009).

The majority of toxicological studies of chemicals have been concerned with the evaluation of exposures to single compounds. In practice, humans are exposed to complex and variable mixtures of chemicals, which may act independently as in a single exposure, but may also interact to modulate the effects of the mixture as a whole (Groten *et al*., 2000).

1.3 AIM AND OBJECTIVES OF THE RESEARCH

The aim of this research was to evaluate the toxicity and oxidative effects of pesticides and their mixture on the liver and gonads of male albino rats.

Specific objectives of the research are:

- To determine the effects of lambda-cyhalothrin, dimethoate and their mixture on the activities of lactate dehydrogenase (LDH) in the liver, gonads and blood of male albino rats.
- To determine the effects of lambda-cyhalothrin, dimethoate and their mixture on the activities of liver function enzymes of male albino rats.
- To determine the effects of lambda-cyhalothrin, dimethoate and their mixture on the antioxidant enzyme system of male albino rats.
- To determine the effects of lambda-cyhalothrin, dimethoate and their mixture on the haematological parameters of male albino rats.
- To determine the effects of lambda-cyhalothrin, dimethoate and their mixture on the reproductive functions of male albino rats.

1.4 HYPOTHESIS

The study was guided by the following alternative hypotheses:

1. Pesticides can induce toxic and oxidative stress in mammals
2. The toxic effect of the pesticide mixture is greater than their individual effects.

1.5 JUSTIFICATION OF THE RESEARCH

Several synthetic pesticides have been released into the environment through the agricultural activities to control insect pests, plant pathogens and weeds in both developed and developing countries. Presently, approximately 1,500 active ingredients have been registered as pesticides

and formulators mix these compounds with one or more of some 900 inert materials to create approximately 50,000 commercial pesticide preparations registered for use. (WHO, 1993). The adverse effects of individual insecticides on a variety of experimental animals have been previously studied by many investigators (Shakoori *et al*., 1990; Stebbins *et al*., 2002; Mansour & Mossa, 2005). In reality, humans are exposed simultaneously or sequentially to large numbers of chemicals via multiple exposure routes. In the environment, most of the chemicals exist as mixtures and their toxicity is mainly attributed to their interactions. However, assessment of the potential health hazard of chemical mixtures (e.g. pesticides) is difficult and a challenging toxicological problem and a major subject of current concern to both the scientific and regulatory communities (Calabrese, 1991).Hence the necessity for this study.

1.6 SCOPE OF THE RESEARCH

This research work evaluated the potential of pesticides singly and in combinations to cause toxic and oxidative effects on adult male albino rats using biochemical techniques.

CHAPTER TWO

LITERATURE REVIEW

2.1 PESTICIDE USAGE

Pesticide can be defined as a substance or mixture of substances used for prevention, controlling or destruction pests, including vectors of human or animal diseases, unwanted plants or animals causing harm during, or otherwise interfering with the production, processing, storage, transport, or marketing of food (FAO, 1986). Pesticide can also be used as vector control and agriculture control agent in public health programmes (WHO, 1990). The development of pesticides became widespread after the Second World War; they were introduced to avert the problems of plant diseases and pest control. A survey on pesticides usage in Nigeria showed that about 15,000 metric tons annually of pesticides comprising about 135 pesticide chemicals marketed locally under 200 different produce brands and formulation were imported during 1983-1990 thus making Nigeria one of the largest pesticides users in sub-Sahara Africa (Osibanjo, 2002).

Although the gains of pesticides cannot be overemphasized, their uses raise a number of environmental concerns such as potential toxicity to humans and other animals (Baba, 2008). Over 98% of sprayed insecticides and 95% herbicides reach a destination other than their target species, including non-target species, air, water and soil. Pesticides usage is one of the causes of water pollution; some are persistent organic pollutant and contribute to soil contamination (David *et al*., 2008). The incidence of pesticides poison can result from the misuse, storage of pesticides close to consumable food stuff, the use of pesticides containers for household, improper disposal of used containers, treatment of food stuff with pesticides, transport of food and pesticides in the same lorry and cars such as in the case of Iraq 1970 (WHO, 1990). The

following categories of people are exposed to pesticides either accidentally or occupationally: pesticides manufacturers, vendors or sellers, mixers, transporters, loaders, operators of application equipment, growers, pickers and clean-up workers and the consumers of food items treated with pesticides.

A joint Food and Agriculture Organization/World Health Organization Food Contamination Monitoring programme found Aldrin, Dieldrin, DDT, Lindane, Heptachlor and Heptachlor epoxide in human breast milk (GEM, 1986). The WHO and UN Environmental program estimated that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides with about 18,000 deaths. According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly (WHO, 2006). Important human diseases in the tropics are transmitted by vectors or intermediate hosts like molluscs or insects that can be killed by molluscicide or insecticides (WHO, 1984). Edwards (1986) identified five main vector – borne diseases for which pesticides are used: malaria, filariasis, onchocerciasis, schistosomiasis and trypanosomiasis. Others include dengue fever, dengue haemorrhagic fever, chagas disease, leishmaniasis, louse-borne typhus etc. This is of great importance to the governments of developing countries as huge amount of pesticides are used indiscriminately for the control of these organisms without proper monitoring.

The adverse effects of pesticides contamination is not limited to the environment but, extended to human health. Hazards arising during the application of pesticides are mainly due to lack of information, knowledge and awareness, poor legislation or enforcement of legislation, and sales in the open market of highly toxic pesticides (WHO, 1990). The severity of any effect from exposure to pesticide depends on the dose, the route of exposure, how easily the pesticide is absorbed, the type of effect of the pesticides and its metabolites, the accumulation and

persistence in the body and lastly, the health status of the individual (WHO, 1990). The adverse effects of pesticide contamination are not limited to human health but extends to the environment, including its biotic and abiotic components.

Government agencies such as National Environmental Standards and Regulations Enforcement Agency (NESREA), National Agency for Foods and Drugs Control (NAFDAC), the Cocoa Research Institute of Nigeria (CRIN), the Nigeria Stored Products Research Institute (NSPRI), etc, should intensify efforts in taking the initiative to ensure safe use of pesticides in Nigeria.

2.2. Chemical Mixtures

A chemical mixture is when two or more chemicals (in this case, insecticides and/or miticides) are combined into a single spray solution (Cloyd, 2001). A pesticide mixture entails exposing individuals in an arthropod (insect and/or mite) pest population to each pesticide simultaneously (Tabashnik, 1989; Hoy, 1998). Pesticide mixtures may be more effective against certain life stages including eggs, larvae, nymphs, and adults of arthropod pests than individual applications (Blümel & Gross, 2001) although this may vary depending on the rates used and formulation of the pesticides mixed together (Blümel & Gross, 2001).

In today's world, human and animal environmental exposures are due to chemicals either at the same time or consecutive (Simmons, 1995). On the contrast, the overwhelming majority of the toxicology studies examined the cancer and non-cancer health effects of solely single chemicals toxicants. To handle issues over health risks from chemical mixtures, the U.S.Environmental Protection Agency developed guidelines for the "Health Risk Assessment of Chemical Mixtures" in 1986 (U.S.EPA, 1986). In addition, the Environmental Protection Agency developed another document to supply additional data on toxicity of whole mixtures and on toxicological

ninteractions between elements in mixtures (U.S.EPA, 1990). Following these tips and also the National analysis Council's recommendations (NRC, 1993), the amount of studies on chemical mixtures multiplied. Within the 1993 report, independent agency counseled that the Environmental Protection Agency (EPA) investigate the chance of synergistic interactions following multiple chemical exposures (prior approach was towards additivity). The EPA most recent document on mixtures is *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S.EPA, 2000).

2.2.1. Model for Binary Toxicity

Isobole Method

M. C. Berenbaum's "What is Synergy" article, published in 1989, gave a thorough treatment of methods for assessing interactions between biologically active agents. He reviewed the literature claiming synergy of biologically active agents and the various approaches for analyzing interaction. He exposed the weaknesses and lack of generality of many of the approaches and recommends the isobole method as a "generally valid procedure for analyzing interactions between agents irrespective of their mechanisms of action or the nature of their dose-response relations" (Berenbaum, 1989). Berenbaum presented a comprehensive proof of the general validity of the isobole method. The isobole method is used to distinguish between the expected increased effect of a combination of active agents and a true interaction (i.e., synergy or antagonism).

The isobole method is based on the dose response of the compounds in a mixture and uses 'isoeffective' or equally effective doses for each of the compounds to build an isobole graph.

Dose response bioassays estimate the biological activity of a test substance by means of the reaction that follows its application to living matter. Increasing concentrations of the test substance are applied to biological subjects. The subject's response is a measure of some characteristic of the subject which indicates the biological activity of the stimulus.

The relationship between dose and response is used to calculate the ECx, the expected concentration of a sample predicted to elicit an X% response. For example, the EC_{50} is the concentration of a compound predicted to elicit a 50% response. A well-designed dose response will yield responses ranging from very little response (e.g., 20% control) to very high response (e.g., 80% control). These ECx's are a measure of the potency of a compound. In crop protection studies, dose response assays effectively characterize the biological activity of a compound, the rate at which highest effect is achieved, the break rate (i.e., the rate where pest control begins to drop) and the rate at which effect is lost (i.e., a steep vs shallow dose response curve). Much more information is gained by a dose response for a compound than by a single rate.

The isobole method works with the potency of a mixture and its individual components, the rate predicted to give a specific level of response (works with the x that gives a specified y). It is important that one not extrapolate outside the range of responses. For example, if the maximum response obtained is only 30% control, do not build an isobole graph using the EC_{50}, rather use an ECx within the range of responses, for example an EC_{20}. Using the isobole method, interaction is detected when the effect of a combination of agents differs from that expected from their individual dose response curves.

The underlying assumption of the isobole method is that:

$$\frac{d_A}{D_A} + \frac{d_B}{D_B} = s \quad \textit{where} \quad \textit{if} \quad s = \begin{cases} < 1 & \textit{implies} \quad \textit{synergy} \\ 1 & \textit{implies} \quad \textit{independent} \quad \textit{action} \\ > 1 & \textit{implies} \quad \textit{antagonism} \end{cases}$$

Where:

D_A = EC_X for pure compound A

DB = ECX for pure compound B

dA = amount of compound A in the ECX of the mixture

dB = amount of compound B in the ECX of the mixture

The sums of the relative potencies of the components determine the overall effect. If the sum is one, then there is independent action in the mixture, just what would be expected if the presence of the other partner does not affect the activity of the first partner, and vice versa.

Synergy:

When agents in combination are more effective than expected from their dose response curves (synergy), smaller amounts are needed to produce the effect under consideration. So d_A and d_B are reduced, while D_A and D_B are the doses of the agents being used alone (Berenbaum, 1989).

$$\frac{d_A}{D_A} + \frac{d_B}{D_B} < 1$$

If there is synergy, s will be less than 1, as it takes less of both mixture partners, when used together to yield that same response as each component used separately. Compounds mixed together give greater than expected level of activity.

Antagonism:

Conversely, when agents in combination are less effective than expected (antagonism), larger amounts are needed to produce the effect under consideration. So d_A and d_B are increased, while D_A and D_B are the doses of the agents being used alone (Berenbaum, 1989).

$$\frac{d_A}{D_A} + \frac{d_B}{D_B} > 1$$

If there is antagonism, s will be greater than 1, as it takes more of both mixture partners to yield a level of activity. Compounds are working against each other. The isobole method is useful for designing mixture studies to efficiently assess a mixture space (i.e., all possible ratios of a mixture of two compounds).

2.3 PESTICIDES.

Pesticide often misunderstood to refer only to insecticides (kill insects and other arthropods), the term pesticide also applies to herbicides (kill weeds and other plants that grow where they are not wanted), fungicides (kill fungi including blights, mildews, molds, and rusts), rodenticides (control mice and other rodents), and various other substances used to control pests.

2.3.1. Definition and classification of insecticides

An insecticide is a pesticide used against insects. They include ovicides and larvicides used against the eggs and larvae of insects, respectively. Insecticides are used in agriculture, medicine, industry and the household. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century (Van Emden & Pealall, 1996).

Insecticides can be classified according to the type of action into organochlorine, organophosphates, carbamates, pyrethroids, neonicotinoids, biological insecticides and antifeedants (Brown, 2006).

2.3.2. Organophosphorus insecticides

Organophosphorus insecticides are highly toxic compounds containing active phosphorus. They are classified into three groups: phosphorothionate group, in which phosphorus is bound to three oxygens and one sulfur (the double bond). Phosphorothionates include chlorpyrifos, parathion, and tebupirimphos. Compounds in the phosphorodithioate group are like the phosphorothionates but with one of the oxygens replaced by sulfur. Phosphorodithioates include malathion, disulfoton, azinphos-methyl, sulprofos, and dimethoate. The atoms bound to the phosphorus of phosphoroamidothiolates are nitrogen, sulfur, and two oxygens; the double bond is to an oxygen. Examples of phosphoroamidothiolates are acephate and methamidophos (Chambers, 1992).

2.4 Dimethoate

2.4.1. Definition

Dimethoate is the ISO common name for O,O-dimethyl Smethylcarbamoylmethyl phosphorodithioate or 2- dimethoxyphosphinothioylthio-N-methylacetamide (IUPAC). Dimethoate belongs to the class of aliphatic amide organothiophosphate insecticides such as omethoate and mecarbam. It belongs also to the classes of organothiophosphate acaricides. Dimethoate was first described by Hoegberg and Cassaday in 1951 and introduced in market in 1956 (Fischer et al., 1997). It was first registered in the United States in 1962 (EPA, 2006). The chemical structure of dimethoate is illustrated in figure 2.1. (Chemfinder, 2006 ; Tomlin, 2006).

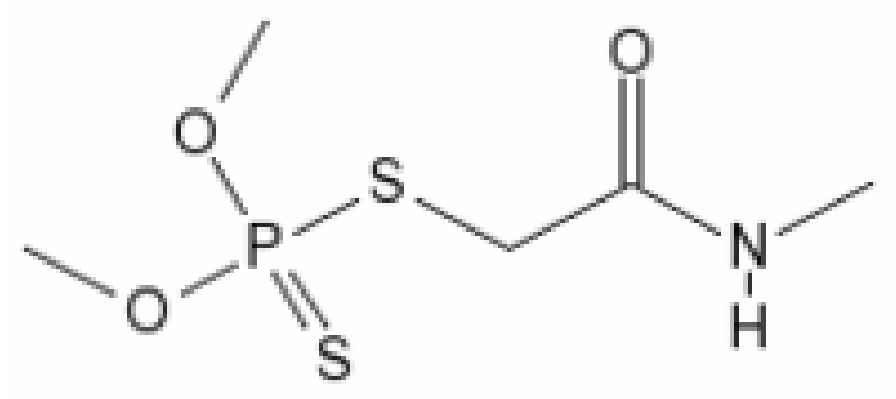


Figure 2.1 Chemical structures of dimethoate (Chemfinder, 2006 ; Tomlin, 2006).

World Health Organization (WHO) hazard classification of dimethoate is Class II, moderately hazardous. (World Health Organization, 2002). EPA has registered dimethoate as a systemic organophosphate insecticide but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements (Bhandare *et al*., 2011). Dimethoate is designed to be effective by direct contact, ingestion, and inhalation.

2.4.2. Physical and chemical properties of dimethoate

Dimethoate is a white crystalline solid with a mercaptan odor (EPA, 2006).Dimethoate is a highly mobile, generally non-persistent organophosphate insecticide (USEPA, 2004). Dimethoate has a low environmental persistence (Extension Toxicology Network, 1996b). The principal chemical properties of dimethoate are compiled in Table 2.1 (Kidd and James, 1994; Tomlin, 2006).

Table 2.1 Physical and chemical properties of dimethoate

Property	Values
Molecular weight	229.30 g/mol
Melting point	50.0ñ51.5 (99.5% purity)^{0}C
Solubility in water	25 g/L at 21^{0}C
Vapor pressure	3 x 10-12 mmHg 1.85 x 10-6 mmHg at 25^{0}C
Density	1.31 g/cm^3 (purity 99.1% w/w)

Source: (Kidd and James, 1994; Young, 2001; European Union Draft Assessment Report, 2005 and Tomlin, 2006).

2.4.3 Mechanism of action of Dimethoate

Dimethoate like other organophosphates inhibits acetylcholinesterase activity; an enzyme that breaks down the neurotransmitter acetylcholine on synapses and neuromuscular junction.

Acetylcholine as a neurotransmitter

Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. The acetylcholine then binds to a protein receptor in the membrane of the nerve synapse (Figure 2.2.A), which then opens/alters an ion channel, which in turn causes changes in the fluxes of ions (Na+, K+, Ca+, and Cl-) ultimately perpetuating the nerve impulse (Lee and Sine, 2005 and Sine and Engel, 2006). There are two types of acetylcholine receptors (AChR) that bind acetylcholine and transmit its signal:

1. Muscarinic receptors (mAChRs) at which muscarine action mimics the stimulatory action of acetylcholine on smooth muscle and gland. Muscarinic receptors are blocked by atropine. There are five subtypes of muscarinic AChRs based on pharmacological activity M1- M5 (Mohamadi *et al*., 2009).

2. Nicotinic receptors (nAChRs) which is stimulated by small amount of nicotine whereas a large amount of nicotine blocks the receptor. This effect mimics the action of acetylcholine on nicotinic receptor. The nicotinic acetylcholine receptors are members of a superfamily of ligand-gated ion channels (Dani, 2001 & Hogg *et al*., 2003). Nicotinic receptors subdivided into those found in muscle at neuromuscular junctions and those found in autonomic ganglia and the central nervous system.

Acetylcholinesterase

As illustrated in Figure 2.2., once acetylcholine makes its action, it is subsequently destroyed by acetylcholinesterase enzyme, and the membrane returns to its normal resting state (Liu and Casida, 1993; Zwart *et al*., 1994).

Acetylcholinesterase as a target for Dimethoate

Dimethoate binds to acetylcholinesterase enzyme in an irreversible manner leading to its inhibition (Figure 2.2.C). Acetylcholinesterase inhibition at synapses results in accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system. This will manifest in convulsions and even tremors leading in severe cases to death (Lotti, 2001 ; Wiener and Hoffman, 2004).

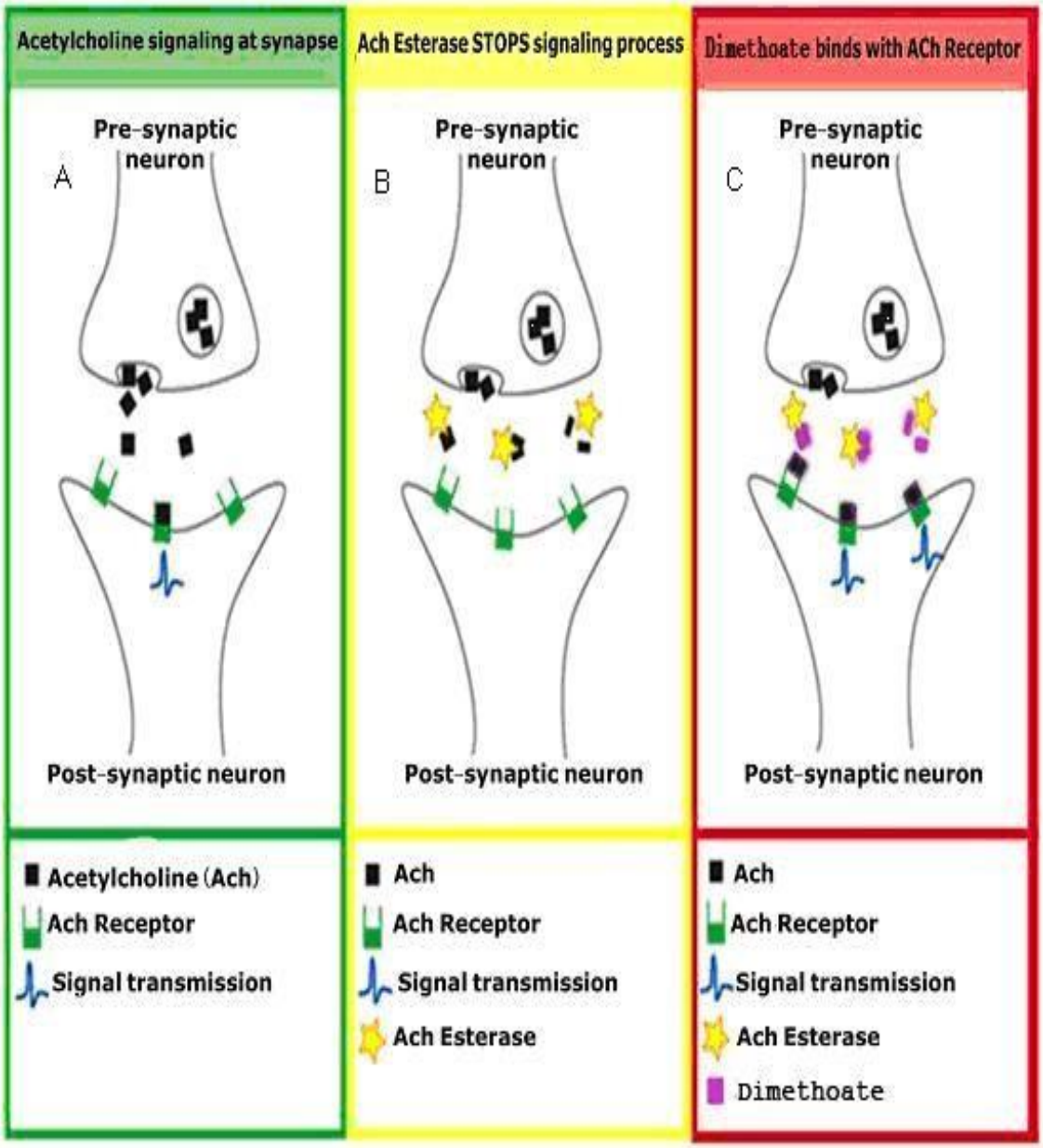


Figure 2.2. Pathophysiology of dimethoate insecticides poisoning**.** (Lotti, 2001 ; Wiener and Hoffman, 2004).

2.4.4 Toxicity symptoms of Dimethoate poisoning

Accumulation of acetylcholine at cholinergic synapses as a result of acetylcholinesterase inhibition producing a range of clinical manifestations, known as the acute cholinergic crisis. The particular clinical features depends on the type of receptors and their location (Eyer 2003; Eddleston *et al*., Karalliedde *et al*., 2006 ; Paudyal, 2008).

A. Muscarinic receptors: diarrhea, urinary frequency, meiosis, bradycardia, bronchorrhoea and bronchoconstriction, emesis, lacrimation, salivation, hypotension and cardiac arrhythmias.

B. Nicotinic receptors: fasciculations and muscle weakness, which may progress to paralysis and respiratory failure, mydriasis, tachycardia and hypertension.

C. Central nervous system: altered level of consciousness, respiratory failure and seizures.

2.4.5. Metabolism of Dimethoate

Dimethoate is rapidly absorbed from the gastrointestinal tract. The radiolabeled dimethoate was found in the liver, bile, kidneys and urine, and not in fat tissues after dosing. The proposed metabolic pathway consisted of hydrolytic (major) and oxidative (minor) pathways (Figure 2.3). The hydrolytic pathway involved cleavage of the C-N bond to yield dimethoate carboxylic acid that was subsequently metabolized to dimethyldithiophosphate, dimethylthiophosphoric acid and dimethyl phosphoric acid. The oxidative pathway involved oxidation of dimethoate to its oxon analogue (Omethoate) that was subsequently metabolized to dimethylthiophosphoric acid and dimethylphosphoric acid. Loss of the methoxy groups of the parent to yield CO_2 was another minor metabolic pathway (USEPA/Office of Pesticide Programs, 1995 ; FAO/WHO, 1997).

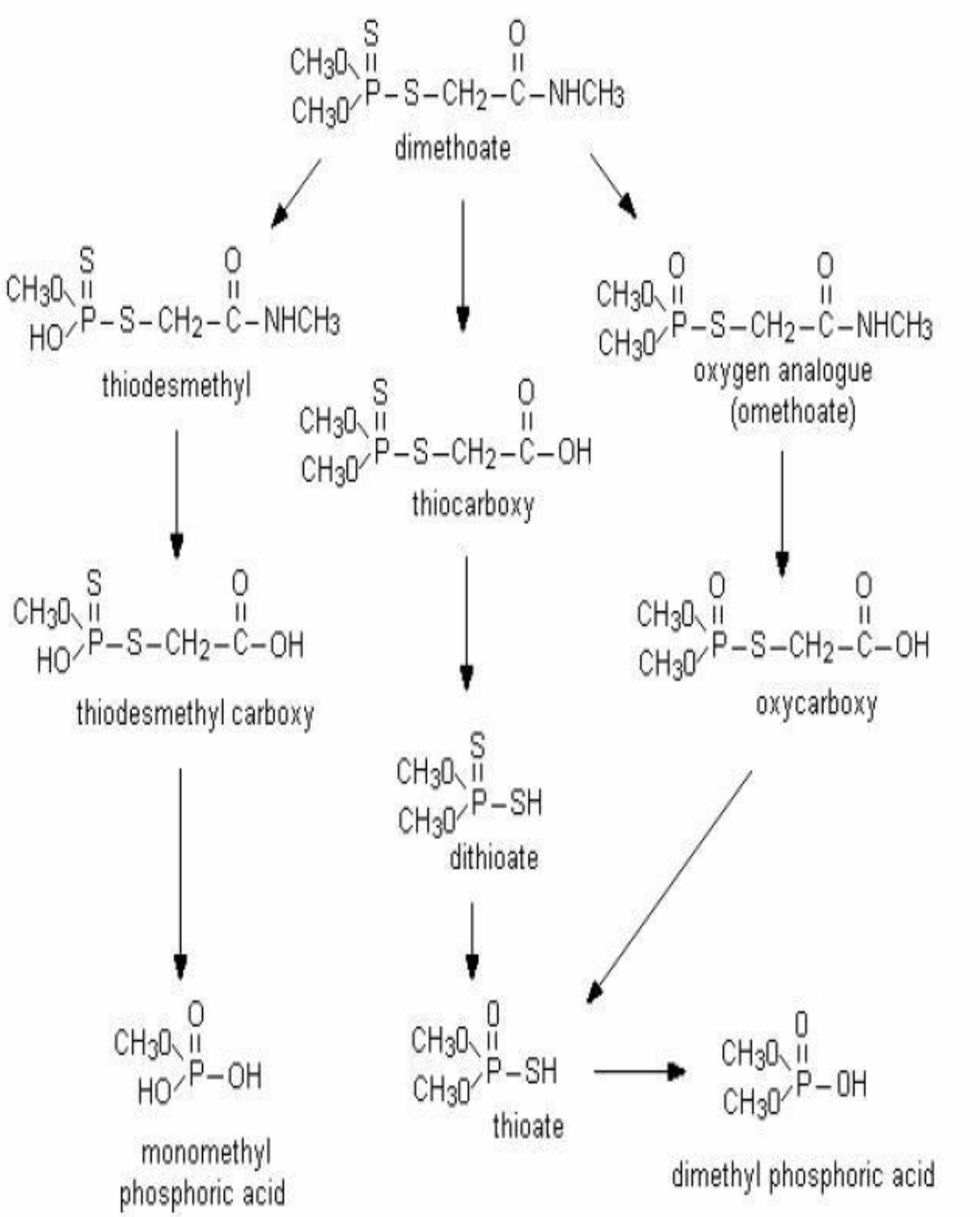


Figure 2.3. Metabolic pathway for dimethoate in rats (Food and Agriculture Organization/ World Health Organization, FAO/WHO, 1997)

2.4.6. Excretion of Dimethoate

About 45% and 5.8% of the radiolabelled dimethoate administered orally were excreted in the urine and the feces, respectively, in rats at 72 h after treatment. The equivalent values in rats after dermal application were 31% and 6.5%, respectively. More than 95% of excreta in the urine after the oral or dermal administration to rats were hydrolytic products (Health Canada Pest Management Regulatory Agency, 2011).

2.4.7. Distribution of Dimethoate

Radiolabeled dimethoate was well absorbed from the gastrointestinal tract following oral administration to rats. Maximum plasma and tissue concentrations were achieved 0.5 hour following dosing and showed a similardistribution in both sexes following a dose of 10 mg/kg body weight. In tissues, radioactivity was primarily detected in the liver and kidney, with the lowest levels found in the brain and fat (Kirkpatrick, 1995).

2.4.8. Uses of Dimethoate

Dimethoate is an organophosphate insecticide more frequently used to kill mites and insects systemically and on contact. This organophosphate is a broad spectrum insecticide effective against aphids, thrips, planthoppers, and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, tobacco, tomatoes, watermelons, wheat, and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. Dimethoate has been administered to livestock for control of botflies (Srivastav *et al*., 2010).

2.4.9 Physiological effects of Dimethoate

Reena *et al*. (1989) investigated chronic effects of a sublethal dose (150 mg/kg body weight) of dimethoate on blood constituents in rats after exposure of 15 and 30 days. After 30 days of exposure, the levels of blood glucose, cholesterol, urea, total bilirubin and the activities of AST, ALT and amylase markedly increased, but the activities of acid phosphatase and cholinesterase significantly decreased. There was no effect on total plasma protein content. The rats exposed to dimethoate for 30 days showed more prominent changes in all the blood constituents than those exposed for 15 days. The effect of dimethoate on Wistar rats was studied (Sivaswamy and Balachandran, 1990 and Sivaswamy, 1991). Alkaline phosphatase activity was found to be increased in liver and kidneys, and decreased in the intestines. In addition, Milillo *et al*. (1993), studied the occasional ingestion of dimethoate by sheep. They found that when 5 sheep were given grass sprayed with dimethoate at 4.5 liters/50 m^2, there was a fall in serum cholinesterase values for 24h. Furthermore, Hassan *et al*. (1994) and Attia (1995) reported significant decrease in body weight in response to dimethoate administration in rats. Abd-Allah (1998) evaluated dimethoate for its mammalian toxicity in albino rats. Dimethoate was administered orally at doses 1/10 and 1/30 LD_{50} daily for 30, 60 and 90 days. The body weight gain of the animals was significantly reduced. The activity of serum cholinesterase was significantly inhibited in all the tested periods. Serum AST, ALT and ALP activities were generally increased at all intervals studied. Albumin and total protein concentrations were decreased at 30 and 60 days intervals. Selmanoglu-Ozmen (2001) reported that ALP and AST were significantly increased whereas cholinesterase was significantly decreased in response to oral administration of dimethoate at a dose of 20.4 mg/kg daily for 3.5 months in Wister albino male rats. Decreased activity of serum cholinesterase was also detected in Wistar rats in response to 12.5 mg/kg body weight of

dimethoate in diet for 4 weeks (Kaspers *et al*., 2004). Hypercalcemia and hypophosphatemia were recorded by Mahjoubi-Samet *et al*. (2005) in rats exposed to 40 mg/kg body weight dimethoate. In addition, Kamath and Rajini (2007) investigated the effect of repeated sublethal doses of dimethoate on glucose homeostasis in adult rats. They found that daily oral administration of dimethoate (20 and 40 mg/kg body weight) for 30 days induced a significant increase in blood glucose levels which was associated with impaired glucose tolerance. Sayim (2007) evaluated the subchronic toxicity of orally administered dimethoate in Wistar albino rats, based on biochemical findings in the liver. The animals of the exposed groups were fed with laboratory chow combined with 2, 8 or 20 mg/kg body weight/day dimethoate for 90 consecutive days under controlled laboratory conditions. Results showed that there were decreases in relative liver weights of exposed rats. Although liver total protein levels were significantly increased, liver cholinesterase activities were decreased in all exposed groups. Mahjoubi-Samet *et al*. (2008) investigated the effect of dimethoate on kidneys of adult rats and their suckling pups. Female Wistar rats were given daily dimethoate in drinking water 0.2 g/l equivalent to 40 mg/kg bw from day zero until day 10 after delivery. In test group the authors have found higher plasma levels and lower urinary levels of creatinine, and urea than in the controls. In addition, Attia and Nasr (2009) recorded significant inhibition of serum cholinesterase and significant increase in the levels of AST, ALT, ALP and □- GT in rats received a single dose of dimethoate (75 mg per kg body weight). Urea, creatinine, uric acid and bilirubin levels were increased, whilst serum total protein, albumin and globulin were significantly decreased. The toxic effects of dimethoate on the biochemical parameters in male rabbits were studied (Salih, 2010). Twenty healthy rabbits (1500-1700 gm) were divided into control group: 10 animals treated with a single daily dose of 5ml corn oil orally for 20 days and dimothoate group: 10 animals treated with a single daily dose

of 1/4 of LD_{50} of dimothoate (20 mg/kg) in 5ml corn oil orally for 20 days. . Data showed that the treatment with 1/4 of LD_{50} of dimethoate resulted in a statistically high significant increase in the levels of serum ALT, AST and ALP as compared to the control. In contrast, total protein and albumin levels were significantly decreased in the serum of rabbits treated with dimethoate. In addition, serum uric acid and creatinine levels were significantly increased in dimethoate treated rabbits compared to controls. Saafi *et al*. (2011) found that daily oral administration of 20 mg/kg body weight dimethoate to males, adult Wistar albino rats caused hepatotoxicity as monitored by the increase in the levels of hepatic markers enzymes (ALT, AST, ALP and □-GT), as well as in bilirubin. Similarly, AL-Awthan *et al*. (2012) demonstrated significant increase in the levels of various serum marker enzymes of liver, including AST, ALT and ALP in response to oral administration of 1/50 LD_{50} dimethoate to guinea pigs. In addition, Saafi-Ben Salah *et al*. (2012) showed that feeding of Wistar rats with dimethoate for two months induced a marked renal failure characterized by a significant increase in serum creatinine and urea levels.

2.5. Lambda-Cyhalothrin

Pyrethroids, derivatives of natural pyrethrins have been well known for their high effectiveness against insects and can be easily biodegraded than other types of pesticides (Sharaf *et al*., 2010). In spite of claims of low mammalian toxicity of pyrethroid, several investigations reported the toxicological evidence of pyrethroid among various species of animals (Khan *et al*., 2016). Pyrethroids are easily absorbed through gastrointestinal and respiratory tract due to their lipophilic nature and also make them easier to be stored in the lipid rich internal tissues like body fat, skin, liver, kidney, central and peripheral nervous systems. Hemato-biochemical studies are important for the analysis of the functional status of animals to suspected toxic agents. It may act as strong evidence against toxicity of contaminated pyrethroid insecticides. Recent reports have

clarified that exposure to pyrethroid leads to a significant modifications in hematological findings (Khan *et al.,* 2009). On the other hand, the liver is the first organ encountered when toxicants enter into the body. The liver was found to accumulate a huge pyrethroid residues as it is the primary site for pyrethroid metabolism. A large number of man-made chemicals such as pesticides stated to produce liver damage (Jaeschke *et al*., 2002). Several investigations also reported that pesticides adversely affect the testicular functions in experimental animals (Lifeng *et al*., 2006) as well as they are potent endocrine disrupters (Solati *et al*., 2007). Pesticides are responsible for oxidative stress that causes free radicals generation, leading to deoxyribonucleic acid (DNA) fragmentation (Anadon *et al*., 2006).

Lambda-cyhalothrin (LC), a type II pyrethroid pesticide, is used worldwide to control pests in a variety of agricultural crops. LC is chemically alpha-cyano-3phenoxybenzy3-(2-chloro-3,3,3 trifluoropropenyl)-2,2,dimethylcyclo- propane carboxylate. It was revealed that lambda-cyhalothrin is moderately toxic for mammals (Anadon *et al*., 2006) and highly toxic for fish, aquatic invertebrates and bees. LC at low concentrations can cause death in these species (Barata *et al*., 2006). The degree of concentration and nature of solvent are important for the toxicity of lambda-cyhalothrin (Meister, 1992). Temperature influences insect paralysis and the toxicity of lambda-cyhalothrin (Toth & Sparks, 1990).

The residues of lambda-cyhalothrin can be found in breast milk, house dust and food (Watts, 2013). It is highly toxic neurotoxin with acute toxicity symptoms such as itching, tingling, burning, prickling sensation of skin especially face; dizziness, headache, nausea, anorexia, fatigue, respiratory irritation, tremors, convulsions, coma, death (US EPA, 2010). It has been reported that lambda-cyhalothrin has chronic toxicity such as damages liver, kidney, lungs, heart, spleen (animals) (Basir *et al*., 2011) while causing Pakinson's disease due brain changes. It is

highly genotoxic in animal and human cells (Fetuori & Zeghai, 2010). It was reported as an endocrine disruptor affecting oestrogenic and androgenic activities while decreasing testosterone and thyroid hormones in rats(Yousef, 2010).It is a male reproductive toxin; decreases semen quality, weight of testes and epididymis (rabbits); 20 sperm abnormalities, reduced sperm count and motility (rats); (Abdallah *et al*., 2012) blocks spermatogenesis, damages seminiferous tubules (Leballi *et al*.,2008).

2.6. HEPATOTOXICITY

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro & Senior, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett, Roth & Walker, 2004; Papay, Clines, Rafi, Yuen, Britt, 2009). Some drugs can cause liver damage when introduced even in right clinical doses. Reactive metabolites formed from primary compounds may elicit hepatotoxicity (Saukkonen, Cohn, Jasmer, Schenker & Jereb, 2006). The expressed hepatotoxic response due to the presence of chemical agents is directly proportional to the toxicant concentrations (Kedderis, 1996).

2.6.1 LIVER- THE TARGET ORGAN

The liver in human body is the largest organ and is found at the upper right corner of the abdomen on top of the stomach. The liver performs more than 500 vital metabolic functions (Naruse *et al*., 2007). It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It

regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile aids in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them (Saukkonen *et al*, 2006). An excess of chemicals hinders the production of bile thus leading to the body's inability to flush out the chemicals through waste. Smooth endoplasmic reticulum of the liver is the principal 'metabolic clearing house' for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury (Saukkonen *et al*., 2006).

2.6.2 BIOTRANSFORMATION OF HEPATOTOXICANTS

Biotransformation and xenobiotics disposition is a crucial role of the liver (Miyai *et al*., 1991). The close association of liver with the small intestine and the systemic circulation enables it to maximize the processing of absorbed nutrients and minimize exposure of the body to toxins and foreign chemicals. The liver may be exposed to large concentrations of exogenous substances and their metabolites. Metabolism of exogenous compounds can modulate the properties of hepatotoxicant by either increasing its toxicity (toxication or metabolic activation) or decreasing its toxicity (detoxification) (Kedderis, 1996). Most of the foreign substances are lipophilic thus enabling them to cross the membranes of intestinal cells. They are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are exported into plasma or bile by transport proteins located on the hepatocyte membrane and subsequently excreted by the kidney or gastrointestinal tract (Tostmann *et al*., 2008).

2.7. MECHANISMS OF HEPATOTOXICITY

Liver pathology serves as an important tool for identifying and characterizing liver injury whether or not clinicobiochemical changes are also identified. Main patterns of liver injury during hepatotoxicity may include zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions, neoplasm and veno-occlusive diseases.

2.8. BIOCHEMICAL MARKERS

Liver toxins can produce several signs of histological and clinical markers of liver damage. Hepatic injury can be assayed using some biochemical indicators such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin. Increase in serum enzyme levels are used as necessary biomarkers of hepatotoxicity but elevations in both conjugated and total bilirubin levels checks the overall hepatic status. An increase in levels of transaminase in conjunction with a rise in bilirubin level to more than double its normal upper level, is considered as an dangerous marker for hepatotoxicity (Reuben, 2004).

Table 2.2. Biochemical Markers of Hepatotoxicity in Blood Plasma and Serum.

	Tissue localization	**Cellular localization**	**Histopathological lesion**	**Reason of abnormality**
Alanine aminotransferase (EC 2.6.1.2)	Primarily liver; trace amounts in skeletal muscles and heart	Cytoplasm and mitochondria	Hepatocellular necrosis	Leakage from damaged tissues
Aspartate aminotransferase (EC 2.6.1.1)	Liver, heart, muscle, brain and kidney	Cytoplasm and mitochondria	Hepatocellular necrosis	Leakage from damaged tissues
Alkaline phosphatase (EC 3.1.3.1)	Liver, bile duct, bone, placenta, kidney and intestine	Cell membrane	Hepatobiliary injury and cholestasis	Overproduction and release in blood
γ-Glutamyl transferase (EC 2.3.2.2)	Kidney, liver, bile duct, pancreas	Cell membrane	Hepatobiliary injury and cholestasis	Overproduction and release in blood
Total bilirubin	Direct (Liver, bile, small intestine, large intestine) Indirect (Reticuloendothelial cells of spleen, serum)	Extracellular fluid	Hepatobiliary injury and cholestasis	Decreased hepatic clearance

Table 2.2 Biochemical Markers of Hepatotoxicity in Blood Plasma and Serum (continued).

	Tissue localization	Cellular localization	Histopathological lesion	Reason of abnormality
Urobilinogen	Large intestine, urine		Hepatocellular dysfunction	An increase in unconjugated bilirubin, due to increased breakdown of RBCs, which undergoes conjugation, excretion in bile and metabolism to urobilinogen
Bile acids	Produced in liver, stored in gall bladder and released into the intestine		Hepatobiliary disease	Regurgitation into blood along with conjugated bilirubin
Prothrombin time			Hepatocellular dysfunction	Decreased synthetic capacity
Lactate dehydrogenase (EC 1.1.1.27)	Liver peroxisomes, muscles, kidney, heart	Mitochondria and sarcoplasmic reticulum	Hepatocellular necrosis	Leakage from damaged tissue
Sorbitol dehydrogenase (EC 1.1.1.14)	Liver, kidney, seminal vesicle, intestine	Cytoplasm, mitochondria	Hepatocellular necrosis	Leakage from damaged tissue
Glutamate dehydrogenase (EC 1.4.1.2)	Liver, kidney	Mitochondrial matrix	Hepatocellular necrosis	Leakage from damaged tissues

Table 2.2 Biochemical markers of hepatotoxicity in blood plasma and serum (continued).

	Tissue localization	Cellular localization	Histopathological lesion	Reason of abnormality
Albumin	Produced in liver	Blood plasma	Hepatic dysfunction	Decreased synthesis
Total protein	Produced in liver and immune system	Blood plasma	Hepatic dysfunction	Decreased synthetic capacity
Serum F protein	Liver, kidney	Primarily cytoplasm	Hepatocellular necrosis	Leakage from damaged tissue
Glutathione-S-transferase (EC 2.5.1.18)	Liver, kidney	Cytoplasm, mitochondrial, centrolobular cells	Early hepatocyte injury; Hepatocellular necrosis	Readily released from hepatocytes in response to injury
Arginase I (EC 3.5.3.1)	Liver	Cytoplasm	Hepatocellular necrosis	Release from injured hepatocytes
Paraoxonase 1 (EC 3.1.8.1)	Liver, kidney, brain, lung	Cytoplasm, microsomal, endoplasmic reticulum	Hepatocellular necrosis	Not a leakage enzyme; reduced hepatic synthesis and secretion

Source: (adapted from Giffen *et al*., 2002)

2.9. DETERMINATION OF HEPATOTOXICITY

The measurement of levels of substances that may be present in the blood helps in the initial detection of hepatotoxicity .The estimation of serum bilirubin, urine bilirubin and urobilinogen helps in knowing the capacity of liver to transport organic anions and to metabolize drugs or xenobiotics. Several enzymes that trigger important chemical reactions in the body are produced in the liver and are normally found within the cells of the liver. However, if the liver is damaged or injured, the liver enzymes spill into the blood, causing elevated liver enzyme levels. The liver enzymes like transaminases, alkaline phosphatase, γ-glutamyl transpeptidase, sorbitol dehydrogenase, glutamate dehydrogenase and lactate dehydrogenase in the blood can be measured to know the normal functioning of liver. These enzymes help in detecting injury to hepatocytes. In case of patients showing hepatotoxicity with elevated liver enzymes due to certain hepatotoxicant, the enzymes levels usually return to normal within weeks or months after stopping the exposure to the hepatotoxicant which is suspected of causing the problem. Another measurable liver function is reflected in the albumin concentration, total protein and the prothrombin time which are the markers of liver biosynthetic capacity. Biochemical markers involved in hepatotoxicity in blood plasma and serum are listed in **Table 2.2**.

2.9.1. Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) is the most generally used clinical biomarker which is regularly used for the non-invasive monitoring of liver diseases in experimental species and in humans (Ozer, Ratner, Shaw, Bailey & Schomaker, 2008). ALT is an enzyme found in higher concentration in the liver with lower activities found in skeletal muscle and heart tissues. It causes the metabolism (transamination) of the amino acid alanine and plays an important role in gluconeogenesis. During hepatocellular injury, ALT escapes from the liver into the extracellular

space and enter the blood causing high serum ALT levels compared to control subjects which shows liver cell damage (Ozer, Ratner, Shaw, Bailey & Schomaker, 2008; Yang, Schnackenberg, Shi & Salminen, 2014).Unfortunately, Extrahepatic injury like muscle injury or cardiac injury can also lead to increase in ALT, making ALT not entirely hepato-specific.

ALT: Alanine + α ketoglutarate = Pyruvate+ Glutamate (Thapa & Walia, 2007).

Regardless of the fact that extrahepatic injury can cause increase in ALT, serum ALT remains the most widely used and generally accepted biomarker for DILI. It is considered to be the clinical chemistry gold standard for DILI detection (Ozer, Ratner, Shaw, Bailey & Schomaker, 2008). Also, newer biomarkers of liver injury with greater specificity to liver can be used in addition with ALT for the safety evaluation of developmental compounds in the pharmaceutical industry.

Recent studies have showed that measuring the ALT isozymes, ALT1 and ALT2helps to differentiate the source of injury. ALT1 is located in human hepatocytes, renal tubular epithelial cells, and salivary gland epithelial cells while ALT2is localized to human adrenal gland cortex, neuronal cells bodies, cardiac myocytes, skeletal muscle fibers, and endocrine pancreas. When compared to ALT1, ALT2 was found to add less to the total serum ALT activity and was probably a reflection of mitochondrial damage (Yang, Schnackenberg, Shi & Salminen, 2014). A novel immunoassay has been developed to discriminate human ALT1 and ALT2 activities and might improve the ALT assay.

2.9.2. Aspartate aminotransferase (AST)

Based on the same explanation as ALT, recent studies has shown aspartate aminotransferase (AST) as a standard biomarker for DILI accepted by clinicians (Ozer, Ratner, Shaw, Bailey &

Schomaker, 2008;Shi, Hong, Senior & Tong, 2010). AST are found in heart, brain, skeletal muscle and liver tissue. Just like ALT, AST is responsible for the metabolism (transamination) of the amino acid aspartate. Despite the fact that the sensitivity of AST test is known to be lower than that of ALT, it is still a generally used liver biomarker. Owing to its widespread expression in extrahepatic organs, such as the heart and muscle, AST is important but has less specificity than ALT in detecting DILI (Ezejiofor, Ezejiofor, Orisakwe, Nwigwe, Osuala & Iwuala, 2014).

AST: Alanine + α ketoglutarate = Oxaloacetate + Glutamate (Thapa & Walia, 2007).

The ratio of serum ALT and AST activity is important in differentiating DILI from extrahepatic organ injury, It also helps in the diagnosis of acute hepatitis and cirrhosis with an AST/ALT ratio at 2:1.AST have been discovered to have at least two isoenzymes, one is cytosolic AST and another is mitochondrial AST (mAST). It is reported that mitochondrial isoenzyme is responsible for an estimated 80% of AST activity in human liver, whereas most of the circulating AST activity in normal people is gotten from cytosolic isoenzyme (Thapa & Walia, 2007). It has not been fully determined that cytosolic AST or mAST adds to serum AST elevation and also if these AST isoenzymes are affected by drug-driven induction or inhibition (Yang, Schnackenberg, Shi & Salminen, 2014).

2.9.3. Alkaline phosphatase

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile. It hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells which line the biliary ducts of the liver. It is also found in other organs including bone, placenta, kidney and intestine. Several isozymes have been identified in humans and preclinical species. Normal levels are in the range of 20-120U/L. It may be elevated if bile excretion is inhibited by liver damage.

Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the duodenum (small intestine). Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepatobiliary effects and cholestasis (Ramaiah, 2007). In humans, increased ALP levels have been associated with druginduced cholestasis (Wright & Vandenberg, 2007).

2.9.4. γ-Glutamyl transferase

γ-Glutamyl transferase (GGT) or transpeptidase (GGTP) is an enzyme which is found in liver, kidney and pancreatic tissues, the enzyme concentration being low in liver as compared to kidney (Ozer, 2008). It catalyzes transfer of γ-glutamyl groups to amino acids and short peptides. It is more useful clinically when compared to ALP. ALP is more sensitive but much less specific than GGT. The comparison of the two enzymes helps in determining the occurrence of bone or liver injury. Normal GGT level with an elevated ALP level is suggestive of bone disease as GGT is not found in bone (Daniel & Marshall, 1999), while an elevated level of both the enzymes is suggestive of liver or bile duct disease. Normal levels are in the range of 0-51 U/L. GGT is a specific biomarker of hepatobiliary injury, especially cholestasis and biliary effects. It was reported as a specific indicator of bile duct lesions in the rat liver. (Dufour *et al*., 2000).

2.9.5. Total bilirubin (TBL)

Bilirubin is an endogenous anion derived when hemoglobin degrades from the red blood cells (Thapa & Walia, 2007). Serum bilirubin is a mixture of α, β, γ and δ fragments which are unconjugated, singly conjugated, doubly conjugated and covalently bound to albumin,

respectively. Even though δ bilirubin measurement is available, it is not widely used because total bilirubin assay is sufficient for LFT, but separation by fractionation may be required in isolated increases in bilirubin and neonatal jaundice (Yap & Aw, 2010).

Serum bilirubin is helpful in differentiating the causes of jaundice. In the case of pre-hepatic jaundice caused by haemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin while in hepatic and post-hepatic jaundice, there is increased conjugated and δ bilirubins(Yap & Aw, 2010). Therefore, serum bilirubin concentration is a liver function biomarker, which measures the ability of liver to clear bilirubin from the blood as it circulates through the liver.

Bilirubin can be classified into direct bilirubin and indirect bilirubin according to original vander Bergh method of measuring bilirubin. Direct bilirubin (DB) refers to the conjugated bilirubinsor water-soluble fractions that react directly with the diazo reagent, while indirect bilirubin is a derived value obtained from the difference of the total bilirubin and DB.

Total bilirubin (TBL) on the other hand is a composite or mixture of unconjugated (extrahepatic) and conjugated (hepatic) bilirubin. Total bilirubin is the factorwhich helps to diagnose Gilbert syndrome in males easily. Increased TBL causes a morbid condition known as jaundice and liver metabolism problems like reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion (Wintrobe & Greer, 2009). In acute human hepatic injury, total bilirubin can be a better indicator of disease severity compared to ALT (Ozer, Ratner, Shaw, Bailey & Schomaker, 2008)

2.9.6. Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme (EC 1.1.1.1.27) found in different types of human tissues. Despite LDH is tetrameric enzyme, only two subunits have been determined which are denoted as H for heart and M for muscle (AL-Janabi, Ali & Noree, 2015). The main duty of LDH is the conversion of pyruvic acid, the final product of glycolysis to lactic acid and vice versa in muscle through production of cellular energy. LDH is localized in most of the active organs in the body in small amounts. Therefore, high level of this enzyme may indicate unusual conditions that can result from liver, muscular disorder (e.g. acute myocardial infarction) and even from cancer disease (AL-Janabi, Ali & Noree, 2015). Moreover, total LDH level may increase in blood of patients suffering from various diseases such as allergy, but not in patients with chronic obstructive pulmonary disease (AL-Janabi, Ali & Noree, 2015). High level of LDH is a biomarker for liver disease.

2.9.7. Albumin (ALB)

Albumin is measurable the most significant protein in plasma synthesized by the liver and is a useful indicator of hepatic function. The serum albumin level is not a good indicator of hepatic protein synthesis in acute liver disease because serum albumin changes slowly due to its long half life of about 20 days (Thapa & Walia, 2007; Yap & Aw, 2010). Albumin synthesis only takes place in the liver and is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. The serum levels are typically down in patients with cirrhosis and ascites. In patients with or without ascites, the serum albumin level corresponds with prognosis also the rate of albumin synthesis has been shown to correlate with the Child-Turcotte or Child- Pugh score (Thapa & Walia, 2007). The serum albumin levels tend to be

normal in diseases like acute viral hepatitis, drug-related hepatotoxicity and obstructive jaundice. Albumin is decreased by trauma, inflammatory conditions and malnutrition (Yap & Aw, 2010).

2.9.8. Total protein

The Assay of total body proteins is helpful in determining a normal and damaged liver function as the majority of plasma proteins like globulins and albumins are produced in the liver (Ozer *et al*., 2008). Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during liver damage.

2.9.9. Glutathione-S-transferase (GST)

This is an inducible phase II detoxification enzymes that catalyzes the conjugation of glutathione with reactive metabolites formed during phase I of metabolism. Induction of GST synthesis is a protective mechanism that occurs in response to xenobiotic exposure. It is released quickly and in large amounts into the bloodstream during liver damage and the increase in its activity are more rapid than AST or ALT. (Ozer *et al*., 2008). GSTα expression is restricted to liver and kidney.

2.10. Reactive Oxygen Species (ROS) / Oxidative Stress

Oxygen is used to carry out metabolism in the body but can sometimes react with a metabolic compound to form a free radical which causes biological damage in a living cell (Manisha, Hasan, Rajak & Jat, 2017). Oxygen is a stable product but when it gains or losses an electron in oxygen molecule or its compound it becomes a reactive oxygen species. Oxygen in form of free radical are produced in large amounts in the body they acts as a substance or molecule which can cause oxidative stress thereby causing disease able effects in a living cell (Bhattacharya, 2015).

Reactive oxygen species (ROS) are very small, highly reactive molecules with unpaired valence shell electrons. Reactive oxygen species include Singlet oxygen, Superoxide, hydroxyl radical, hydrogen peroxide, hydroperoxyl radical, ozone etc (Manisha, Hasan, Rajak & Jat, 2017). ROS is formed as a natural secondary product of the normal metabolism of oxygen which plays important roles in cell signaling. Though, ROS levels can increase excessive during environmental stress which can lead to significant damages to cell structures (Khan, Garg, Singh & Kumar, 2018). ROS helps in wound repair and blood homeostasis by addition of platelets to already existing platelets in the sites of injury. Also, the harmful effects of reactive oxygen species on the cell are most often like DNA damage, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors (Khan, Garg, Singh & Kumar, 2018).

Oxidative stress can be defined as an imbalance between the production of reactive oxygen species or reactive nitrogen species and the levels of antioxidants used as protective or defensive mechanisms (Amiri, 2018; Khan, Garg, Singh & Kumar, 2018). Oxidative stress can also be as a result of the fast production of reactive forms of oxygen than they can be neutralized by antioxidants i.e free radicals exceeding the oxidant system of the body. Hence, increasing RONs and decreasing or lack of antioxidants induces oxidative Stress (Amiri, 2018). Any prolonged imbalance results to oxidative damage to cells, tissues and organs.

Generally, oxidative stress are caused by series of activities like alcohol consumption, use of drugs (anti-inflammation, anti-analgesic, anti- cancer and anti-depressants), environmental pollution by pollutants like mercury chloride, lead and other factors like mobile phone/radio frequency radiation exposure, UV radiation, x-Ray exposure, temperature (cold stress), maternal high-fat diet, Pesticides etc (Amiri, 2018).

Oxidative stress is said to be the cause of cancer, Alzheimer's diseases, Parkinson's disease, amyotrophic lateral sclerosis and so many other lifestyle related disorders such as arterial sclerosis, coronary heart diseases, atherosclerosis, stroke, high blood pressure, myocardial infarction, cerebral apoplexy, dementia, diabetes, cataract, asthma, obesity, lung cancer etc (Halder & Bhattacharya, 2014; Amiri, 2018). Oxidative stress is one of the major causes of cardiac diseases like congestive heart failure, hypertension, and atherosclerosis (Sugamura & Keaney, 2011). The Smooth muscles and cardiac muscles during the regulation of metabolic process release free oxygen radicals as a form of the byproducts of this process which interrupts the supply of blood to the heart which sometimes leads to myocardial infarction (Cardiac infarction, heart attack)of the heart (Manisha, Hasan, Rajak & Jat, 2017).

Oxidative stress also plays some beneficial roles in the body system such as assisting in birth delivery by inducing apoptosis to prepare the birth canal for delivery. It also plays a role in strengthening of the biological defense mechanisms during ischemia and physical exercise (Yoshikawa &Naito, 2002). Oxidative stress activates the aging process because aging is known to be the progressive loss of tissue and organ function over a period of time (Liguori, Russo, Curcio, Bulli, Aran *et al*., 2018).

2.11. REPRODUCTIVE EFFECTS OF PESTICIDES

Organophosphate

Organophosphates are among the most widely used synthetic insect pesticides. The widespread use of organophosphates has stimulated research into the possible existence of effects related with their reproductive toxic activity. Ngoula *et al*., (2007) investigated the effects of pirimiphos-methyl (0, 2- diethylamino-6-methylpirimidin-4-yl O, O-dimethyl phosphorothioate), an

organophosphothioate pesticide, on male rat reproductive performances. Results from the study showed decrease in serum total protein, sperm density and motility, fertility. Histological findings also indicated enlargement of interstitial space, inhibition of spermatogenesis, rarefaction of Leydig cells and oedema in testes compared to control animals.

Dimethoate

Sayým, (2007) investigate subchronic effect of dimethoate on the testes of rats. The animals of exposed groups were fed with laboratory chow combined with 2, 8 and 20 mg/kg dimethoate for 90 days. When compared to control, there was a statistically decrease in relative testis weights of rats treated with dimethoate. In light microscopic examinations, histopathological observation of the treated rats revealed that dimethoate caused doserelated testicular damage characterized by moderate to severe seminiferous tubule degeneration as sloughing, atrophy, germ cell degeneration and by partial arrest of spermatogenesis. Farag *et al*. (2007) demonstrated the adverse effects of dimethoate on the reproductive performance of male mice. The sperm viability, motility and density were reduced in dimethoate treated mice.

Pyrethroid

Yao & Wang, (2008) observed a new type of pesticides and because of their high performance and low toxicity, pyrethroid insecticides are widely used in place of organochlorine insecticides both in agriculture and in the home. In the recent years, more and more evidence indicates that pyrethroid insecticides can reduce sperm count and motility, cause deformity of the sperm head, increase the count of abnormal sperm, damage sperm DNA and induce its aneuploidy rate, as well as affect sex hormone levels and produce reproductive toxicity. Meeker *et al*., 2008 observed that reduced semen quality and increased sperm DNA

Lambda-cyhalothrin

Sanhez *et al*. (2004) observed reduction in the testicular weight on LCT exposure which was perhaps due to decreased tubule size, reduced number of germ cells and enlarged spermatids. Decline in sperm motility after oral administration of LCT was either by androgen deprivation effect of the pyrethroid or by low spermatogenesis. Another possibility was enhanced reactive oxygen species ROS production by pyrethroid exposure. Similar results were also reported (Joshi *et al*., 2011)

Multiple pesticide exposure

A study conducted by Rope *et al*. (1991) among male workers who were exposed to various mixtures of pesticides such as DDT, BHC, endosulfan; and organophosphorus pesticides i.e. malathion, methylparathion, dimethote, monocrotophos, phosphamidon and quinalphos; synthetic pyrethroids such as fenvelrate and cypermethrin during mixing and spraying showed male mediated adverse reproductive outcome such as abortion, stillbirths, neonatal deaths, congenital defects, etc. Studies of males exposed to DDT have found decrements in serum bioavailable testosterone levels, (Martin *et al*., 2002) and reduced semen volume on ejaculation and reduced sperm counts. Ben *et al*., (2001) evaluated damage in relation to urinary metabolites of pyrethroid insecticides. Meeker *et al*., (2008) evaluated pyrethroid reduced semen quality and increased sperm DNAdamage in relation to urinary metabolites of pyrethroid insecticides.

CHAPTER THREE

MATERIALS AND METHODS

3.1. INSECTICIDES

Dimethoate (DM) (40% EC; Jiangsu Tenglong Biological and Medicinal Co. Ltd. China.) and Lambda-cyhalothrin (LC) (2.5% EC; Bretmont Ltd. England) insecticides were used in the present research. A Mixture of both insecticides (20:1) was contained in MagicForce; a commercial EC formulation produced by Anhui Zhongshan Chemical Industries Co. Ltd. China. These chemicals were purchased from Alaba International market in Owerri, Imo state, Nigeria. The LD_{50} of the mixture (Magicforce) was determined to be 23 mg/kg according to method adopted by Locke *et al* (1983) while that of Dimethoate and Lambda-cyhalothrin were 215 mg/kg and 79 mg/kg respectively as obtained from the work of Blümel & Gross (2001).

Determination of LD_{50} of Pesticide (Magicforce)

The index of acute toxicity LD_{50} is the dose of a substance capable of producing death in 50% of the population of animal exposed to the substance. Modification of Locke's method (1983) was used. This method involved two phases, that is, phase I and phase II

Phase I

Three groups of mice containing three mice each were used. Pesticide was administered at concentrations of 10mg/kg body weight (b.w) to group I mice, 100 mg/kg b.w to group II mice and 1000 mg/kg b.w into group III mice that formed the phase one of LD_{50}. Administration was done orally and monitored for 24 hours for abnormal reaction or death.

Phase II (This was carried out depending on the result obtained from phase one). After 24 hours, death was recorded as low as in group that received 100mg/kg dose of pesticide, thus this result informed our design in phase two of the LD_{50} test.

In this phase, two groups of three mice each was used and the following treatment was carried out; Group IV received 50 mg/kg b.w. of pesticide, while Group V received 75 mg/kg b.w. of pesticide and were monitored for 24 hours for any abnormal reaction or death.

$$LD_{50} = \sqrt{minimal\ dose\ at\ which\ death\ occured\ \times\ maximal\ dose\ at\ which\ no\ death\ was\ recorded}$$

3.2. ANIMALS

Forty male albino rats (3–4 month's old; 160–180g b.wt.) were supplied by the breading animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in individual cages and allowed to acclimatize under laboratory conditions at room temperature for one week prior to commencement of the experiment. The animals were kept under hygienic and favorable conditions, and maintained under a 12 h light/12 h dark cycle, with pelletized rat feeds (UAC Vital Feeds, Nigeria) and water available *ad libitum.*

3.3. Experimental Design

Sublethal dose of the insecticides (5% of the LD_{50}) was used in this research administered through oral gavage. The animals were divided into 4 groups of 10 animals each with the mean weights equalized as nearly as possible. Dimethoate group (DM) received 10.7 mg/kg body weight. Lambda-cyhalothrin group (LC) received 3.95 mg/kg body weight. MagicForce group (MF) received 1.2 mg/kg body weight. Control group (C) received food and distilled water only. The experimental animals were offered normal diet and water *ad libitum* throughout the 28 day period of the experiment. During the period, all the animals were observed for clinical signs and mortality patterns once daily. The body weights of the animals were taken weekly using sensitive balance. At the end of the 28 day feeding period, an overnight fast was imposed on the animals and then their weights taken to obtain the final body weights of the rats.

3.4. Blood and Tissue Sampling

At the end of the post feeding fast, the rats were subjected to light diethyl ether to induce anesthesia. Blood was collected by cardiac puncture into clean and dry test-tubes without anticoagulant, allowed to stand to separate serum and then preserved in a refrigerator until used for analyses. Similarly, some quantity of the blood was collected into EDTA containers for hematological studies. Then the liver and testes were collected, cleaned, weighed (absolute weight) and separately homogenized in 5mL cold buffer (0.1 M-phosphate buffer, pH 7.4) per gram tissue. The homogenates were further vortexed for 15 minutes, allowed to stand and the supernatant collected into sterile tubes and preserved in a refrigerator until used for biochemical analyses.

3.5. Relative organ weight of the rats

The relative organ weight of each animal was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight of the rat (g)}} \times 100$$

3.6. BIOCHEMICAL ANALYSES OF SAMPLES.

3.6.1 Determination of serum lactate dehydrogenase (LDH):

Serum LDH was determined according to the method of McQueen, (1975) as reported by Sherif *et al*. (2014)

Principle

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is proportional to the LDH activity in the sample.

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\textbf{LDH}} \text{L- Lactate} + NAD^{+}$$

Procedure

Reagents used were from Agappe diagnostic kit. 0.01ml of serum was added to 1 ml of working reagent prepared by mixing 4 volume of Reagent 1 [Tris buffer (pH 7.4, 80mmpl/L), Pyruvate (1.6 mmol/L), Sodium Chloride (200 mmol/L)] with 1 volume of reagent 2 [NADH (240 µ mol/L)] provided in the kit in a test tube. The content of the test tube was well mixed and incubated for 1 min at 37^{o} C. Then the absorbance was measured every minute for 3 min at 340 nm.

LDH activity (U/L) = (Δ OD / min) x 16030

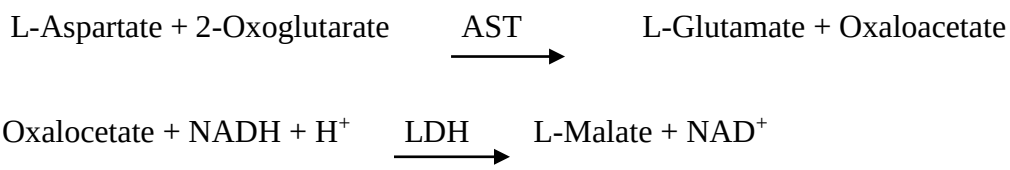
Where: (Δ OD / min) = Change optical density/minute.
16030 = Dilution faction

3.6.2. Determination of Serum Aspartate Aminotransferase

Serum aspartate aminotransferase (AST) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to Thomas (Thomas, 1998) using DiaSys reagent kits.

Principle

The principle of the test is that AST catalyzes the reversible transamination of L-aspartate and ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the AST activity in the sample.



Reagents

Components	**Concentration**
Reagent 1	
TRIS pH 7.65	80 mmol/l
L-Aspartate	240 mmol /l
MDH (Malate dehydrogenase) LDH (Lactate dehydrogenase)	≥ 600 U/l ≥ 900 U/l
Reagent 2	
2-Oxoglutarate	12 mmol/l
NADH	0.18 mmol/l

Monoreagent preparation

Four parts of R1 was mixed with1 part of R2 (800µl R1 + 200µl R2) = Monoreagent

Procedure

	Quantity
Monoreagent	1000 **µl**
Sample	100 **µl**

The reagents and the sample were pipette into labeled test tubes, well mixed and incubated for 1 minute at room temperature. Then the absorbance was measured every minute for 3 minutes at 340nm.

Calculation

The change in absorbance reading ΔA /min was calculated and multiplied by the corresponding dilution factor:

ΔA /min X factor (1745) = AST activity [U/l]

3.6.3. Determination of Serum Alanine aminotransferase (ALT) Activity

Principle

This was based on the method of Reitman and Frankel (1957) as reported by Sherif *et al*. (2014). The principle of the test is that ALT catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. ALT is

measured by monitoring the concentration of pyruvate hydrazone formed when pyruvate is reacted with 2,4-dinitrophenylhydrazine.

Procedure

At room temperature, the reagents were pipetted into labeled test tubes and treated as follows:

Reagents	**Reagent Blank**	**Sample**
Sample	-	0.1 ml
Solution R1 (100mmol/l phosphate buffer pH 7.4, 200mmol/l L-alanine and 2.0mmol/l α-oxoglutarate	0.5 ml	0.5 ml
Distilled water	0.1 ml	-
Mixed and incubated for Exactly 30mins at 37^{o}C		
Solution R2 (2mmol/l 2,4-dinitrophenyl hydrazine)	0.5 ml	0.5 ml
Mixed and incubated for Exactly 20mins at room temperature 25^{o}C)		
Sodium Hydroxide (0.4mol/L)	5.0 ml	5.0 ml

The content of the test tubes were mixed thoroughly and left at room temperature for 5 minutes, absorbance of each sample was read in a spectrophotometer against the reagent blank at 540nm wavelength. The activity of ALT in the serum was read from the calibration curve in U/L.

3.6.4. Determination of Serum Alkaline Phosphatase (ALP) activity

ALP activity was determined according to the method described by Englehardt *et al*., 1970 as reported by Sherif *et al*. (2014).

Principle

ALP catalyzes the hydrolysis of p-nitrophenylphosphate to p-nitrophenol - with the simultaneous release of phosphate group. The rate of reduction of p-nitrophenylphosphate, measured by determining the rate of increase in absorbance at 405nm, is directly proportional to the ALP activity.

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate+ p-nitrophenol

Reagent Contents

Buffer (1M diethanolamine buffer, 0.5M $MgCl_2$), Subtrate (10M p-nitrophenylphosphate).

Procedure: At room temperature, the reagents were pipetted into labeled cuvettes as follows:

	Reagent blank	Sample
Sample	-	50 µl
Reagent	3.0 ml	3.0 ml

The content of each of the cuvettes was mixed, initial absorbance read against the blank and subsequently read at 405nm after 1, 2 and 3 minutes.

Calculations:

ALP activity (U/L) = (ΔA 405 nm/min) X 3300

Where; ΔA= Change in absorbance, min = minute, 3300 = dilution factor

3.7. OXIDATIVE EFFECTS OF THE PESTICIDES

3.7.1 Determination of lipid peroxidation

The levels of lipid peroxidation in samples were measured as malondialdehyde (MDA) according to the method of Buege and Aust (1978) as reported by Sherif *et al*. (2014).

Principle

The malondialdehyde (MDA) is formed mainly from the peroxidation of poly unsaturated fatty acids (PUFAs). MDA is a thiobarbituric acid (TBA) reacting substance (TBARs) and the product (a chromogen) formed between the reaction of MDA and TBA is extracted into an organic layer and absorbance estimated at 532 nm.

Procedure:

A 0.1 ml of tissue supernatant, 0.9 ml of distilled water and 1 ml TBA reagent (TBA (0.375 %), TCA (15 %), 11N HCl (0.003 %) and EDTA (0.22 %) were pipette into sterile labeled test tubes and incubated in boiling water bath for 30 minutes. After 30 minutes, the reaction mixture was removed from the water bath, cooled and centrifuged at 1000 rpm for 15 minutes. Absorbance of the clear supernatant was measured at 532 nm against TBA reagent. The MDA was calculated with the help of a standard graph prepared by using different

concentrations (1-10 nmol) of 1'1'3'3'- tetramethoxypropane in 1 ml distilled water and is expressed as nmol of MDA/mg protein.

3.7.2. Determination of Total Protein Concentration

The Biuret method as described by Tietz, 1995 was employed for the determination of protein concentration as reported by Sherif *et al*. (2014).

Principle: Copper (II) ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. The test was carried out using a protein test-kit (Randox, UK) that utilizes the Biuret method for protein determination.

Procedure: At room temperature the reagents were pipetted into labeled test tubes as follows:

Reagents	Reagent Blank	Standard	Sample
Distilled water	0.02 ml	-	-
Standard (66 g/l bovine serum albumin)	-	0.02 ml	-
Sample (Serum)	-	-	0.02 ml
Solution1(Biuret Reagent) 6 mmol/l of copper (II) acetate, 12mmol/l of potassium iodide, 1.15 mmol/l of sodium hydroxide, and detergent	1.0 ml	1.0 ml	1.0 ml

The contents of each of the test tubes was mixed thoroughly and incubated for 30 mins at room temperature

- The absorbance (A) of samples or standard was read against the reagent blank at 546nm in a spectrophotometer.

Calculations: The total protein concentration in the sample was calculated as follows

$$C_{sample} = \frac{A_{sample} \times C_{standard}}{A_{standard}}$$

3.7.3. Determination of Serum albumin Concentration

This was determined by method employing bromocresol green as described by Doumas *et al* (1971) as reported by Sherif *et al*. (2014).

Principle: Albumin, in the sample, reacts with bromocresol green in an acid medium forming a coloured complex that absorbs maximally at 630 nm.

Procedure: The reagents were brought to room temperature and then were pipetted into labeled test tubes as follows:

Reagents	Blank	Standard	Sample
Albumin standard	-	10 µl	-
Sample	-	-	10 µl
Reagent A-Bromocresol reagent (100mmol/l acetate buffer, 0.27 mmol/l bromocresol green, detergent), pH 4.1	3.0 ml	3.0 ml	3.0 ml

The test tubes were mixed thoroughly and incubated for 5minutes at room temperature.

The absorbance (A) of samples or standard was read against the blank at 630 nm in a spectrophotometer.

Calculations: The albumin concentration in the sample was calculated as follows

$$C_{sample} = \frac{A_{sample} \times C_{standard}}{A_{standard}}$$

3.7.4. Determination of Serum Total Bilirubin Concentration

The method that exploits the use of diazotized sulphanilic acid as described by Jendrassik and Grof (1938) was used in the assay as reported by Sherif *et al*. (2014).

Principle: Direct (Conjugated) bilirubin in the sample reacts with diazotized sulphanilic acid forming azobilirubin which is converted to a blue coloured complex by addition of alkaline solution that can be measured by spectrophotometry. Total bilirubin is determined in the presence of caffeine (solubilizing agent), which releases albumin bound bilirubin (unconjugated) and then the conjugated and unconjugated estimated by the reaction with diazotized sulphanilic acid.

Procedure: The reagents were pipetted into labeled test tubes at room temperature as follows:

Reagents	Sample blank	Sample
Reagent 1 (29mmol/l sulphanilic acid,0.17N HCl)	200 µl	200 µl
Reagent 2 (38.5mmol/l Sodium nitrite)	-	50 µl
Reagent 3 (0.26mol/l Caffeine, 0.52mol/l Sodium benzoate)	1000 µl	1000 µl
Sample	200 µl	200 µl
Mixed and Incubated for 10mins at 20-25^{0}C		
Reagent 4 (0.93mol/l Tartrate, 1.9N NaOH)	1000 µl	1000 µl

The test tubes were mixed thoroughly by vortexing and incubated further for 15 min at 25^0 C.

The absorbance (A) of samples was read in a spectrophotometer at 578nm against the blank.

The total bilirubin concentration was calculated as follows:

Total bilirubin (mg/dl) = 10.8 x A_{sample} (A_{sample} is the absorbance of sample at 578 nm).

3.7.5. Determination of superoxide dismutase (SOD) activity in the samples:

SOD activity was measured using the technique of Mc Cord and Fridovich (1969) reported by Sherif *et al*. (2014)

Principle

The photo-illumination of riboflavin solution in the presence of EDTA causes a reduction of the flavin. It then re-oxidizes and simultaneously reduces oxygen to $O2^-$, which is allowed to react with nitroblue tetrazolium (NBT), a detector molecule. Upon reaction NBT is reduced to a formazan blue. The SOD in the sample inhibits the formazan production.

Procedure:

A 0.01 ml of each sample supernatant was pipetted into sterile labeled test tubes, then mixed with 0.2 ml of 0.1 M EDTA (containing 0.0015% NaCN), 0.1 ml of 1.5 mM NBT and made up to final volume of 2.95ml with phosphate buffer (67 mM, pH 7.8). Then, 0.05 ml of riboflavin was added and the absorbance of the solution was measured against distilled water at 560 nm. All the tubes were illuminated uniformly with an incandescent lamp for 15 min and absorbance was measured again at 560 nm. Percent of inhibition was calculated after

comparing absorbance of sample with the absorbance of control (the tube containing no enzyme activity). The volume of the sample required to scavenge 50 % of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/ mg protein.

3.7.6. Determination of catalase (CAT) activity in the samples:

Catalase activity in the blood was determined by the method of Aebi (1983) as reported by Sherif *et al*. (2014).

Principle

The catalase activity was assayed by measuring the decomposition of hydrogen peroxide (H_2O_2). The H_2O_2 has absorption maxima at 240 nm and absorption decreases with the decomposition of H_2O_2. The difference in extinction per unit time is a measure of the catalase activity.

Procedure:

A 0.01 ml of each sample supernatant was pipetted into sterile labeled test tubes then mixed with 1.9 ml of the phosphate buffer (0.5 M, pH 7). The decrease in extinction was measured at 240 nm, 15 sec interval for 1 minute immediately after adding 1 ml of H_2O_2 (30 mM in the buffer, fresh every time). A sample control was placed in the reference cuvette containing 0.1 ml of sample supernatant and 2.9 ml of the buffer. Activity of catalase was calculated using the formula given below and expressed as U/mg protein.

Calculations

$$\text{Amount of Catalase} = \frac{\Delta A/\text{min} \times 1000 \times 3}{40 \times \text{mg protein in sample}}$$

Where ΔA/min = Change in absorbance per time; Dilution = 1000

3.7.7. Determination of glutathione peroxidase (GPx) activity in the samples:

Glutathione peroxidase activity was measured using the technique of Hafemann *et al*. (1974) as reported by Sherif *et al*. (2014).

Principle

The GPx enzyme degrades the hydrogen peroxide (H_2O_2) in presence of GSH by the following reaction.

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$$

The remaining GSH is measured by its reaction with dithionitrobenzene (DTNB)

Procedure:

A 0.01 ml of each samples supernatant was pipetted into sterile labeled test tubes then mixed with 0.1 ml of 5 mM GSH, 0.1 ml of 1.2 mM H_2O_2, 0.1 ml of 25 mM NaN_3 and made up to final volume of 2.5 ml with phosphate buffer (1M, pH 7.0) at 37^0 C for 5 min. The reaction was stopped by adding 2 ml of 1.67 % mM H_3PO_4 and then centrifuged for 10 minutes in order to sediment the precipitate at 3000 rpm. A 2 ml of the supernatant was mixed with 2 ml 0.4 M Na_2HPO_4 and 1 ml of 1 mM DTNB (in buffer). The absorbance of the yellow coloured complex was measured at 412 nm after incubation for 10 min at 37^0 C against distilled water. A sample without the tissue homogenate supernatant was processed in the same way and kept as the blank. The activity is expressed as U/mg protein for tissue homogenate supernatant.

$$\text{The GPx activity} = \frac{\text{O.D of blank} - \text{O.D of sample}}{0.01 \text{ X mg protein X } 10}$$

Where

O.D = Optical density

3.7.8. Determination of Glutathione-S-transferase (GST) in the samples.

Glutathione-s-transferase (GST) was measured by the technique of Habig *et al.,* (1974) as reported by Sherif *et al*. (2014).

Principle

This test is based on the GST-catalyzed reaction between reduced glutathione and GST substrate, 1-chloro-2, 4-dinitrobenzene (CDNB). The GST activity is determined by measuring the rate of produced conjugation between reduced glutathione and CDNB, which is proportional to the increase in absorbance at 340nm over time (ΔOD_{340nm}/min).

Procedure:

A 0.04 ml of each sample supernatant was pipetted into sterile labeled test tubes then mixed with 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml of GSH (30 mM). The reaction was initiated by addition of 0.06 ml of 1-chloro-2, 4-dinitrobenzene (CDNB) (0.01 M dissolved in 50% ethanol). The activity was calculated by using milli molar extinction coefficient of CDNB-GSH conjugate as 9.6. The activity was expressed as μmoles of CDNB-GSH conjugate formed/min/mg protein.

3.7.9. Determination of Glutathione (GSH) content in the samples.

Reduced glutathione in the samples was determined according to the method of Moron *et al*. (1979) as reported by Sherif *et al*. (2014).

Principle

Reduced glutathione forms a yellow colored complex with dithionitrobenzene (DTNB) with an absorbance at 412 nm.

Procedure:

A 0.1ml of each sample supernatant was pipetted into sterile labeled test tubes then mixed with 0.063 ml of 25 % trichloroacetic acid (TCA) and placed on ice for 5 minutes followed by further dilution of the mixture with 0.3ml of 5 % TCA and these were then subjected to centrifugation at 3000 r.p.m for 5 minutes to sediment the precipitate. 0.15ml of the supernatant was mixed with 0.35 ml of sodium phosphate buffer (0.2 M, pH 8.0) and 1.0 ml of DTNB (0.6 mM in 0.2 M, phosphate buffer, pH 8.0). The yellow colour obtained was measured at 412 nm against a blank which contained 5 % TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 nM) of GSH. The GSH content of the sample was calculated with reference to standard and expressed as mg/g protein.

3.8. Hematological Analyses.

3.8.1. Determination of Full blood count

Principle

The machine employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HB detector block measures the haemoglobin concentration using a non-cyanide haemoglobin method. Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes.

Procedure:

The full blood count was done with the sysmex K-21n automatic multi-parameter blood cell counter for *in vitro* diagnostic use in clinical laboratories. Counting of blood cells is based on the volumetric impedance method, directly measuring white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), platelets, mean corpuscular volume(MCV), and mean platelet volume, and automatically calculating hematocrit (HCT), mean corpuscular haemoglobin

(MCH), MCH concentration(MCHC), RBC distribution width, plateletcrit, and platelet distribution width. The instrument differentiates the subpopulations of lymphocytes, granulocytes and the mid-cell fraction (eosinophils, basophils, monocytes,and precursors of WBCs) by electronic sizing. Specially formulated reagents cause the WBC membrane to shrink around the nucleus while keeping the cell intact, allowing separation of white cells according to their volume. Lymphocytes fall within the small-cell region, neutrophils within the large-cell region, and the remaining cells into the mid-size cell region.

3.9. Effects of the Pesticides on Reproductive Functions of Male Albino Rats.

3.9.1. Sperm Evaluation

Rat spermatozoa were obtained by the method of Cancel *et al*. 2000. A 5g of testes was minced in 2 ml of physiological saline and incubated at 37^0c for 45 minutes to allow dispersion of spermatozoa.

Sperm Motility: Sperm motility was evaluated by placing a drop of the sperm suspension on a microscopic slide and observed for sperm motility at 400x magnification using a light microscope. Ten microscopic fields and a total of 200 sperms were observed in each case. Sperm motility was expressed as a percentage of motile sperm relative to the total sperm count according to WHO (1999).

Sperm Count: 10µL of the diluted sperm suspension made by adding 5µl of diluted buffer (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue) was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min.

This chamber was then placed under a binocular light microscope using an adjustable light source. The calibrated part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was then calculated multiplied by 5 and expressed as $[X]_X10^6$ ml^{-1}, where [x] is the number of spermatozoa in a 16-celled square. Counting in the microscope was done at 400_x magnification (WHO, 1999).

Sperm Viability: To determine the sperm viability, 0.2ml of 1%trypan blue stain was mixed with a drop of the semen in grease free slide for 5 min, then a drop of the solution was pipetted into a Neubauer hemocytometer, covered with a slip and allowed to stand for 2 min. Then observation was under a light microscope at 400X magnification. The numbers of stained and unstained sperms were scored in ten different microscope fields. Dead sperm were stained pink while live ones were not stained.

3.9.2. Determination of Testosterone concentration

Testosterone level was measured according to the method of Tietz, (1995).

Principle of the Assay:

The Testosterone ELISA is based on the principle of competitive binding between Testosterone in the test specimen and testosterone-horseradish peroxidase (HRP) conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with testosterone standards, controls, patient samples, testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the amount of testosterone-HRP immunologically bound to the well progressively

decreases as the concentration of Testosterone in the specimen increases. Unbound testosterone-peroxidase conjugate is then removed and the wells washed, followed by addition of TMB Reagent resulting in the development of blue color. The color development is stopped and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

Procedure:

All reagents used were at room temperature. The microplate wells were formatted for serum reference, control and sample. Then 0.01 ml of the appropriate sample was pipetted into respective wells and 0.1 ml (100µl) of Testosterone-HRP Conjugate was added to the wells. Thereafter, 0.05ml (50µl) of rabbit anti-Testosterone reagent was dispensed into each well. Then, the set-up was gently swirled for 30 sec to mix. It was then incubated for 90 min at 37^0 C. Thereafter, the incubated mixture was removed by flicking the plate contents into a waste container. The microtiter wells were rinsed and flicked 5 times with distilled water and blotted dry with absorbent paper to remove all residual water droplets. A 0.1ml (100µl) TMB reagent was dispensed into each well and gently mixed for 5 sec then incubated at room temperature for 20 min. The reaction was stopped by the addition of 0.1ml (100µl) of stop solution to each well and gently mixed for 30 sec. The absorbance in each well was read at 450nm 9 using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. The result was read within 30 min of adding the stop solution.

3.10. Statistical Analysis

The data obtained was analyzed with one-way analysis of variance (ANOVA) using the statistical package for the social science (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. GENERAL HEALTH OF RATS

Observation of the animals for mortality revealed that no rat died in the control group during the 28 day experiment recording mortality rate of 0 (0%). However, the overall mortality rate recorded for rats treated with 1/20 LD_{50} (5%) dimethoate (DM), lambda-cyhalothrin (LC) and magicforce (MF) were 2/10 (20%), 3/10 (30%), 1/10 (10%) respectively. In addition, rats in the control group did not show any sign of toxicity. However, pesticide-treated rats showed varying degrees of clinical signs few days after administration. The signs included disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea. The rats treated with LC showed most signs of toxicity followed by the DM-treated then the MF.

4.1.2. BODY WEIGHT OF THE RATS

There was general decrease in body weight of the rats exposed to the sublethal dose of the pesticides compared to the control group as shown in Fig 4.1. The DM group with initial average weight of 104.7 g had increase in body weight in day 7 (116.7 g) and subsequently had steady decrease from day 14 to the 28 day (98.8g, 91.5g, 87.4g). LC group with initial average body weight of 111.2g had steady decrease from day 7 to the 28 day (103.3g, 92.7g, 89.3g, 89.0g). MF group with initial average body weight of 115.3g had an increase in the day 7 (128.9g) and steady decrease to the expiration of the treatment (117.2g, 112.7g, 110.9g). The C group which is

control group with initial average body weight of 120.2g had an increase in day 7 (127.0g), then reduction in day 14 (121.4g), thereafter had steady increase from day 21 to 28 (128.4g, 140.0g).

4.1.3. AVERAGE ORGAN WEIGHT OF THE RATS.

There was general increase in the average weight of the liver of rats exposed to the pesticides. The treated groups (DM, LC and MF) had 4.67g, 5.50g and 4.66g respectively as against the control group (C) 4.25g. The weight of the testes was significantly reduced. DM, LC and MF groups had the following weights 1.83g, 1.89g, and 2.62g respectively as against the control group with 2.62g. The testes weight had no significant difference while the liver weight of the pesticide treated groups against the control group was significant at $p \leq 0.05$ as shown in Fig 4.2.

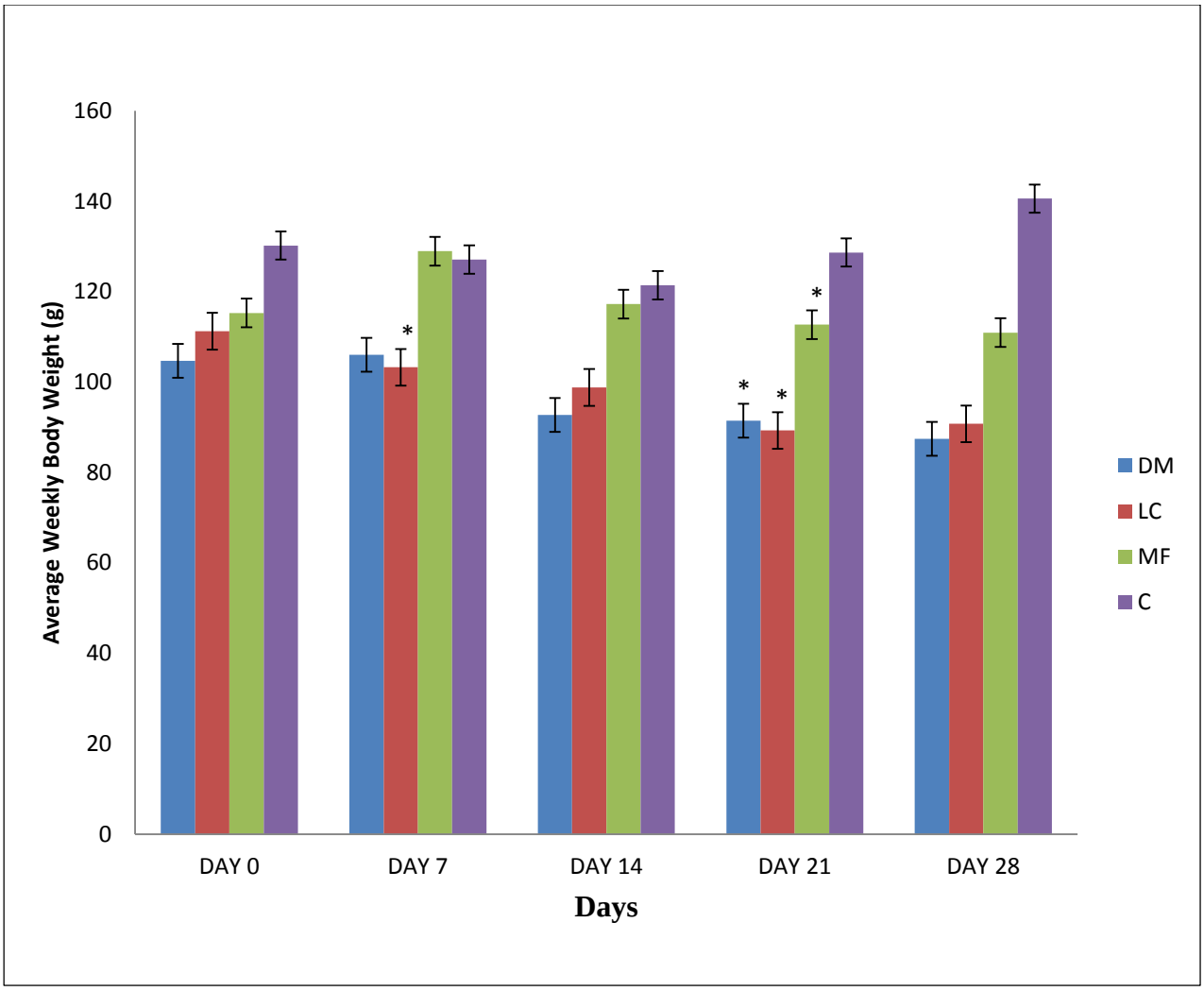


Fig.4.1.Average Weekly Body Weight of Male Albino Rats (g).

All values were expressed as mean ±SEM.

LSD* shows the significant difference of treatments compared to control (Group C) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce(MF), Control(C)

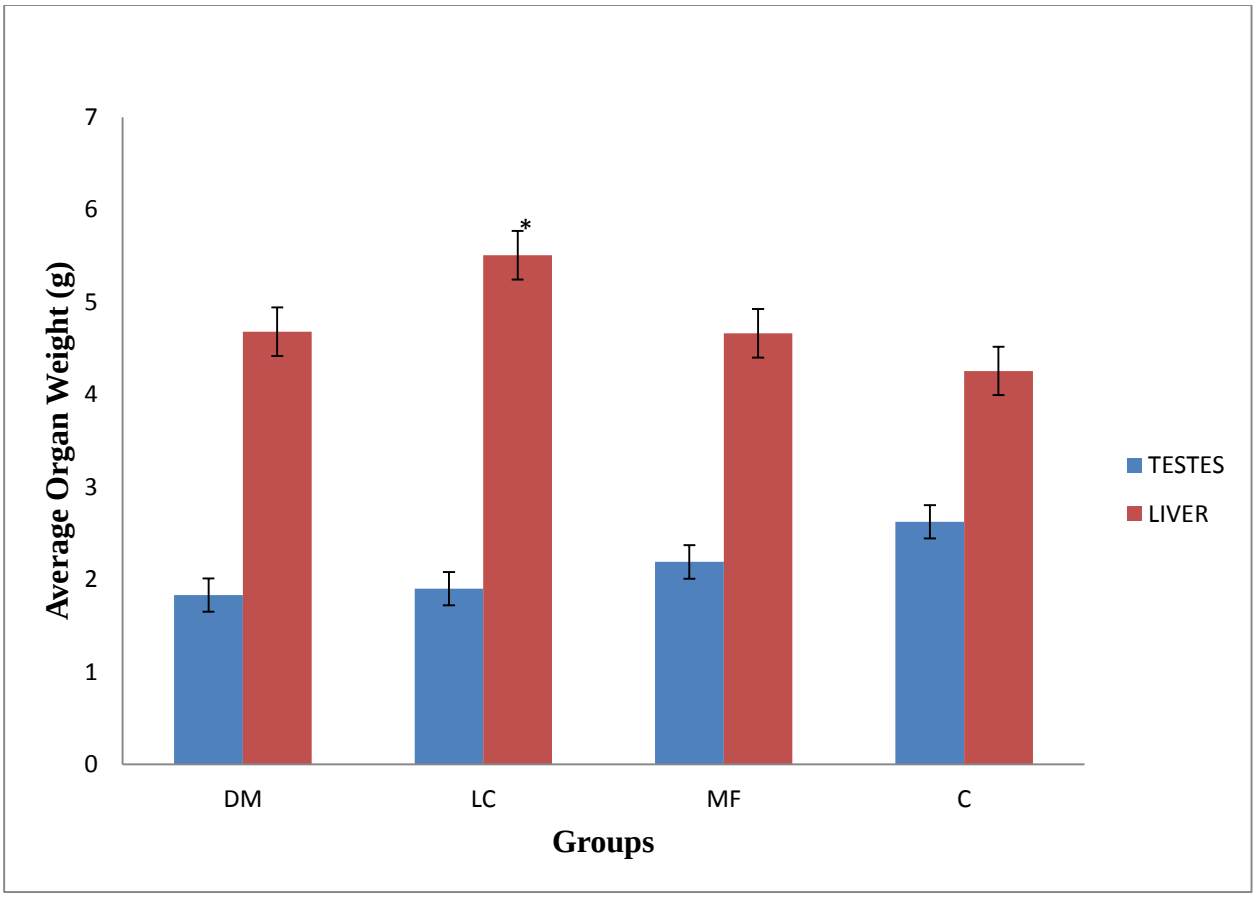


Fig.4.2. AVERAGE ORGAN WEIGHT OF MALE ALBINO RATS

All values were expressed as mean ±SEM.

LSD* shows the significant difference of treatments compared to control (Group C) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce(MF), Control(C)

4.1.4. TOXIC EFFECT OF THE PESTICIDES AND THEIR MIXTURE ON LIVER, GONAD AND BLOOD CELLS

Lactate Dehydrogenase Activity.

Lactate dehydrogenase activity was employed to monitor the cytotoxic impacts of the agricultural chemicals singly and their combinations on liver, testes and serum of the male albino rats after 28 days of daily oral administration of sublethal dose (0.25ml) and compared against the control as shown in Fig 4.3. There was significant difference in the lactate dehydrogenase activity in the liver and testes but that of the serum was not significant at $p \leq 0.05$. For LDH liver activity, the LC-treated group had the highest activity (73.83µ/L) followed by the MF-treated with 66.17µ/L, then the DM-treated group (65.68) against the C group (55.56µ/L). The LDH testes activity in the C group was 261.57 µ/L. There was increase in the enzyme activity in pesticide treated groups with highest observed in LC treated group (352.35 µ/L), the MF-treated group was 333.53 µ/L while the least was the DM-treated group (285.58 µ/L).

4.1.5. THE EFFECTS OF THE PESTICIDES AND THEIR MIXTURE ON LIVER FUNCTION ENZYMES.

From the results of the liver function tests as shown in Fig 4.4., there exist variations in the liver function enzymes due to pesticide exposure of the male albino rats for 28 days experimental period. All the parameters measured at $p \leq 0.05$ had significant difference. For AST, the control (C) group enzyme activity was 107.13µ/L. The LC treated group was highest (138.13 µ/L), followed by the DM treated group (120.62 µ/L) while the MF treated group was the least (119.77 µ/L). ALT activity of the control group (C) was 38.76µ/L with its activity elevated in all the pesticide-treated groups. 44.73 µ/L was recorded for the LC treated group as the highest

followed by the MF treated group (42.75 µ/L), then the least was the DM treated group (41.75 µ/L).

The control group for the ALP activity was recorded as 14.54µ/L. There was general increase in all the pesticide treated groups. Most elevated was the LC treated group with 29.92 µ/L, followed by the DM-treated group (26.21 µ/L) and the least was the MF treated group (25.30µ/L).

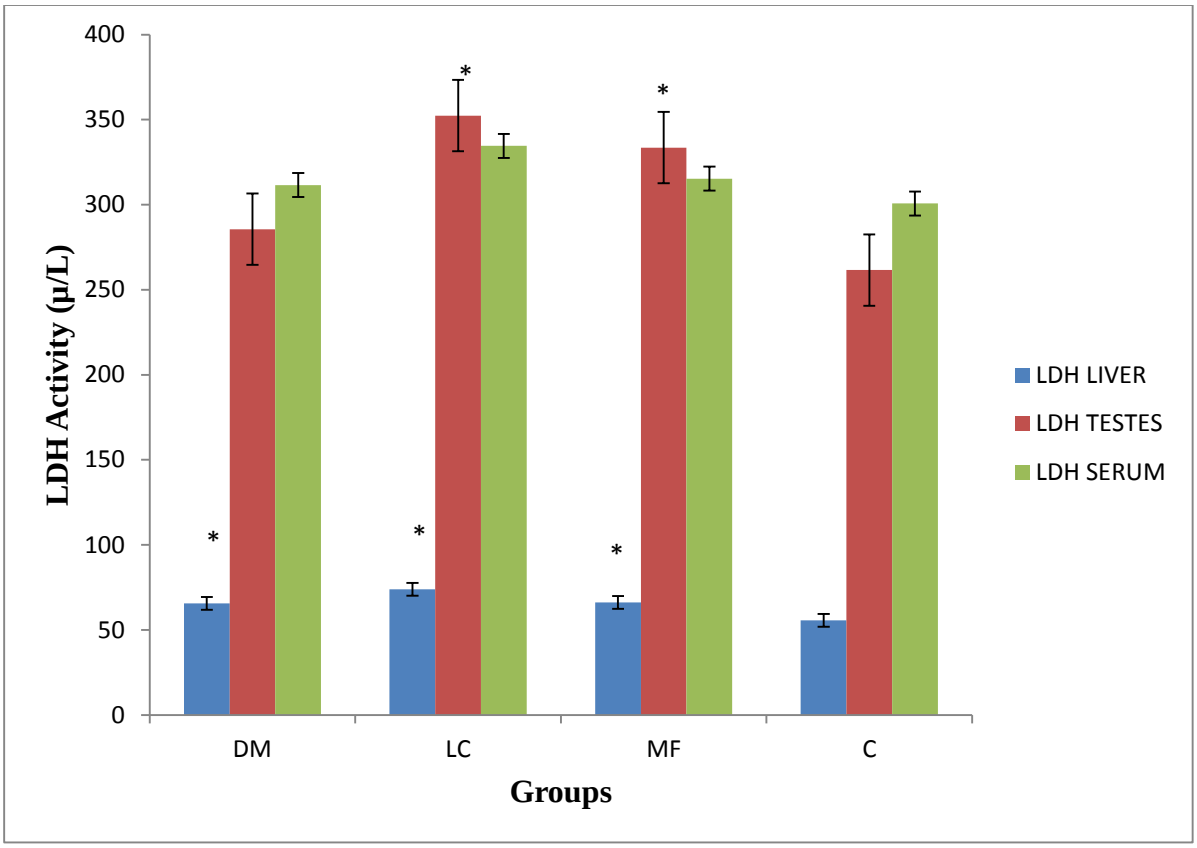


Fig.4.3. EFFECTS OF THE PESTICIDES AND THEIR MIXTURE ON LACTATE DEHYDROGENASE ACTIVITY

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (Group IV) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control (C)

LDH = Lactate dehydrogenase

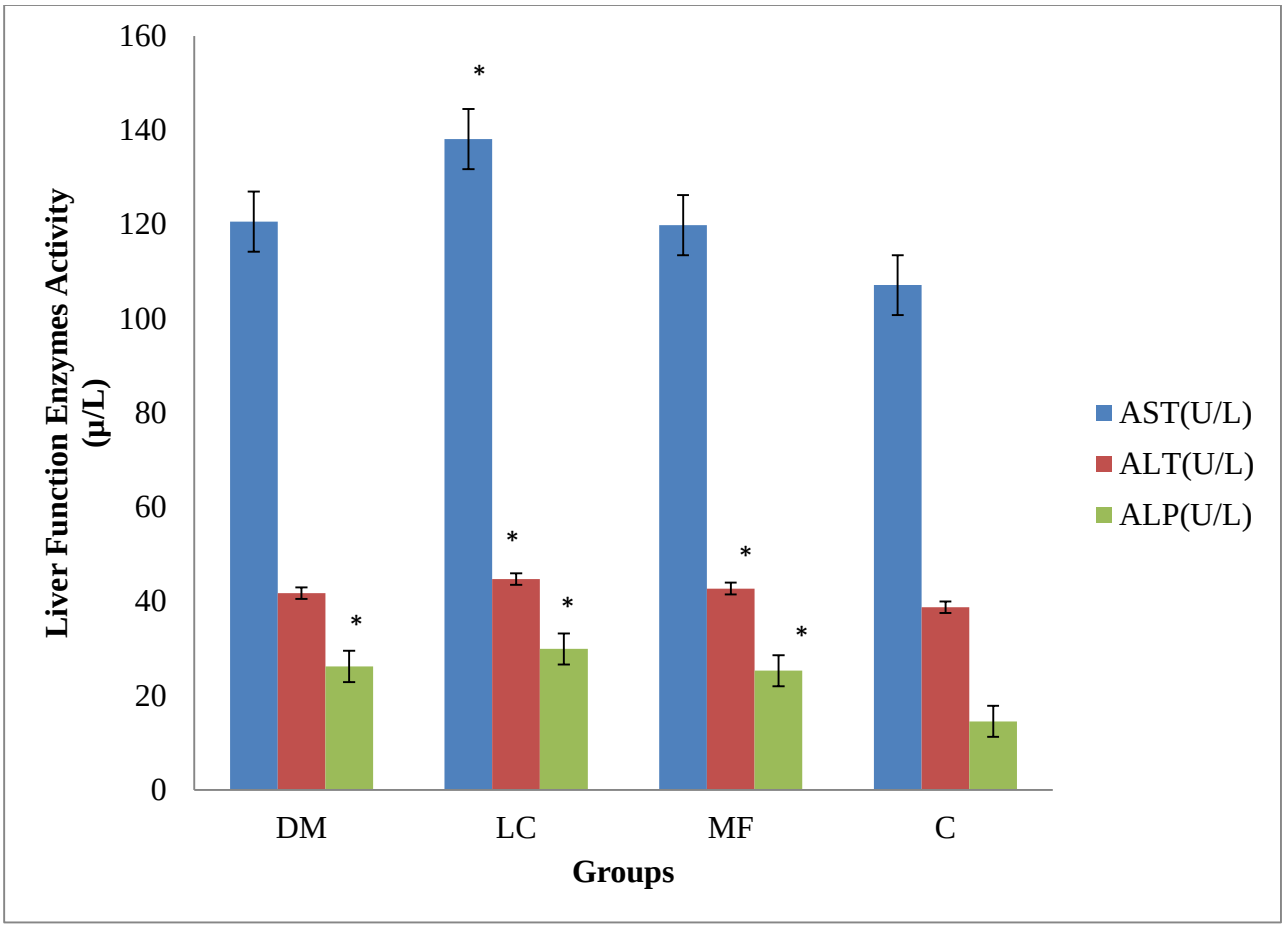


Fig.4.4. EFFECTS OF THE PESTICIDES AND THEIR MIXTURE ON LIVER FUNCTION ENZYMES.

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (Group IV) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control (C)

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.

4.1.6. OXIDATIVE EFFECTS OF THE PESTICIDES.

The Effects of the Pesticides and Their Mixture on Serum Protein Profile.

The result of the serum protein profile as shown in Fig 4.5 revealed a general decrease in Total protein and albumin levels while increase was observed in the Total bilirubin in the pesticide treated groups against the control. There was significant difference in all the parameters measured at $p \leq 0.05$. The control group for the Total protein level was 74.23 g/L. The most reduced was the LC treated group with 58.12 g/L followed by the MF treated group (62.88), then, the least was the DM treated group (64.29 g/L). The Total Albumin level for the control (C) group was 36.66 g/L. The group exposed to LC was the most reduced with 17.19 followed by the DM treated group (24.89 g/L), then, the least was the MF treated group (30.48 g/L). For the Total bilirubin levels, the control (C) group was measured as 1.18 g/L. The most elevated was the LC treated group (1.71) followed by the MF treated group (1.62 g/L) with the least being the DM treated group (1.52g/L)

A) Oxidative Effects of the Pesticides on the Liver

There exist variations in results of the oxidative stress parameters on the liver of the male albino rats exposed to pesticides within the 28 day experimental period as depicted in Table 4.1. There was a general decrease in SOD activity of the pesticide group against the control (8.12E-05 IU/L). LC group was the most decreased (3.95E-05 IU/L) followed by DM treated group with 4.36E-05 IU/L. The control (C) group for Catalase (CAT) activity was recorded as 8.82E-06U/L with most decreased being the LC treated group (5.31E-06 U/L) followed by the DM treated group (7.14E-06 U/L) while the least was the MF treated group (7.77E-06 U/L). The GSH level for the control group was 2.62mg/dL. The pesticide treated groups had general decrease across

the treatments compared to the control with the most reduction recorded in LC treated group (0.62mg/dL). GPx activity in the pesticide treated groups reduced as against the control (6.59) with the most reduction observed in LC treated group (3.32) followed by the DM treated group (4.03) and the MF treated group (4.50). The MDA level of the control was 0.28 which elevated in the entire pesticide treated groups with highest elevation recorded as 0.50 for DM followed by the MF treated group (0.42) and LC treated group (0.30) was the least. The liver protein level had a general decrease in the pesticides treated groups compared to the control (83.8 g/L). Most decreased was the LC treated group (59.6 g/L) followed by the DM treated group (64.5 g/L) and the MF treated group (65.3g/L). All measured parameters were significant at $p \leq 0.05$ except GST.

B) Oxidative Effects of the Pesticides on the Testes

The results of the oxidative stress parameters on the testes of the male albino rats exposed to sublethal dose of the pesticides and their mixture shows that there was no significant difference in CAT, GSH and GPx but SOD, GST, MDA and Protein had significant different at $p \leq 0.05$ as shown in Table 4.7. There was general decrease in SOD activity in the pesticide treated groups against the control (8.12E-05 IU/L) with the most reduction observed in LC treated group (3.92E-05IU/L) followed by the MF treated group (4.36E-05IU/L) and the least was the DM treated group (5.33E-05IU/L). The GST activity was observed to have variations across the treatment groups against the control group (2.62E-06). The LC treated group was the least elevated (1.01E-05) while the MF treated group was (2.31E-05) with the highest elevation in DM treated group (3.55E-05). MDA level for the control group was recorded as 0.12 with significant increase in the pesticides treated groups. The LC treated group was most increased (0.51) followed by the MF treated group (0.17) with the least elevation in the DM treated group (0.15). The level of the testes protein decreased across the pesticides treated groups compared to the

control (92.0g/L). The most reduction was recorded in the LC treated group (44.9g/L) followed by the MF treated group (89.7g/L) and the DM treated group (87.6g/L)

C) Oxidative Effects of the Pesticides on the Serum

The results of the oxidative stress status on the serum of the male albino rats exposed to sublethal dose of the pesticides showed that all the parameters measured had significant difference except GPx and MDA which were not significant at $p \leq 0.05$ as presented in table 4.8.There was general decrease in the SOD activity across the pesticides treated groups against the control group (3.86E-05). The most reduced was the LC treated group (2.06E-05) followed by the MF treated group (2.16E-05) and the least was the DM treated group (2.31E-05).The CAT activity in the control group was 2.50E-05, while the pesticide exposed groups recorded general decrease. The most decreased was the LC treated group (3.05E-06) followed by the MF treated group (3.44E-06), then, the DM treated group (7.08E-06). GSH level decreased across the pesticide treated groups against the control (0.58). LC treated group was most reduced (0.44) followed by the MF treated group (0.48) and the DM treated group was the least (0.50). GST activity in control was recorded as 1.2E-06 while the most decrease was observed in the LC treated group ((7.59E-07) followed by the MF treated group (1.13E-06). There was an increase in GST activity in the DM treated group (1.62E-06). Serum protein level of the control was recorded as 74.2 while the pesticide exposed groups had significant reduction. LC treated group was the most reduced (58.1g/L) followed by the MF treated group (62.9g/L) and the least reduced was the DM treated group (64.3g/L)

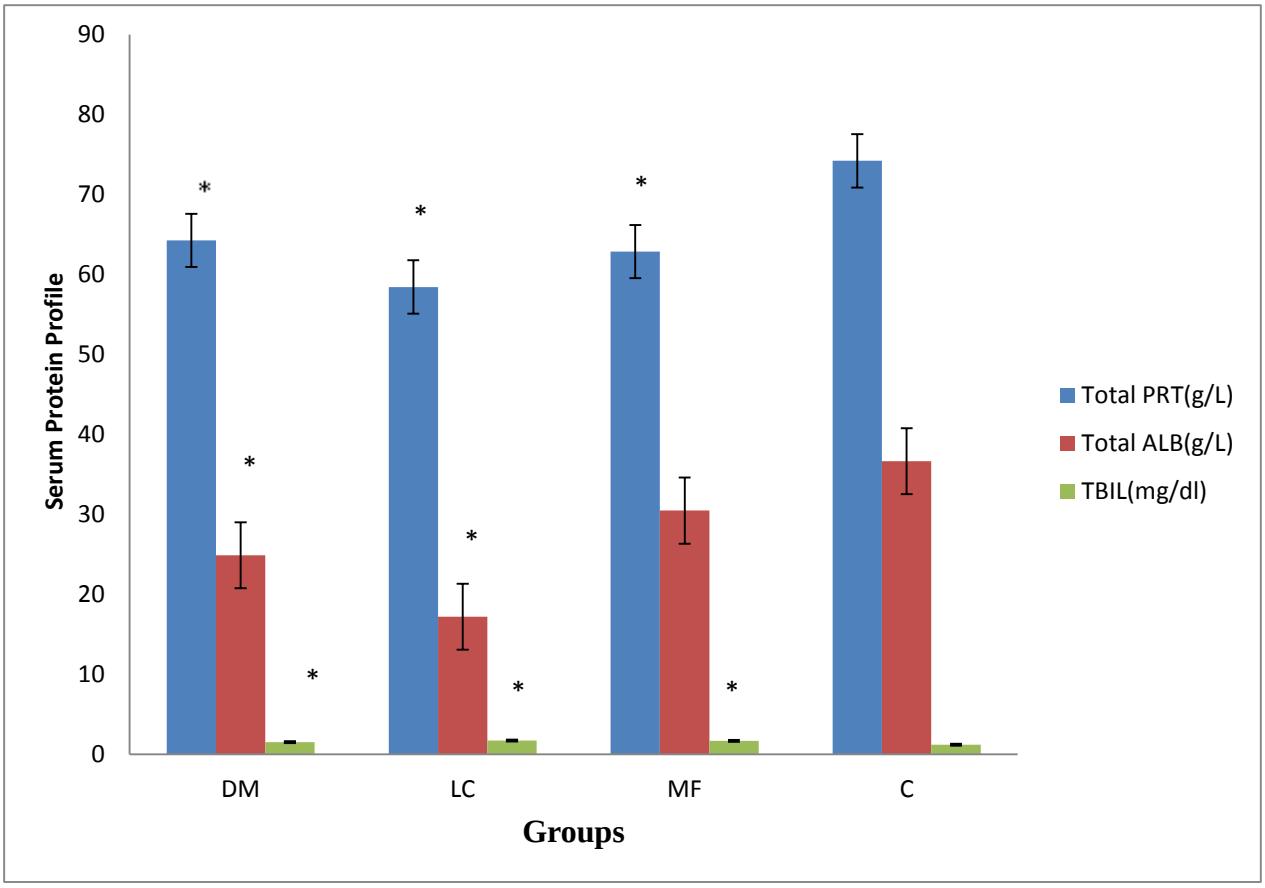


Fig.4.5. THE EFFECTS OF THE PESTICIDES AND THEIR MIXTURE ON SERUM PROTEIN PROFILE

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control (C)

Table 4.1. **Effects on the Oxidative Stress Parameters on the Liver**

PARAMETER	DM	LC	MF	C
SOD(IU/L)	4.16E-05±1.9E-06*	3.92E-05±1.2E-07	4.36E-05±4.4E-06*	8.12E-06±3.3E-08
CAT(U/L)	7.14E-06±6.8E-07	5.31E-06±2.7E-07*	7.77E-06±2.1E-07	8.82E-06±1.1E-06
GSH(mg/dL)	0.92±0.3*	0.67±0.1*	1.58±0.1*	2.62±0.7
GST(µmol/g.tissue)	5.42E-06±1.6E-07	4.27E-06±2.5E-07	3.96E-06±0.0	4.14E-06±6.3E-07
GPx(mg/g.tissue)	4.03±0.2*	3.32±0.4*	4.50±0.5*	6.59±0.6
MDA(nmol/g.tissue)	0.50±0.0*	0.30±0.0	0.42±0.0*	0.28±0.0
Protein(g/L)	64.5±5.4*	59.6±3.1*	65.3±7.6*	83.8±0.5

All values were expressed as mean ±SEM. LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$. Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, GST = Glutathione-S-transferase, GPx = Glutathione peroxidase, MDA = Malondialdehyde.

Table 4.2. Effects on the Oxidative Stress Parameters on the Testes

PARAMETER	DM	LC	MF	C
SOD(IU/L)	5.33E-05±2.3E-06	3.92E-05±2.6E-06*	4.36E-05±1.1E-06	8.12E-05±2.2E-06
CAT(U/L)	6.48E-06±1.2E-07	4.92E-06±1.1E-06	6.38E-06±1.3E-07	6.64E-06±1.1E-07
GSH(mg/dL)	2.32±01	2.92±01	2.54±03	3.52±08
GST(µmol/g.tissue)	3.55E-06±6.E-07	1.01E-05±5.9E-07*	2.31E-05±4.3E-08	2.62E-06±3.5E-07
GPx(mg/g.tissue)	5.05±0.5	4.79±0.9	5.4±0.7	6.59±0.6
MDA(nmol/g.tissue)	0.15±0.0	0.51±0.1*	0.17±0.0	0.12±0.0
Protein(g/L)	87.6±3.0	44.9±1.4*	82.7±0.9*	92.0±0.8

All values were expressed as mean ±SEM.

LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$

Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, GST = Glutathione-S-transferase, GPx = Glutathione peroxidase, MDA = Malondialdehyde.

Table 4.3. Effects on the Oxidative Stress Parameters on the Serum

PARAMETER	DM	LC	MF	C
SOD(IU/L)	2.31E-05±7.2E-07*	2.06E-05±3.5E-07*	2.16E-05±8.8E-08*	3.86E-05±7.8E-07
CAT(U/L)	7.08E-06±0.0*	3.05E-06±6.7E-07*	3.44E-06±2.8E-07*	2.50E-05±4.8E-06
GSH(mg/dL)	0.5±0.0*	0.44±0.0*	0.48±0.0*	0.58±0.0
GST(µmol/g.tissue)	1.62E-06±2.3E-07	7.59E-07±5.3E-08*	1.13E-06±8.8E-09	1.26E-06±1.9E-07
GPx(mg/g.tissue)	3.76±0.5	3.35±0.3	3.70±0.4	4.47±0.7
MDA(nmol/g.tissue)	0.24±0.0	0.20±0.0	0.19±0.0	0.17±0.0
Protein(g/L)	64.3±1.9*	58.1±0.9*	62.9±3.9*	74.2±3.8

All values were expressed as mean ±SEM. LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$. Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, GST = Glutathione-S-transferase, GPx = Glutathione peroxidase, MDA = Malondialdehyde.

4.1.7. Haematological Analyses.

The results on the haematological status of the male albino rats exposed to oral administration of sublethal dose of pesticides showed that all the parameters measured had significant difference except PLT which was not significant at $p \leq 0.05$ as depicted in table 4.9. There was general decrease in WBC of the pesticide treated groups compared to the control (10.02). The most reduced was the LCT treated group (6.70) followed by the MF treated group (7.30) and the least was the DM treated group (7.60). The control group for RBC was recorded as 7.43. There was general reduction in the pesticide treated groups with highest in the LCT treated group (5.23) followed by the MF treated group (5.53) and the DM treated group (6.38). HGB was 13.30 in the control group while it reduced in the pesticide treated groups with the LCT treated group (10.90) being the most reduced, followed by the MF treated group (11.90) and the DM treated group (12.40). MCHC was 36.70 in the control group with little reduction in the pesticide exposed groups with LCT treated being the most reduced (35.90) and the least was the DM treated group (36.20).

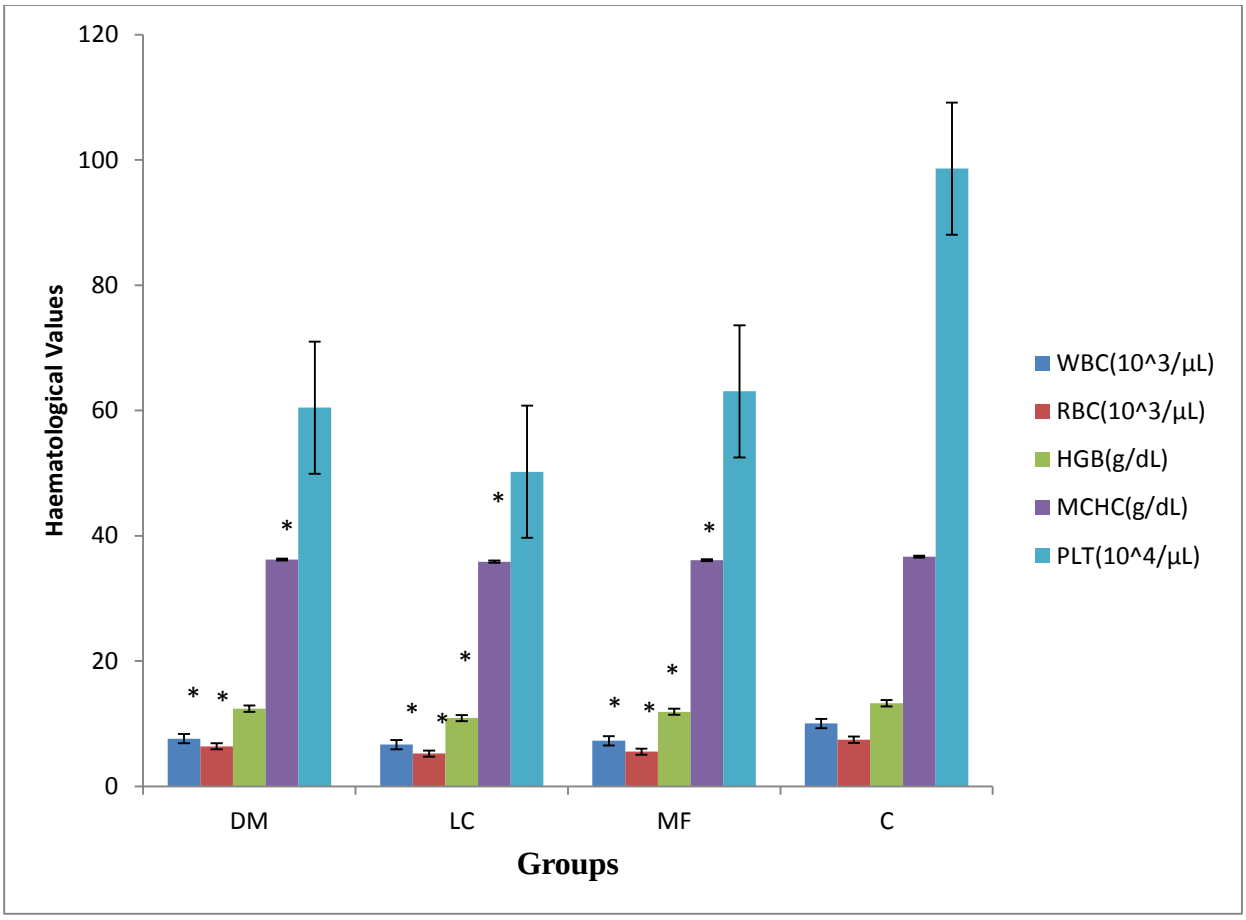


Fig.4.6. Effects of the Pesticides on the Haematological Status

All values were expressed as mean±SEM.

LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control (C)

WBC = White blood cells, RBC = Red blood cells, HGB = Haemoglobin, MCHC =Mean corpuscular haemoglobin concentration, PLT = Platelets

4.1.8. **THE EFFECTS OF THE PESTICIDES AND THEIR MIXTURE ON THE REPRODUCTIVE STATUS ON THE RATS.**

The results on the reproductive status of the male albino rats exposed to sublethal dose of the pesticides showed that all the parameters measured had no significant difference except the testosterone which was significant at $p \leq 0.05$ as depicted in table 4.4. There was general decrease in the testosterone levels of the pesticide treated groups against the control group (0.55pg/mL). The LC treated was most reduced (0.35pg/mL) followed by the DM treated group (0.43pg/mL) and the least was the MF treated group (0.48pg/mL).

Table 4.4. Effects the Pesticide Mixtures on the Reproductive Status of the Rats.

PARAMETER	DM	LC	MF	C
Testosterone (pg/mL)	0.43	0.35*	0.48	0.55
Sperm Count (x10^6)	39.77	32.93	35.53	51.21
Sperm Motility (%)	44.03	41.05	42.79	45.32
Sperm Viability (%)	40.75	38.41	38.20	45.67

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (C) at $p \leq 0.05$

Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control (C)

4.2 DISCUSSION

The mechanism through which pesticides cause damage can range according to the structure of the pesticides. The current trend towards increased marketing and usage of organophosphate-pyrethroid mixtures is likely to result in creating new patterns of mixed toxicity. Accordingly, this research was employed to evaluate the toxicological impact of the oral administrations of pesticides singly and in combination on liver, serum and testes of adult male albino rats.

General Health of the Rats

The present study demonstrated that oral treatment of rats with 1/20 LD_{50} (5%) of pesticides dimethoate (10.7 mg/kg body weight), Lambda-cyhalotrin (3.9 mg/kg body weight) and magic force (1.2 mg/kg body weight) caused an overall mortality rate of 20%, 30% and 10% respectively throughout the 28 day experimental period. Such mortality was mostly attributed to diarrhea which may be related to neurotoxic crisis. The outcome of the dimethoate –treated group is in agreement with finding of Saafi *et al*. (2011) and Noor *et al*. (2012) who observed diarrhea in mature male rats in response to chronic exposure of dimenthoate. The result of LC-treated rat is line with the observations of Farag *et al*. (2010) who observed tremors and diarrhea in mature male rats in response to chronic exposure of LC. Concerning the remarkable less toxicity showed by MagicForce (DM + LC), it is in line with the findings of Latuszynska *et al*. (2001), who observed that dimethoate had antagonistic effect in commercial mixtures with pyrethroids. The control showed no sign of toxicity. These coincide with the level of protein decrease observed throughout the groups.

Average body weight of the Rats.

As indicated in the current data, the body weight of all the pesticides treated groups showed significant decrease from the 14th day of the experimental period which shows that animals were exposed to toxic substances, this is in agreement with the findings of El-Damaty *et al.* (2012) and Teo *et al,*. (2002).The control group on the contrary showed continuous weight gain. The reduction in body weight in response to the pesticides in take may be as a result of the combined action of neurotoxic and oxidative stress and/or due to increase degredation of lipids and proteins as a direct effect of the toxic compounds (Heikal *et al*., 2012; Salama *et al*., 2013). The DM and LC groups showed more decrease in body weight, than the MF which is the commercial mixture indicating antagonistic interaction.

Average organ weight of the Rats.

The data obtained in the organ weight shows that there was significant increase in the liver weight of all the pesticide treated groups compared to the control. The LC treated group had the highest weight followed by the DM group and the MF. This agrees with the observations of Waggas, 2013, saying that the liver is an important vital organ in the animal body as it is the site of detoxification and elimination of toxic materials. A foreign body in form of a chemical stress is sufficient enough to cause severe hepatic dysfunction.

Consequently, there was no significant difference the testes weight of the pesticide exposed rats compared to the control. This might be due to the dosing of the pesticides (1/20 LD_{50}) since the dose makes the poison.

Toxic effects of the pesticides and their mixture on liver, testes and serum.

Results presented in this study revealed that there was no significant increase in the lactate dehydrogenase activity in the serum of the pesticide treated groups against the control group. This suggests that there was no significant toxic effect in the serum.

The liver and the testes showed significant increase in lactate dehydrogenase activity in the pesticide-treated groups compared to the control group. The high lactate dehydrogenase activity shows that the plasma membrane of the affected cells must have been damaged or ruptured causing the cytosolic enzyme to leak out due to the presence of the toxicants (Cain, 2000).

The toxic effects of the pesticides on the liver and testes showed that they are metabolized in the liver by an enzymatic system (Jaeschel *et al*., 2002). The increased level of its cellular metabolites could induce oxidative and inflammatory tissue damages (Cain, 2000).The LC treated-group had the highest LDH activity followed by the MF–treated group, then the DM group. This is in agreement that dimethoate is easily metabolized and excreted 95% in the urine after oral or dermal administration (Health Canada Pest Management Regulatory Agency, 2011) while synthetic-pyrethroid are known to cause serve liver damage (Jaesche *et al*., 2002). Their mixture in the Magicforce at 1/20 LD_{50} dose seems antagonistic.

The toxic effect on the testes were exhibited by the LC and the MF treated groups compared to control group which agrees with reports that man-made pyrethroids adversely affect the testicular functions in experimental animals (Lifeng *et al*., 2006) as well as they are potent endocrine disrupters (Solati *et al.,* 2011).

The effect of pesticides and their mixture on liver function enzymes.

In the present study, the pesticide treated rats showed significant elevation of marker enzymes namely ALT, AST and ALP compared to the group. The LC-treated group had higher level of the marker enzymes followed by the DM-treated group, the MF group which is their mixture. Aminotransferases (ALT and AST) are sensitive indicators of liver cell damage for both acute and chronic hepatocellular injury (Barth *et al*., 2009). Therefore levels of these enzymes reflect the state of hepatic function (Konarn *et al*., 2007). The elevated levels of ALT indicate a possible hepatotoxicity that would have resulted in the leakage of the enzyme into the serum. Similarly, Shakoori *et al*., (1994) reported that the increase in the activity of AST is mainly due to the leakage of this enzyme from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function. On the other hand, Al-Haj *et al*., (2005) observed that ALP is often employed to assess the integrity of the plasma membrane of the liver. The significant increase in serum activity in ALP due to treatment with 1/20 LD_{50} of the pesticide may be as a result of disruption of the plasma membrane.

Furthermore, the increased level of the total bilirubin in the treated rats may be an indicator of hyper-bilirubinemia, a useful index for the severity of hepato cellular dysfunction (Pimple *et al*., 2007; Fakurazi *et al*., 2008). The higher bilirubinemia may be due to excessive haem destruction and blockage of billary tract. This however, may have led to a mass inhibition of conjugation reaction and release of unconjugation bilirubin from damaged and dead hepatocytes (Kingsley & Iniobong, 2014). It is well known that one of the major functions of the liver is to synthesis serum protein and albumin. However, the significant decrease in the level of serum albumin and protein observed in the pesticide-treated rats may have resulted to hypoproteinemia, indicating liver damage which may have caused a significant fall in protein synthesis (Anitha *et al*., 2012).

Since the liver is the site of protein synthesis, the oxidative damage of some amino acids may be attributed to the decrease in protein synthesis (Dawn, 1994).

The effect of the pesticides and their mixture on the oxidative stress parameters on male albino rats.

Liver

The current study revealed that the exposure of the rats to the pesticides resulted in decrease in antioxidant defense mechanisms due to a state of oxidative stress in the liver. The activity of SOD, CAT, GP_x and the levels of GSH and MDA were decreased across the treatment groups compared to the control. The LC treated group had more decreased activity of SOD, CAT, GP_x and levels of GSH and protein followed by the DM treated group, then the MF. MDA was most elevated in DM-treated group followed by the MF, then the LC treated group compared with the control. On the other hand, GST activity in the liver was insignificant compared with control. Pesticides have been implicated to cause oxidative stress and change in antioxidant status system (Solati *et al*., 2013). Similarly effects of organophosphate pesticides were previously reported by Yousef (2010) in liver. They may induce oxidative stress through their "redox-cycling" activity, where they generate superoxide anions and hydrogen peroxide, or through ROS generation via change in normal antioxidant homeostasis that results in depletion of antioxidants (Altuntas *et al*., 2011). Most pesticides have been shown to induce inflammation and cell infiltration (Elhaway & Zaki, 2009). Decrease in GSH concentrations may be through low production or non-enzymatic oxidation of GSH to glutathione disulfide (GSSH) due to oxidative stress in the pesticide-treated rats' liver (Banerjee *et al*., 1999). Oxidative stress can be monitored by observing the elevation of lipid peroxidation products (Goel *et al*., 2005). The elevated MDA

level in the intoxicated rat livers was in agreement with the above statement. The oxidative stressed state of the liver had led to the fall in protein synthesis.

Testes

The results from the oxidative state of the testes of male albino rats exposed to 1/20 LD_{50} of the pesticides and their mixtures showed that the activity or levels of CAT, GSH, GP_x were not statistically significant compared to the control while that of SOD, GST, MDA and protein were significant compared to the control at ($p \leq 0.05$). The LC-treated group had more decreased activity of SOD, GST and protein followed by the MF-treated group, and DM-treated group. There was an increase in MDA concentration in the LC-treated group followed by the MF, then, the DM. An increase in MDA, the most likely used biomarker of lipid peroxidation, indicates cellular damage, inhibition of several enzymes and cellular functions (Thirunarukkarasu *et al*., 2001). This result is in agreement with the findings of Cain *et al*. (2010). He reported that decrease in SOD and GST is indicating some levels of increased production of free radical species (ROS) that led to lipid peroxidation in liver and testes. Since the testes is not the site for metabolism and detoxification, the oxidative stress caused by 1/20 LD_{50} of the pesticides is expected to be low unlike the liver.

Serum

Data obtained from this study revealed that the serum of the pesticide treated rats experience some level of oxidative stress since the analysis on activity or levels of SOD, CAT, GSH, GST and serum were all significant compared with the control. Nevertheless, those of MDA and GPx were not significant. LC treated group had highest decreased activity of SOD, CAT, as well as reduced levels of GSH and protein followed by the MF-treated group and then, the DM-treated

group. The high significant decrease in the activity of SOD, CAT, GSH, GST and serum protein in lamba-cyhalothrin intoxicated rats is in consonance with the report of Manna *et al*. (2015) and Tuzmen *et al*. (2008). They observed a significant decrease in the parameters when rats were exposed to sublethal doses of deltamethrim which is a a pyrethroid for 30 days. However, the significant decrease in activities or level of SOD, CAT and GSH recorded in the present results contradicts the results of Altuntas *et al*. (2004) and Buyukokuroglu *et al*. (2008). They recorded significant elevation in SOD, CAT activities as well as GSH level due to exposure to dimethoate, an organophosphate. Nevertheless, GST activity elevation was observed by Timur *et al*. (2003) and Khan (2006) in tissues of animals fed organophosphate psticides. However combined lamba-cyhalothrin and dimenthoate induced the previous trend of toxicity exhibited by lamba-cyhalothrin alone, thus describing an antagonistic effect of dimethoate against lambda-cyhalothrim effect in their commercial product (Magicforce).

The effect of the pesticides and their mixture on the haematological parameters of the male albino rats.

Following exposure, toxicants are transported by the blood to various organs including the liver and kidney where they eventually cause harmful effects. Blood can act as a pathological and physiological indicator of animal health (Jorum *et al*., 2016). The results of this study showed that WBC, RBC, HGB and MCHC were significantly decreased in all the pesticide treated rats compared to the control. The LC-treated group had the highest reduction followed by the MF-treated group and then, DM-treated group. The effect of the pesticides on the WBC lowered the defense mechanism of the rats there by compromising the immune system due to oxidative stress (Yousef *et al*., 2003). The decrease in MCHC and HGB along with the decrease in the RBC might be due to the effect of pesticides on blood forming organ (bone marrow and liver) and

inhibition of many steps of heme biosynthesis in rats as a result of pesticide exposure (Heikal, *et al*., 2012). The poisoning by pesticide residue leads to the development of anemia due to interference of Hb Biosynthesis (Tyotsana *et al*., 2003). These effects could be due to the ability of the pesticides to form free radicals (Hazarika *et al*. 2003; Vidyasagar *et al.*, 2004).

The effects of the pesticides and their mixture on the reproductive status on the male albino rats.

Data obtained from the present study revealed that sperm count, sperm motility and sperm viability of the pesticide treated rats were not significant compared to their control. However, Testosterone analysis was significant with much decrease observed in the LC-treated group. The above result is in line with the report of Joshi *et al*. (2011) who stated that serum testosterone levels decreased in male mice following sub lethal exposure to pyrethroid pesticide. The high decrease in testosterone might be due to high production of reactive oxygen species (ROS) by the pyrethroid exposure. Similar results were reported by Demerdash *et al*., (2004). The toxic effect was antagonized by dimethoate in their commercial product. The overall integrity of the reproductive status of the exposed rats was not general compromised due to the dose (1/20 LD_{50}) the rats were exposed.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

According to the results obtained from the present study, it can be concluded that chronic exposure to sublethal dose (1/20 LD_{50}) of dimethoate and lambda-cyhalothrin singly and in combination (magicforce) cause significant neurotoxic, cytototic and hepatic effects in rats thereby affecting the liver, gonads and blood adversely. The antioxidant defense system was significantly lowered due to high oxidative stress of the toxicants. However, the singly effects of the pesticides were higher when compared with their combined effects. This suggests that there is an antagonistic interaction in the novel commercial product (Magicforce).

5.2 RECOMMENDATIONS

The data obtained from this study revealed that the LD_{50} of the Magicforce is 23 mg/kg body weight of the rat which is very high. More research should be done on this new commercial product to reduce its toxicity on non-target species while still maintaining good efficiency on the target species.

The predomination of antagonistic effect observed in the present study are in harmony with some findings in literature, however, more work needs to be done to ascertain the interaction mechanisms of the pesticides in their combination.

Finally, the indiscriminate application of pesticides should be regulated since they cause health problems.

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APPENDICES

Appendix 1

BODY WEIGHT

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: BODYWEIGHT

GROUP	WEEK	Mean	Std. Deviation	N
1	1	104.6800	5.37562	10
	2	106.0200	7.00060	10
	3	92.7000	33.28523	10
	4	91.4500	33.55616	10
	5	87.4200	47.18681	10
	Total	96.4540	29.84485	50
2	1	111.2300	17.38985	10
	2	103.2600	39.46079	10
	3	98.7900	37.97243	10
	4	89.2900	49.65477	10
	5	90.7600	65.94158	10
	Total	98.6660	43.87647	50
3	1	115.2800	12.73044	10
	2	128.9200	19.11339	10
	3	117.2200	13.50504	10
	4	112.6900	12.27341	10
	5	110.9200	39.97604	10
	Total	117.0060	22.19247	50
4	1	130.1800	24.88649	10
	2	127.0700	20.73248	10
	3	121.4000	20.63950	10
	4	128.6400	22.85793	10
	5	140.5600	25.34812	10
	Total	129.5700	22.90697	50
Total	1	115.3425	18.62031	40
	2	116.3175	26.37094	40
	3	107.5275	29.63434	40

	4	105.5175	35.39369	40
	5	107.4150	49.92819	40
	Total	110.4240	33.62420	200

Tests of Between-Subjects Effects

Dependent Variable: BODYWEIGHT

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	47214.787[a]	19	2484.989	2.516	.001
Intercept	2438691.955	1	2438691.955	2469.256	.000
GROUP	37165.175	3	12388.392	12.544	.000
WEEK	4017.701	4	1004.425	1.017	.400
GROUP * WEEK	6031.911	12	502.659	.509	.907
Error	177771.998	180	987.622		
Total	2663678.740	200			
Corrected Total	224986.785	199			

a. R Squared = .210 (Adjusted R Squared = .126)

Estimated Marginal Means

1. GROUP * WEEK

Dependent Variable: BODYWEIGHT

GROUP	WEEK	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	104.680	9.938	85.070	124.290
	2	106.020	9.938	86.410	125.630
	3	92.700	9.938	73.090	112.310
	4	91.450	9.938	71.840	111.060
	5	87.420	9.938	67.810	107.030
2	1	111.230	9.938	91.620	130.840
	2	103.260	9.938	83.650	122.870
	3	98.790	9.938	79.180	118.400
	4	89.290	9.938	69.680	108.900
	5	90.760	9.938	71.150	110.370
3	1	115.280	9.938	95.670	134.890
	2	128.920	9.938	109.310	148.530
	3	117.220	9.938	97.610	136.830

	4	112.690	9.938	93.080	132.300
	5	110.920	9.938	91.310	130.530
4	1	130.180	9.938	110.570	149.790
	2	127.070	9.938	107.460	146.680
	3	121.400	9.938	101.790	141.010
	4	128.640	9.938	109.030	148.250
	5	140.560	9.938	120.950	160.170

2. WEEK

Dependent Variable: BODYWEIGHT

			95% Confidence Interval	
WEEK	Mean	Std. Error	Lower Bound	Upper Bound
1	115.342	4.969	105.538	125.147
2	116.317	4.969	106.513	126.122
3	107.527	4.969	97.723	117.332
4	105.518	4.969	95.713	115.322
5	107.415	4.969	97.610	117.220

3. GROUP

Dependent Variable: BODYWEIGHT

			95% Confidence Interval	
GROUP	Mean	Std. Error	Lower Bound	Upper Bound
1	96.454	4.444	87.684	105.224
2	98.666	4.444	89.896	107.436
3	117.006	4.444	108.236	125.776
4	129.570	4.444	120.800	138.340

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: BODYWEIGHT

	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1	2	-2.2120	6.28529	.985	-18.5110	14.0870
		3	-20.5520*	6.28529	.007	-36.8510	-4.2530
		4	-33.1160*	6.28529	.000	-49.4150	-16.8170
	2	1	2.2120	6.28529	.985	-14.0870	18.5110
		3	-18.3400*	6.28529	.021	-34.6390	-2.0410
		4	-30.9040*	6.28529	.000	-47.2030	-14.6050
	3	1	20.5520*	6.28529	.007	4.2530	36.8510
		2	18.3400*	6.28529	.021	2.0410	34.6390
		4	-12.5640	6.28529	.192	-28.8630	3.7350
	4	1	33.1160*	6.28529	.000	16.8170	49.4150
		2	30.9040*	6.28529	.000	14.6050	47.2030
		3	12.5640	6.28529	.192	-3.7350	28.8630
Dunnett t (2-sided)[b]	1	4	-33.1160*	6.28529	.000	-48.0058	-18.2262
	2	4	-30.9040*	6.28529	.000	-45.7938	-16.0142
	3	4	-12.5640	6.28529	.118	-27.4538	2.3258

Based on observed means.

The error term is Mean Square(Error) = 987.622.

*. The mean difference is significant at the .05 level.

b. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

BODYWEIGHT

	GROUP	N	Subset 1	Subset 2
Tukey HSD[a,b]	1	50	96.4540	
	2	50	98.6660	
	3	50		117.0060
	4	50		129.5700
	Sig.		.985	.192

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 987.622.

a. Uses Harmonic Mean Sample Size = 50.000.

b. Alpha = .05.

WEEK

Multiple Comparisons

Dependent Variable: BODYWEIGHT

	(I) WEEK	(J) WEEK	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1	2	-.9750	7.02717	1.000	-20.3393	18.3893
		3	7.8150	7.02717	.800	-11.5493	27.1793
		4	9.8250	7.02717	.630	-9.5393	29.1893
		5	7.9275	7.02717	.791	-11.4368	27.2918
	2	1	.9750	7.02717	1.000	-18.3893	20.3393
		3	8.7900	7.02717	.721	-10.5743	28.1543
		4	10.8000	7.02717	.540	-8.5643	30.1643
		5	8.9025	7.02717	.712	-10.4618	28.2668
	3	1	-7.8150	7.02717	.800	-27.1793	11.5493
		2	-8.7900	7.02717	.721	-28.1543	10.5743
		4	2.0100	7.02717	.999	-17.3543	21.3743
		5	.1125	7.02717	1.000	-19.2518	19.4768
	4	1	-9.8250	7.02717	.630	-29.1893	9.5393
		2	-10.8000	7.02717	.540	-30.1643	8.5643
		3	-2.0100	7.02717	.999	-21.3743	17.3543
		5	-1.8975	7.02717	.999	-21.2618	17.4668
	5	1	-7.9275	7.02717	.791	-27.2918	11.4368
		2	-8.9025	7.02717	.712	-28.2668	10.4618
		3	-.1125	7.02717	1.000	-19.4768	19.2518
		4	1.8975	7.02717	.999	-17.4668	21.2618
Dunnett t (2-sided)[a]	1	5	7.9275	7.02717	.620	-9.3850	25.2400
	2	5	8.9025	7.02717	.522	-8.4100	26.2150
	3	5	.1125	7.02717	1.000	-17.2000	17.4250
	4	5	-1.8975	7.02717	.996	-19.2100	15.4150

Based on observed means.

The error term is Mean Square(Error) = 987.622.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

BODYWEIGHT

			Subset
	WEEK	N	1
Tukey HSD[a,b]	4	40	105.5175
	5	40	107.4150
	3	40	107.5275
	1	40	115.3425
	2	40	116.3175
	Sig.		.540

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 987.622.

a. Uses Harmonic Mean Sample Size = 40.000.

b. Alpha = .05.

Appendix 2

ORGAN WEIGHT

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: ORGANWEIGHT

GROUP	ORGANPART	Mean	Std. Deviation	N
1	ORGAN FOR TESTE	1.8307	.79833	3
	ORGAN FOR LIVER	4.6793	.46559	3
	Total	3.2550	1.66617	6
2	ORGAN FOR TESTE	1.8990	.57430	3
	ORGAN FOR LIVER	5.5080	.51524	3
	Total	3.7035	2.03607	6
3	ORGAN FOR TESTE	2.1893	.22519	3
	ORGAN FOR LIVER	4.6633	.37797	3
	Total	3.4263	1.38334	6
4	ORGAN FOR TESTE	2.6247	.68907	3
	ORGAN FOR LIVER	4.2553	.18015	3
	Total	3.4400	1.00032	6
Total	ORGAN FOR TESTE	2.1359	.61485	12
	ORGAN FOR LIVER	4.7765	.58791	12
	Total	3.4562	1.47142	24

Tests of Between-Subjects Effects

Dependent Variable: ORGANWEIGHT

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	45.496[a]	7	6.499	24.181	.000
Intercept	286.689	1	286.689	1066.631	.000
GROUP	.617	3	.206	.765	.530
ORGANPART	41.836	1	41.836	155.652	.000
GROUP * ORGANPART	3.043	3	1.014	3.774	.032
Error	4.300	16	.269		
Total	336.486	24			
Corrected Total	49.797	23			

a. R Squared = .914 (Adjusted R Squared = .876)

Estimated Marginal Means

GROUP

Dependent Variable: ORGANWEIGHT

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	3.255	.212	2.806	3.704
2	3.704	.212	3.255	4.152
3	3.426	.212	2.978	3.875
4	3.440	.212	2.991	3.889

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: ORGANWEIGHT

Dunnett t (<control)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Upper Bound
1	4	-.1850	.29932	.493	.4816
2	4	.2635	.29932	.950	.9301
3	4	-.0137	.29932	.733	.6530

Based on observed means.

The error term is Mean Square(Error) = .269.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 4

LDH

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	LDH_ACTIVITY	Mean	Std. Deviation	N
GROUP 1	LDH SERUM	311.570000000	66.8600000000	3
	LDH LIVER	65.680000000	3.1400000000	3
	LDH TESTES	285.580000000	30.4900000000	3
	Total	220.943333333	122.6340597061	9
GROUP 2	LDH SERUM	334.536666667	3.1650013165	3
	LDH LIVER	73.820000000	1.2750294114	3
	LDH TESTES	352.346666667	22.3550001864	3
	Total	253.567777778	135.5038091511	9
GROUP 4	LDH SERUM	315.280000000	13.5400000000	3
	LDH LIVER	66.170000000	1.0800000000	3
	LDH TESTES	333.526666667	20.7850002005	3
	Total	238.325555556	129.9525627980	9
GROUP 5	LDH SERUM	300.686666667	15.2957652091	3
	LDH LIVER	55.560000000	.6500000000	3
	LDH TESTES	261.566666667	51.9550000802	3
	Total	205.937777778	117.2196729199	9
Total	LDH SERUM	315.518333333	32.4557071136	12
	LDH LIVER	65.307500000	6.9493336829	12
	LDH TESTES	308.255000000	47.6268771522	12
	Total	229.693611111	122.3340463100	36

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	504853.926[a]	11	45895.811	58.149	.000
Intercept	1899329.579	1	1899329.579	2406.406	.000
GROUP	11568.539	3	3856.180	4.886	.009
LDH_ACTIVITY	486726.820	2	243363.410	308.336	.000
GROUP * LDH_ACTIVITY	6558.567	6	1093.095	1.385	.261
Error	18942.735	24	789.281		
Total	2423126.240	36			
Corrected Total	523796.661	35			

a. R Squared = .964 (Adjusted R Squared = .947)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
229.694	4.682	220.030	239.358

2. GROUP

Dependent Variable: VALUE

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
GROUP 1	220.943	9.365	201.616	240.271
GROUP 2	253.568	9.365	234.240	272.896
GROUP 4	238.326	9.365	218.998	257.653
GROUP 5	205.938	9.365	186.610	225.266

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP 1	GROUP 5	15.005555556	13.2437039316	.542	-18.192049980	48.203161091
GROUP 2	GROUP 5	47.630000000*	13.2437039316	.004	14.432394464	80.827605536
GROUP 4	GROUP 5	32.387777778	13.2437039316	.057	-.809827758	65.585383313

Based on observed means.

The error term is Mean Square(Error) = 789.281.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

LDH_ACTIVITY

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) LDH_ACTIVITY	(J) LDH_ACTIVITY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
LDH SERUM	LDH TESTES	7.263333333	11.469384044 9	.754	-19.682927835	34.209594502
LDH LIVER	LDH TESTES	-242.947500000*	11.469384044 9	.000	-269.893761168	-216.001238832

Based on observed means.

The error term is Mean Square(Error) = 789.281.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 5

LIVER FUNCTION ENZYMES

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	ENZYMES_FUNCTION	Mean	Std. Deviation	N
GROUP 1	AST(u/L)	120.623333333	13.6879375120	3
	ALT(u/L)	41.756666667	2.6914927704	3
	ALP(u/L)	26.210000000	3.2300000000	3
	Total	62.863333333	44.4207170136	9
GROUP 2	AST(u/L)	138.136666667	10.2779975352	3
	ALT(u/L)	44.736666667	2.1364066404	3
	ALP(u/L)	29.920000000	3.2400000000	3
	Total	70.931111111	51.1069206283	9
GROUP 4	AST(u/L)	119.863333333	4.1381316235	3
	ALT(u/L)	42.720000000	1.0222035022	3
	ALP(u/L)	25.300000000	4.1400000000	3
	Total	62.627777778	43.6855118368	9
GROUP 5	AST(u/L)	107.136666667	2.5935368386	3
	ALT(u/L)	38.766666667	1.7715623989	3
	ALP(u/L)	14.540000000	3.5201704504	3
	Total	53.481111111	41.6533970537	9
Total	AST(u/L)	121.440000000	13.7947466944	12
	ALT(u/L)	41.995000000	2.8211425796	12
	ALP(u/L)	23.992500000	6.7031812325	12
	Total	62.475833333	43.8182952250	36

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	66434.903[a]	11	6039.537	189.080	.000
Intercept	140516.271	1	140516.271	4399.143	.000
GROUP	1373.130	3	457.710	14.330	.000
ENZYMES_FUNCTION	64526.453	2	32263.227	1010.065	.000

GROUP * ENZYMES_FUNCTION	535.320	6	89.220	2.793	.033
Error	766.602	24	31.942		
Total	207717.776	36			
Corrected Total	67201.505	35			

a. R Squared = .989 (Adjusted R Squared = .983)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
62.476	.942	60.532	64.420

2. GROUP

Dependent Variable: VALUE

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
GROUP 1	62.863	1.884	58.975	66.752
GROUP 2	70.931	1.884	67.043	74.819
GROUP 4	62.628	1.884	58.740	66.516
GROUP 5	53.481	1.884	49.593	57.369

Post Hoc Tests
GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP 1	GROUP 5	9.382222222*	2.6642378986	.005	2.703854957	16.060589488
GROUP 2	GROUP 5	17.450000000*	2.6642378986	.000	10.771632735	24.128367265
GROUP 4	GROUP 5	9.146666667*	2.6642378986	.006	2.468299401	15.825033932

Based on observed means.

The error term is Mean Square(Error) = 31.942.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

Appendix 6

ENZYMES_FUNCTION

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) ENZYMES_FUNCTION	(J) ENZYMES_FUNCTION	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
AST(u/L)	ALP(u/L)	97.447500000*	2.3072977019	.000	92.026716615	102.868283385
ALT(u/L)	ALP(u/L)	18.002500000*	2.3072977019	.000	12.581716615	23.423283385

Based on observed means.

The error term is Mean Square(Error) = 31.942.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 7

SERUM PROTEIN PROFILE

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	SERUM_PROTEIN_PROFILE	Mean	Std. Deviation	N
GROUP 1	Total PRT(g/L)	64.296666667	3.2520506351	3
	Total ALB(g/L)	24.890000000	3.4229081203	3
	TBIL(mg/dl)	1.529600000	.2275332064	3
	Total	30.238755556	27.5748665755	9
GROUP 2	Total PRT(g/L)	58.453333333	1.5022094838	3
	Total ALB(g/L)	17.196666667	10.3021664388	3
	TBIL(mg/dl)	1.715133333	.0435221017	3
	Total	25.788377778	25.9273024043	9
GROUP 4	Total PRT(g/L)	62.886666667	6.7701206292	3
	Total ALB(g/L)	30.483333333	1.1350036711	3
	TBIL(mg/dl)	1.688933333	.1282911273	3
	Total	31.686311111	26.7360582785	9
GROUP 5	Total PRT(g/L)	74.236666667	6.5291832057	3
	Total ALB(g/L)	36.660000000	1.1779643458	3
	TBIL(mg/dl)	1.186466667	.0508512864	3
	Total	37.361044444	31.8094915146	9
Total	Total PRT(g/L)	64.968333333	7.3977242610	12
	Total ALB(g/L)	27.307500000	8.8314460105	12
	TBIL(mg/dl)	1.530033333	.2482792392	12
	Total	31.268622222	27.1974487816	36

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25445.749[a]	11	2313.250	125.099	.000
Intercept	35198.162	1	35198.162	1903.489	.000

GROUP	615.472	3	205.157	11.095	.000
SERUM_PROTEIN_PROFILE	24428.936	2	12214.468	660.549	.000
GROUP * SERUM_PROTEIN_PROFILE	401.341	6	66.890	3.617	.011
Error	443.793	24	18.491		
Total	61087.705	36			
Corrected Total	25889.543	35			

a. R Squared = .983 (Adjusted R Squared = .975)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

		95% Confidence Interval	
Mean	Std. Error	Lower Bound	Upper Bound
31.269	.717	29.789	32.748

2. GROUP

Dependent Variable: VALUE

			95% Confidence Interval	
GROUP	Mean	Std. Error	Lower Bound	Upper Bound
GROUP 1	30.239	1.433	27.280	33.197
GROUP 2	25.788	1.433	22.830	28.747
GROUP 4	31.686	1.433	28.728	34.645
GROUP 5	37.361	1.433	34.403	40.319

3. SERUM_PROTEIN_PROFILE

Dependent Variable: VALUE

			95% Confidence Interval	
SERUM_PROTEIN_PROFILE	Mean	Std. Error	Lower Bound	Upper Bound
Total PRT(g/L)	64.968	1.241	62.406	67.530
Total ALB(g/L)	27.308	1.241	24.745	29.870
TBIL(mg/dl)	1.530	1.241	-1.032	4.092

4. GROUP * SERUM_PROTEIN_PROFILE

Dependent Variable: VALUE

				95% Confidence Interval	
GROUP	SERUM_PROTEIN_PROFILE	Mean	Std. Error	Lower Bound	Upper Bound

GROUP 1	Total PRT(g/L)	64.297	2.483	59.173	69.421
	Total ALB(g/L)	24.890	2.483	19.766	30.014
	TBIL(mg/dl)	1.530	2.483	-3.594	6.654
GROUP 2	Total PRT(g/L)	58.453	2.483	53.329	63.577
	Total ALB(g/L)	17.197	2.483	12.073	22.321
	TBIL(mg/dl)	1.715	2.483	-3.409	6.839
GROUP 4	Total PRT(g/L)	62.887	2.483	57.763	68.011
	Total ALB(g/L)	30.483	2.483	25.359	35.607
	TBIL(mg/dl)	1.689	2.483	-3.435	6.813
GROUP 5	Total PRT(g/L)	74.237	2.483	69.113	79.361
	Total ALB(g/L)	36.660	2.483	31.536	41.784
	TBIL(mg/dl)	1.186	2.483	-3.938	6.311

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

					95% Confidence Interval	
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
GROUP 1	GROUP 5	-7.122288889*	2.0271157671	.005	-12.203600730	-2.040977047
GROUP 2	GROUP 5	-11.572666667*	2.0271157671	.000	-16.653978508	-6.491354825
GROUP 4	GROUP 5	-5.674733333*	2.0271157671	.026	-10.756045175	-.593421492

Based on observed means.

The error term is Mean Square(Error) = 18.491.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

SERUM_PROTEIN_PROFILE

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) SERUM_PROTEIN_PROFILE	(J) SERUM_PROTEIN_PROFILE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Total PRT(g/L)	TBIL(mg/dl)	63.438300000*	1.7555337507	.000	59.313835186	67.562764814
Total ALB(g/L)	TBIL(mg/dl)	25.777466667*	1.7555337507	.000	21.653001852	29.901931481

Based on observed means.

The error term is Mean Square(Error) = 18.491.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 8

OXIDATIVE STRESS LIVER

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	OXIDATIVE_STRESS_PARAMETER	Mean	Std. Deviation	N
GROUP 1	PROT LIVER(g/L)	65.546666667	9.4011825497	3
	SOD LIVER	.000021600	.0000033000	3
	MDA LIVER	.506666667	.0650640710	3
	GSH LIVER	.920000000	.5129327441	3
	GST LIVER	.000005420	.0000002700	3
	GPx LIVER	4.030000000	.4200000000	3
	CAT	.000007137	.0000011750	3
	Total	10.143338213	23.4074208189	21
GROUP 2	PROT LIVER(g/L)	59.626666667	5.4350007666	3
	SOD LIVER	.000014800	.0000002000	3
	MDA LIVER	.306666667	.0152752523	3
	GSH LIVER	.670000000	.1509966887	3
	GST LIVER	.000004270	.0000004400	3
	GPx LIVER	3.320000000	.6260191690	3
	CAT	.000005307	.0000004950	3
	Total	9.131908244	21.2242735897	21
GROUP 4	PROT LIVER(g/L)	65.326666667	13.2250003151	3
	SOD LIVER	.000022267	.0000075501	3
	MDA LIVER	.426666667	.0850490055	3
	GSH LIVER	1.583333333	.2396525262	3
	GST LIVER	.000134637	.0002263475	3
	GPx LIVER	4.500000000	.8758424516	3
	CAT	.000007767	.0000003650	3
	Total	10.262404477	23.4641706029	21
GROUP 5	PROT LIVER(g/L)	83.815000000	.9050000000	3
	SOD LIVER	.000012267	.0000000577	3

	MDA LIVER	.280000000	.0700000000	3
	GSH LIVER	2.626666667	1.2850032425	3
	GST LIVER	.000004140	.0000010900	3
	GPx LIVER	6.590000000	1.0539924098	3
	CAT	.000008820	.0000018800	3
	Total	13.330241699	29.5817935614	21
Total	PROT LIVER(g/L)	68.578750000	11.9978797606	12
	SOD LIVER	.000017733	.0000057036	12
	MDA LIVER	.380000000	.1103712743	12
	GSH LIVER	1.450000000	.9937989553	12
	GST LIVER	.000037117	.0001130217	12
	GPx LIVER	4.610000000	1.4362704228	12
	CAT	.000007258	.0000016556	12
	Total	10.716973158	24.2198669264	84

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48091.765[a]	27	1781.176	167.304	.000
Intercept	9647.695	1	9647.695	906.195	.000
GROUP	207.423	3	69.141	6.494	.001
OXIDATIVE_STRESS_PARAMETER	47070.832	6	7845.139	736.883	.000
GROUP * OXIDATIVE_STRESS_PARAMETER	813.510	18	45.195	4.245	.000
Error	596.197	56	10.646		
Total	58335.657	84			
Corrected Total	48687.962	83			

a. R Squared = .988 (Adjusted R Squared = .982)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
10.717	.356	10.004	11.430

2. GROUP

Dependent Variable: VALUE

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
GROUP 1	10.143	.712	8.717	11.570
GROUP 2	9.132	.712	7.706	10.558
GROUP 4	10.262	.712	8.836	11.689
GROUP 5	13.330	.712	11.904	14.757

Post Hoc Tests
GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP 1	GROUP 5	-3.186903486*	1.0069462688	.007	-5.618081961	-.755725011
GROUP 2	GROUP 5	-4.198333455*	1.0069462688	.000	-6.629511930	-1.767154980
GROUP 4	GROUP 5	-3.067837222*	1.0069462688	.010	-5.499015697	-.636658747

Based on observed means.

The error term is Mean Square(Error) = 10.646.

Multiple Comparisons

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) OXIDATIVE_STRESS_PARAMETER	(J) OXIDATIVE_STRESS_PARAMETER	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PROT LIVER(g/L)	CAT	68.578742743*	1.3320647054	.000	65.052585959	72.104899526
SOD LIVER	CAT	.000010476	1.3320647054	1.000	-3.526146307	3.526167259
MDA LIVER	CAT	.379992743	1.3320647054	1.000	-3.146164041	3.906149526
GSH LIVER	CAT	1.449992742	1.3320647054	.764	-2.076164041	4.976149526
GST LIVER	CAT	.000029859	1.3320647054	1.000	-3.526126924	3.526186642
GPx LIVER	CAT	4.609992743*	1.3320647054	.006	1.083835959	8.136149526

Based on observed means.

The error term is Mean Square(Error) = 10.646.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

OXIDATIVE STRESS TESTES

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	OXIDATIVE_STRESS_PARAMETER	Mean	Std. Deviation	N
GROUP 1	PROT TESTES(g/L)	27.500003093	47.5188556765	3
	SOD TESTES	2.756668203	2.8822943335	3
	MDA TESTES	14.176678163	24.5546969922	3
	GSH TESTES	1.043337033	1.4664316392	3
	GST TESTES	29.476668927	47.4305369301	3
	GPx TESTES	1.086671867	1.7538575716	3
	CAT TESTES	1.666669460	2.8867489269	3
	Total	11.100956678	25.6840284783	21
GROUP 2	PROT TESTES(g/L)	30.280001450	52.3253004605	3
	SOD TESTES	3.456667337	3.0044011692	3
	MDA TESTES	30.956669400	53.6185171325	3
	GSH TESTES	.780000830	1.1656749366	3

	GST TESTES	17.543335420	25.9398713087	3
	GPx TESTES	1.240016900	1.6479190310	3
	CAT TESTES	2.206670703	3.8220552862	3
	Total	12.351908863	28.3096744915	21
GROUP 4	PROT TESTES(g/L)	27.093337233	46.7885308205	3
	SOD TESTES	2.890000743	3.4365816672	3
	MDA TESTES	31.103339553	53.8725482314	3
	GSH TESTES	.740001073	1.1877279222	3
	GST TESTES	31.496668813	48.7668989223	3
	GPx TESTES	.833335113	1.2906311957	3
	CAT TESTES	1.370003343	2.3729067110	3
	Total	13.646669410	30.9712460954	21
GROUP 5	PROT TESTES(g/L)	15.150013100	25.8001454377	3
	SOD TESTES	2.146670033	1.8815450173	3
	MDA TESTES	27.553339540	47.7237678761	3
	GSH TESTES	.903334103	1.4282966242	3
	GST TESTES	32.170002127	51.8633444232	3
	GPx TESTES	1.216669377	1.9958014535	3
	CAT TESTES	2.510003087	4.3474448539	3
	Total	11.664290195	26.9965291464	21
Total	PROT TESTES(g/L)	25.005838719	38.2672224559	12
	SOD TESTES	2.812501579	2.4857945210	12
	MDA TESTES	25.947506664	40.3324731281	12
	GSH TESTES	.866668260	1.1317015960	12
	GST TESTES	27.671668822	38.6160820661	12
	GPx TESTES	1.094173314	1.4520930694	12
	CAT TESTES	1.938336648	2.9746139926	12
	Total	12.190956287	27.5634150149	84

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13863.398[a]	27	513.459	.584	.935
Intercept	12484.031	1	12484.031	14.211	.000
GROUP	75.820	3	25.273	.029	.993

OXIDATIVE_STRESS_PARAMETER	12450.793	6	2075.132	2.362	.042
GROUP * OXIDATIVE_STRESS_PARAMETER	1336.785	18	74.266	.085	1.000
Error	49195.176	56	878.485		
Total	75542.604	84			
Corrected Total	63058.573	83			

a. R Squared = .220 (Adjusted R Squared = -.156)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

		95% Confidence Interval	
Mean	Std. Error	Lower Bound	Upper Bound
12.191	3.234	5.713	18.669

2. GROUP

Dependent Variable: VALUE

			95% Confidence Interval	
GROUP	Mean	Std. Error	Lower Bound	Upper Bound
GROUP 1	11.101	6.468	-1.856	24.058
GROUP 2	12.352	6.468	-.605	25.308
GROUP 4	13.647	6.468	.690	26.603
GROUP 5	11.664	6.468	-1.292	24.621

Post Hoc Tests
GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference	Std. Error	Sig.	95% Confidence Interval

		(I-J)			Lower Bound	Upper Bound
GROUP 1	GROUP 5	-.563333517	9.1468718509	1.000	-22.647608167	21.520941133
GROUP 2	GROUP 5	.687618668	9.1468718509	1.000	-21.396655983	22.771893318
GROUP 4	GROUP 5	1.982379215	9.1468718509	.993	-20.101895435	24.066653865

Based on observed means.

The error term is Mean Square(Error) = 878.485.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets
OXIDATIVE_STRESS_PARAMETER

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) OXIDATIVE_STRESS_PARAMETER	(J) OXIDATIVE_STRESS_PARAMETER	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PROT TESTES(g/L)	CAT TESTES	23.067502071	12.1001740958	.244	-8.963307540	55.098311681
SOD TESTES	CAT TESTES	.874164931	12.1001740958	1.000	-31.156644680	32.904974541
MDA TESTES	CAT TESTES	24.009170016	12.1001740958	.211	-8.021639595	56.039979626
GSH TESTES	CAT TESTES	-1.071668388	12.1001740958	1.000	-33.102477999	30.959141222
GST TESTES	CAT TESTES	25.733332173	12.1001740958	.160	-6.297477437	57.764141784
GPx TESTES	CAT TESTES	-.844163334	12.1001740958	1.000	-32.874972945	31.186646276

Based on observed means.

The error term is Mean Square(Error) = 878.485.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

OXIDATIVE STRESS ON THE SERUM

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: OXIDATIVE_VALUE

GROUP	OXIDATIVE_STRESS_PARAMETER	Mean	Std. Deviation	N
GROUP 1	SOD SERUM	.240008133	.2505875979	3
	MDA SERUM	1.000003037	1.7320481777	3
	GSH SERUM	.216673333	.2311471396	3
	GST SERUM	1.296667577	2.2458917585	3
	GPx SERUM	.230007167	.2406139131	3
	CAT SERUM	1.203334687	2.0842332998	3
	Total	.697782322	1.3110524949	18
GROUP 2	SOD SERUM	.246673333	.2553985124	3
	MDA SERUM	1.553344963	2.6904421826	3
	GSH SERUM	.253340633	.2500555648	3
	GST SERUM	1.536669430	2.6615823478	3

	GPx SERUM	.206673733	.2050096392	3
	CAT SERUM	1.140001626	1.9745365128	3
	Total	.822783953	1.5963446885	18
GROUP 3	SOD SERUM	.240007267	.2399891001	3
	MDA SERUM	.993335020	1.7205023415	3
	GSH SERUM	.250005767	.3421924533	3
	GST SERUM	1.036672543	1.7955542479	3
	GPx SERUM	.246674400	.2500552156	3
	CAT SERUM	1.376669567	2.3844541003	3
	Total	.690560761	1.2843621683	18
GROUP 4	SOD SERUM	.213340200	.2202928478	3
	MDA SERUM	.916667937	1.5877121404	3
	GSH SERUM	.236673900	.2400587383	3
	GST SERUM	1.503334857	2.6038483948	3
	GPx SERUM	.250006233	.2981531907	3
	CAT SERUM	1.886675420	3.2677949430	3
	Total	.834449758	1.6853501091	18
Total	SOD SERUM	.235007233	.2067627550	12
	MDA SERUM	1.115837739	1.7113889542	12
	GSH SERUM	.239173408	.2303856393	12
	GST SERUM	1.343336102	2.0167881173	12
	GPx SERUM	.233340383	.2145242491	12
	CAT SERUM	1.401670325	2.1372208719	12
	Total	.761394198	1.4496181089	72

Tests of Between-Subjects Effects

Dependent Variable: OXIDATIVE_VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22.730[a]	23	.988	.375	.994
Intercept	41.740	1	41.740	15.842	.000
GROUP	.327	3	.109	.041	.989
OXIDATIVE_STRESS_PARAMETER	20.435	5	4.087	1.551	.192
GROUP * OXIDATIVE_STRESS_PARAMETER	1.968	15	.131	.050	1.000
Error	126.469	48	2.635		

Total	190.939	72			
Corrected Total	149.199	71			

a. R Squared = .152 (Adjusted R Squared = -.254)

Estimated Marginal Mean

1. Grand Mean

Dependent Variable: OXIDATIVE_VALUE

		95% Confidence Interval	
Mean	Std. Error	Lower Bound	Upper Bound
.761	.191	.377	1.146

2. GROUP

Dependent Variable: OXIDATIVE_VALUE

			95% Confidence Interval	
GROUP	Mean	Std. Error	Lower Bound	Upper Bound
GROUP 1	.698	.383	-.071	1.467
GROUP 2	.823	.383	.054	1.592
GROUP 3	.691	.383	-.079	1.460
GROUP 4	.834	.383	.065	1.604

Post Hoc Tests
GROUP

Multiple Comparisons

Dependent Variable: OXIDATIVE_VALUE

Dunnett t (2-sided)[a]

					95% Confidence Interval	
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
GROUP 1	GROUP 4	-.136667435	.5410664280	.989	-1.449090015	1.175755144
GROUP 2	GROUP 4	-.011665805	.5410664280	1.000	-1.324088384	1.300756775
GROUP 3	GROUP 4	-.143888997	.5410664280	.987	-1.456311577	1.168533582

Based on observed means.

The error term is Mean Square(Error) = 2.635.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets
OXIDATIVE_STRESS_PARAMETER

Multiple Comparisons

Dependent Variable: OXIDATIVE_VALUE

Dunnett t (2-sided)[a]

(I) OXIDATIVE_STRESS_PARAMETER	(J) OXIDATIVE_STRESS_PARAMETER	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SOD SERUM	CAT SERUM	-1.166663091	.6626683327	.284	-2.890127844	.556801661
MDA SERUM	CAT SERUM	-.285832586	.6626683327	.991	-2.009297338	1.437632167
GSH SERUM	CAT SERUM	-1.162496916	.6626683327	.287	-2.885961669	.560967836
GST SERUM	CAT SERUM	-.058334223	.6626683327	1.000	-1.781798975	1.665130530
GPx SERUM	CAT SERUM	-1.168329941	.6626683327	.283	-2.891794694	.555134811

Based on observed means.

The error term is Mean Square(Error) = 2.635.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix

HEMATOLOGY STUDIES

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	HAEMATOLOGICAL_PARAMETER	Mean	Std. Deviation	N
GROUP 1	WBC	7.600000000	1.8000000000	3
	RBC	6.376666667	.0850490055	3
	HGB	12.400000000	.2000000000	3
	MCHC	36.200000000	.3000000000	3
	MPV	5.900000000	.9000000000	3
	PLT	604.500000000	75.5000000000	3
	Total	112.162777778	228.2945882177	18
GROUP 2	WBC	6.666666667	.4509249753	3
	RBC	5.230000000	.0600000000	3
	HGB	10.900000000	1.3000000000	3
	MCHC	35.866666667	.2516611478	3
	MPV	5.450000000	.0500000000	3
	PLT	502.000000000	201.0000000000	3
	Total	94.352222222	200.1611050116	18
GROUP 4	WBC	7.266666667	.1527525232	3
	RBC	5.536666667	.3763420430	3
	HGB	11.900000000	.9000000000	3
	MCHC	36.100000000	.3000000000	3
	MPV	6.133333333	.5033222957	3
	PLT	630.500000000	158.5000000000	3
	Total	116.239444444	243.0597391219	18
GROUP 5	WBC	10.033333333	1.6563010998	3
	RBC	7.433333333	.9315220520	3
	HGB	13.266666667	.6506407099	3
	MCHC	36.666666667	.0577350269	3
	MPV	6.766666667	.3511884584	3
	PLT	986.000000000	309.0000000000	3
	Total	176.694444444	387.3551431898	18

Total	WBC	7.891666667	1.7085125553	12
	RBC	6.144166667	.9909908571	12
	HGB	12.116666667	1.1566671033	12
	MCHC	36.208333333	.3704010930	12
	MPV	6.062500000	.6799481932	12
	PLT	680.750000000	258.2834507070	12
	Total	124.862222222	270.4075963035	72

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4858106.779[a]	23	211222.034	30.407	.000
Intercept	1122521.367	1	1122521.367	161.595	.000
GROUP	69355.216	3	23118.405	3.328	.027
HAEMATOLOGICAL_PARAMETER	4457661.065	5	891532.213	128.343	.000
GROUP * HAEMATOLOGICAL_PARAMETER	331090.499	15	22072.700	3.178	.001
Error	333432.259	48	6946.505		
Total	6314060.405	72			
Corrected Total	5191539.038	71			

a. R Squared = .936 (Adjusted R Squared = .905)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
124.862	9.822	105.113	144.611

2. GROUP

Dependent Variable: VALUE

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound

GROUP 1	112.163	19.645	72.664	151.661
GROUP 2	94.352	19.645	54.854	133.851
GROUP 4	116.239	19.645	76.741	155.738
GROUP 5	176.694	19.645	137.196	216.193

Post Hoc Tests
GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP 1	GROUP 5	-64.531666667	27.7818993637	.063	-131.920050317	2.856716984
GROUP 2	GROUP 5	-82.342222222*	27.7818993637	.013	-149.730605873	-14.953838572
GROUP 4	GROUP 5	-60.455000000	27.7818993637	.088	-127.843383651	6.933383651

Based on observed means.

The error term is Mean Square (Error) = 6946.505.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

HAEMATOLOGICAL_PARAMETER

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) HAEMATOLOGICAL_PARAMETER	(J) HAEMATOLOGICAL_PARAMETER	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
WBC	PLT	-672.858333333*	34.0257387632	.000	-761.352318244	-584.364348423
RBC	PLT	-674.605833333*	34.0257387632	.000	-763.099818244	-586.111848423
HGB	PLT	-668.633333333*	34.0257387632	.000	-757.127318244	-580.139348423
MCHC	PLT	-644.541666667*	34.0257387632	.000	-733.035651577	-556.047681756
MPV	PLT	-674.687500000*	34.0257387632	.000	-763.181484910	-586.193515090

Based on observed means.

The error term is Mean Square (Error) = 6946.505.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 10

REPRODUCTIVE STATUS

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable: VALUE

GROUP	REPRODUCTIVE_STATUS	Mean	Std. Deviation	N
	TESTOSTERONE	.470000000	.	1
	SPERM MOTILITY	68.300000000	.	1
	Total	34.385000000	47.9630529679	2
GROUP 1	TESTOSTERONE	.430000000	.0900000000	3
	SPERM COUNT	39.666666667	23.7920855188	3
	SPERM MOTILITY	44.033333333	36.5964934568	3
	SPERM VIABILITY	40.750000000	34.9883194795	3
	Total	31.220000000	30.2748509493	12
GROUP 2	TESTOSTERONE	.350000000	.0800000000	3
	SPERM COUNT	32.933333333	27.9249589674	3
	SPERM MOTILITY	41.050000000	34.7681506554	3
	SPERM VIABILITY	38.413333333	31.7746838432	3
	Total	28.186666667	28.9182419098	12
GROUP 4	TESTOSTERONE	.480000000	.0100000000	3
	SPERM COUNT	35.533333333	28.7070258531	3
	SPERM MOTILITY	42.790000000	34.6068533675	3
	SPERM VIABILITY	38.203333333	30.5505815547	3
	Total	29.251666667	29.0801281207	12
GROUP 5	TESTOSTERONE	.590000000	.0565685425	2
	SPERM COUNT	51.213333333	43.2020200145	3
	SPERM MOTILITY	33.830000000	45.6366716578	2
	SPERM VIABILITY	45.666666667	39.1170465824	3
	Total	35.948000000	37.0699362467	10
Total	TESTOSTERONE	.452500000	.0980839158	12
	SPERM COUNT	39.836666667	28.0578063464	12
	SPERM MOTILITY	43.298333333	30.7425860488	12
	SPERM VIABILITY	40.758333333	29.3922459884	12

Total	31.086458333	30.4753253863	48

Tests of Between-Subjects Effects

Dependent Variable: VALUE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16609.437[a]	17	977.026	1.084	.411
Intercept	35516.738	1	35516.738	39.402	.000
GROUP	507.215	4	126.804	.141	.966
REPRODUCTIVE_STATUS	14824.668	3	4941.556	5.482	.004
GROUP * REPRODUCTIVE_STATUS	963.709	10	96.371	.107	1.000
Error	27041.600	30	901.387		
Total	90036.695	48			
Corrected Total	43651.036	47			

a. R Squared = .381 (Adjusted R Squared = .029)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: VALUE

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
30.817[a]	4.618	21.385	40.249

a. Based on modified population marginal mean.

2. GROUP

Dependent Variable: VALUE

GROUP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
	34.385[a]	21.230	-8.972	77.742
GROUP 1	31.220	8.667	13.520	48.920
GROUP 2	28.187	8.667	10.486	45.887
GROUP 4	29.252	8.667	11.551	46.952
GROUP 5	32.825	9.690	13.036	52.614

a. Based on modified population marginal mean.

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	GROUP 5	-1.563000000	23.2557949481	1.000	-61.827661388	58.701661388
GROUP 1	GROUP 5	-4.728000000	12.8551243407	.989	-38.040544990	28.584544990
GROUP 2	GROUP 5	-7.761333333	12.8551243407	.936	-41.073878324	25.551211657
GROUP 4	GROUP 5	-6.696333333	12.8551243407	.961	-40.008878324	26.616211657

Based on observed means.

The error term is Mean Square(Error) = 901.387.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Homogeneous Subsets

REPRODUCTIVE_STATUS

Multiple Comparisons

Dependent Variable: VALUE

Dunnett t (2-sided)[a]

(I) REPRODUCTIVE_STATUS	(J) REPRODUCTIVE_STATUS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TESTOSTERONE	SPERM VIABILITY	-40.305833333*	12.2568801390	.007	-70.625793053	-9.985873613
SPERM COUNT	SPERM VIABILITY	-.921666667	12.2568801390	1.000	-31.241626387	29.398293053
SPERM MOTILITY	SPERM VIABILITY	2.540000000	12.2568801390	.994	-27.779959720	32.859959720

Based on observed means.

The error term is Mean Square(Error) = 901.387.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Boby weights

	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
DM	104.68	106.02	92.7	91.45	87.42
LC	111.23	103.26	98.79	89.29	90.76
MF	115.28	128.92	117.22	112.69	110.92
C	130.18	127.07	121.4	128.64	140.56

Average organ weights

	TESTES	LIVER
DM	1.830667	4.679333
LC	1.899	5.508
MF	2.189333	4.663333
C	2.624667	4.255333

LDH activities

	LDH LIVER	LDH TESTES	LDH SERUM
DM	65.68	285.58	311.57
LC	73.82	352.34667	334.536667
MF	66.17	333.52667	315.28
C	55.56	261.56667	300.686667

Liver Function Enzyme

	AST(U/L)	ALT(U/L)	ALP(U/L)
DM	120.6233	41.75667	26.21
LC	138.1367	44.73667	29.92
MF	119.8633	42.72	25.3
C	107.1367	38.76667	14.54

Serum Protein Profile

	DM	LC	MF	C
Total PRT(g/L)	64.29	58.45	62.88	74.23
Total ALB(g/L)	24.89	17.2	30.48	36.66
TBIL(mg/dl)	1.53	1.72	1.69	1.19

Haemetological Status

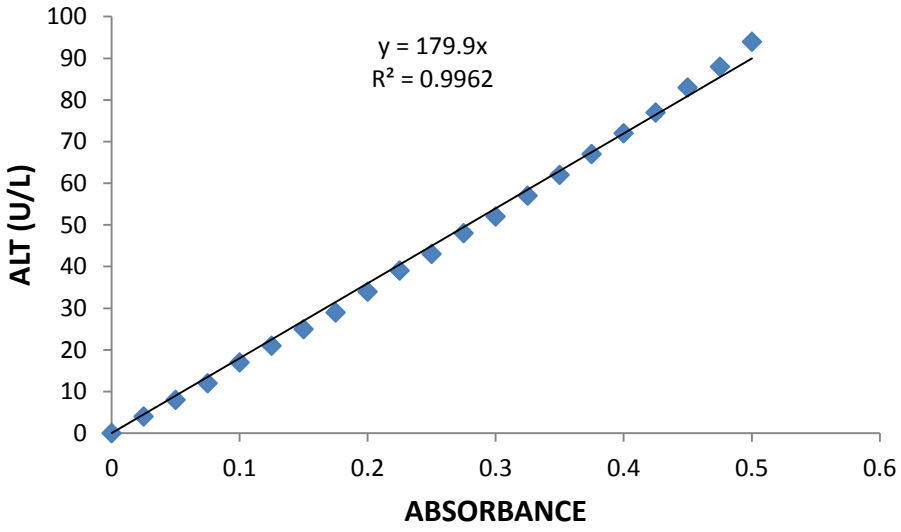
	WBC(10^3/µL)	RBC(10^3/µL)	HGB(g/dL)	MCHC(g/dL)	PLT(10^4/µL)
DM	7.6	6.376667	12.4	36.2	60.45
LC	6.666667	5.23	10.9	35.86667	50.2
MF	7.266667	5.536667	11.9	36.1	63.05
C	10.03333	7.433333	13.26667	36.66667	98.6

Reproductive Status

LC	0.35	32.93333333	41.05	38.41333333
MF	0.48	35.53333333	42.79	38.20333333
C	0.55	51.21333333	45.32	45.66666667

Appendix 11

Calibration curve for Alanine aminotransferase



Calibration curve for Aspartate aminotransferase