EVALUATION OF THE EFFECTS OF ASCORBIC ACID AND ACETYL-L-CARNITINE ON SUBACUTE CHLORPYRIFOS POISONING IN WISTAR RATS

BY

UCHENDU, CHIDIEBERE

MSc/VET-MED/00276/08-09

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY, ZARIA. NIGERIA.**

IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY TOXICOLOGY

**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA. NIGERIA.**

MARCH, 2011

DECLARATION

I declare that the work in the thesis entitled, ‘Evaluation of the Effects of Ascorbic Acid and Acetyl-L-carnitine on Subacute Chlorpyrifos Poisoning in Wistar Rats.’ has been performed by me in the Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, under the supervision of Dr. S. F. Ambali, Prof. J. O. Ayo and Prof. K.A.N. Esievo.

The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been presented for the award of a degree or diploma at any university.

--- ----------------------

UCHENDU, CHIDIEBERE DATE

CERTIFICATION

This thesis, entitled "Evaluation of the Effects of Ascorbic Acid and Acetyl-L-carnitine on Subacute Chlorpyrifos Poisoning in Wistar Rats" by Uchendu, Chidiebere meets the regulations governing the award of Master of Science degree in Veterinary Toxicology of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

-- ---------------------
Dr S. F. Ambali Date
Chairman, Supervisory Committee

-- ---------------------
Prof. J. O. Ayo Date
Member, Supervisory Committee

-- --------------------
Prof. K.A.N. Esievo Date
Member, Supervisory Committee

-- ------------------
Dr M. U. Kawu Date
Head of Department

--- -------------------
Prof. A.A. Joshua
Dean, Postgraduate School Date

DEDICATION

This work is dedicated to God Almighty for the strength He gave me, for His favour and blessings to me, and to my parents Mr. and Mrs. I.O. UCHENDU. I could not have been what I am today without you.

ACKNOWLEDGEMENT

I want to firstly and rightly acknowledge the contribution of my supervisory committee without which this work would have been impossible, Dr S. F. Ambali, Prof. J. O. Ayo and Prof. K.A.N. Esievo, God bless you, sirs. I cannot overemphasize my sincere gratitude to Dr S. F. Ambali for his intelligent and constructive criticism as well as his tireless efforts, encouragement and understanding. My sincere appreciation and love go out to my parents and siblings, Mr. and Mrs. I.O. Uchendu, Charles, Kelechi, Ikechukwu, Okechukwu and Moses, whom I cannot repay for their understanding, concern, love and support. To Dr. and Mrs. A.C. Odunze and the family, I want to appreciate you for your love, encouragement and support. I want to also appreciate Prof. M.Y. Fatihu and Dr. M. Bisalla for their patience in reading my histopathology slides.

To my colleagues, Drs M. Shittu, L.S. Yaqub, Veronica Ochigbo, Blessed Madaki, Oludare Omoniwa and especially Dr Oluwole Lasisi, I cannot thank you enough for being there. God bless you. To Richard Edeh and Chinedu Orieji, thank you for your support and contribution to make this work a success. I also want to thank the entire technical staff of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, headed by Chief Emmanuel Nwosu, for their assistance.

To the friends who stood by me and encouraged me, Oluwatoyin Iyun, Jibil Jibril, Martins Churchil, Num Bulus, Maidodo Usha, Sepke Maria, Mohammed Bala, Osu Chidi, Momoh Asabe, Adole Benny, Ese Joel, Moore Akin, Aregbe Bunmi, Drs Olayinka Asala, Joel Atuman and Tizhe Vandi, I say thanks a lot.

Finally, I want to thank the entire 2008-2009 Masters class, you are the best, and I could not have done it without you. God bless you all.

In case your name wasn’t mentioned, then I am sorry, just remember you will always be dear to my heart.

ABSTRACT

Chlorpyrifos (CPF) toxicity has been shown to be partly mediated via induction of oxidative stress. In the present study, studies were conducted with the aim of evaluating the effect of subacute CPF exposure on haematological and biochemical changes and the ameliorating effects of acetyl-L-carnitine (ALC) and vitamin C (VC) in Wistar rats. Fifty-six adult male Wistar rats divided into 8 groups of 7 animals each were used for this study. Rats in group I (Soya oil group) received soya oil (2 ml/kg). Rats in groups II (VC) and III (ALC) were administered with vitamin C (100 mg/kg), and ALC (300 mg/kg), while the rats in group IV (VC+ALC) received the combination of VC and ALC at 100 mg/kg and 300 mg/kg. Rats in group V (CPF) received CPF only (8.5 mg/kg ~ 1/10th of the LD_{50}). Rats in groups VI (VC + CPF) and VII (ALC + CPF) were pretreated with VC (100 mg/kg) and ALC (300 mg/kg), respectively and then exposed to CPF (8.5 mg/kg), 30 minutes later. Rats in group VIII (VC+ALC+CPF) were pretreated with VC (100 mg/kg), ALC (300 mg/kg) and then exposed to CPF (8.5 mg/kg), 30 minutes later. The regimens were administered orally by gavage once daily for a period of 28 days. During this period, the rats were observed for signs of toxicity and death. At the end of the dosing period, the rats were sacrificed; blood samples collected and analyzed for total red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb) concentration, platelet count, white blood cells (WBC), absolute white blood count and neutrophil/lymphocyte ratio. Sera obtained from the blood sample were analyzed for Na^+, K^+, Cl^-, HCO_3^-, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), glucose, total protein, albumin and globulin. Hepatic tissues were also examined for malonaldehyde (MDA) concentration and antioxidant enzymes (catalase and superoxide dismutase). Gross and histopathological examinations were also carried out. The results showed that pretreatment with ALC and/or VC alleviated the alterations in toxic signs, haematological, serum biochemical and hepatic tissues changes induced by subacute CPF exposure in rats. In conclusion, the study has shown oxidative stress is partly involved in the molecular mechanisms of subacute CPF-induced alteration in haematological and biochemical parameters in Wistar rats. The amelioration of the CPF-induced alteration in haematological, biochemical and pathological changes by VC and/or ALC may be due to their antioxidant properties. VC was shown to be more potent than ALC in ameliorating the CPF-induced changes in the haematological and biochemical profiles. Their combination also gave a better result in some of the experiment than when either of them was used alone, demonstrating their synergism. Therefore, it is conceivable that farmers, pesticide applicators and individuals who are repeatedly exposed to low-dose CPF and, perhaps other organophosphates may be protected from the pesticide-evoked haematological and biochemical toxicity by pretreatment with ALC and VC alone or their combination.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

2,4-D -	2,4- dichlorophenoxyacetic acid
ACh -	Acetylcholine
AChE -	Acetylcholinesterase
ALC -	Acetyl-L-carnitine
ALP -	Alkaline phosphatase
ALT -	Alanine aminotransferase
ANOVA -	Analysis of variance
AST -	Aspartate aminotransferase
ATP -	Adenosine triphosphate
BUChE -	Butyrylcholinesterase
CAT -	Catalase
Cl^- -	Chloride ion
CK -	Creatine kinase
CK -B-	Isozyme of creatine kinase
COPIND -	Chronic organophosphate-induced neuropsychiatric disorder
CPF -	Chlorpyrifos
CPF -oxon-	Chlorpyrifos oxygen analogue
Cu^{2+} -	Cuprous ion
CYP450 -	Cytochrome P_{450} mixed-function oxidase
DDT -	Dichlorodiphenyltrichloroethane
DEP -	Diethylphosphate
Fe^{2+}-	Ferrous ion
FR -	Free radicals

GPx - Glutathione peroxidase
GR - Glutathione reductase
GST - Glutathione-S-transferase
H_2O_2 - Hydrogen peroxide
Hb - Haemoglobin
HOCl - Hypochlorous acid
IMS - Intermediate syndrome
K^+ - Potassium ion
LC - L-carnitine
LD_{50} - Median lethal dose
LDL - Low density lipoprotein
LPO - Lipid peroxidation
MCH - Mean corpuscular haemoglobin
MCHC - Mean corpuscular haemoglobin concentration
MCPA - Dinitrocresol-4-chloro-2-methyloxyacetic acid
MCV - Mean cell volume
MDA - Malonaldehyde
Na^+ - Sodium ion
NaCl - Sodium chloride
NO^- - Nitric oxide radical
$O2^-$ - Superoxide anion radical
OH^- - Hydroxyl radical
$ONOO^-$ - Peroxynitrite anion
OP - Organophosphates

OPICN -	Organophosphorus ester-induced chronic neurotoxicity
OPIDN -	Organophosphate-induced delayed neurotoxicity
OPIDP -	Organophosphate-induced delayed polyneuropathy
PCV -	Packed cell volume
PUFA -	Polyunsaturated fatty acid
RBC -	Red blood cell count
ROS -	Reactive oxygen species
SEM -	Standard error of the mean
SH -	Sulfhydryl group
SOD -	Superoxide dismutase
SVCT1 -	Sodium dependent vitamin C transporter type 1
TBA -	Thiobarbituric acid
TBARS -	Thiobarbituric reactive substances
TCA -	Trichloroacetic acid
TCP -	3, 5, 6-trichloro-2-pyridinol
TP -	Total protein
UNEPA -	United Nation Environmental Programe
USEPA -	United States Environmental Programe
VC -	Vitamin C
WBC -	White blood cell

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Pesticides are ubiquitous with significant economic, environmental and public health impacts. Their usage has resulted in significant improvement in human and animal nutrition through greater availability, longer storage life, and lower costs of food (Weiss *et al*., 2004). Pesticide poisoning remains a serious public health problem world-wide. According to the World Health Organization, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (Yang and Deng, 2007; Chheteri *et al.,* 2008). Among the insecticides, organophosphates (OPs) remain the most widely used, accounting for 50% of all insecticide applications world-wide (Casida and Quistad, 2004; Fishel, 2005). OP poisoning is a progressively worrying phenomenon as worldwide pesticide production and consumption have doubled since the last three decades (Ali and Chia, 2008).

Chlorpyrifos (CPF) (*0,0 –diethyl-0-{3,5,6-trichloro-2-pyridyl*) phosphorothionate) is a broad spectrum, chlorinated OP insecticide that was first registered in 1965 to control foliage and soil-borne insect pests on a variety of food and feed crops (Odenkirchen and Eisler, 1988; Smegal, 2000; Mitra *et al.,* 2008; Aly *et al*., 2010). The most common trade names of CPF are; Dursban®, Empire 20®, Termicot®, Whitemire PT 270® and Lorsban® (Smegal, 2000; Timchalk *et al.,* 2002). CPF is a widely used OP pesticide with relatively low acute toxicity compared to other insecticides in the same class (Zheng *et al*., 2000). Like other OPs, it elicits toxicity by irreversibly inhibiting acetylcholinesterase (AChE) in the central and peripheral nervous system (Timchalk *et al*., 2002), allowing accumulation of acetylcholine (ACh) at the cholinergic synapses with consequent neurotoxicity (Zheng *et al.,* 2000). However, recent evidence has shown that other mechanisms may be implicated in its toxicity (Slotkin, 2004; Slotkin *et al*., 2006). Since toxicity occurs at doses that do not inhibit AChE (Pope *et al*., 1992: Chakraborti *et al*., 1993).

One of the mechanisms implicated in both acute and chronic CPF poisoning is oxidative stress, which is associated with the enhanced production of reactive oxygen species (ROS) and a decrease in the antioxidant or oxygen free-radical scavenging systems of cells (Almeida *et al*., 1997; Kalender *et al.*, 2004; Gultekin *et al.,* 2006; Ambali *et al.,* 2007; Durak *et al.,* 2008; Greter *et al.,* 2008). Oxidative stress occur when the production of ROS exceeds the body's natural antioxidant defence systems, causing damage to macromolecules such as DNA, proteins and lipids (Vandana *et al.*, 2006; Aly *et al*., 2010). This leads to increased lipoperoxidative damage to cell membrane, and a decrease in the antioxidant enzymes, including superoxide dismutase (SOD) and catalase, and antioxidant molecules like vitamins C and E and β-carotene (Verma *et al.,* 2007).

L-carnitine (LC) (trimethylamino-β-hydroxybutyrate) and its ester, acetyl-L-carnitine (ALC), are vital co-factors for the mitochondrial oxidation of fatty acids that result in ATP production in peripheral tissues (Cetinkaya *et al.,* 2006; Gülcin, 2006; Onem *et al.,* 2006; Picconi *et al*., 2006). LC is a naturally occurring compound that is widely distributed in the body, but is mainly synthesized in the brain (Kidd, 1999). It is a carrier of long-chain fatty acids and plays an important role in branched-chain amino acid metabolism, ketone body utilization, peroxisomal oxidation and erythrocyte membrane phospholipid turnover (Rani and Pannerselvan, 2001). However, ALC penetrates cells and crosses the blood-brain barrier more efficiently than LC (Kidd, 1999). Its transport through the lipid component of the intestinal barrier suggests possible better bioavailability of the ester than LC (Mansour, 2006). ALC promotes the oxidation of fatty acids, prevents their toxic accumulation in the cell, and ease energy availability at the cellular level (Ilias *et al*., 2004). It has an antioxidant activity and may be involved in ameliorating cellular dysfunction via an inhibition of the increase in lipid hydroperoxidation (Mansour, 2006; Gülcin, 2006), or by preventing the accumulation of end-products of lipid peroxidation (Cetinkaya *et al.,* 2006).

Acetyl-L-carnitine regulates the activities of enzymes involved in the defence against oxidative damage and protects the antioxidant enzymes, glutathione peroxidase, catalase and SOD, from peroxidative damage (Gülcin, 2006). Some studies have suggested neuroprotective role of ALC (Ilias *et al.,* 2004; Picconi *et al.,* 2006), as it provides protection from lesions induced by neurotoxic agents (Virmani *et al.,* 1995; Binienda, 2003). It is effective in various pathologic conditions characterized by increased oxidative stress, and also ameliorates oxidative injury of organs in animal models via its free radical scavenging and antioxidant properties (Calo *et al*., 2005).

Ascorbic acid or Vitamin C (VC) is a naturally occurring antioxidant and currently is the most widely used vitamin supplement throughout the world (Naidu, 2003; Adenkola *et al.,* 2009). It is the most effective and least toxic antioxidant molecule identified in mammalian system (Sauberlich, 1994). VC is a water-soluble, chain-breaking antioxidant molecule capable of scavenging/neutralizing an array of ROS like hydroxyl, alkoxyl, peroxyl, superoxide anion and hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide and peroxynitrite at very low concentration (Sauberlich*,* 1994; Naidu, 2003). It has been shown that VC completely protects plasma lipids and low-density lipoprotein (LDL) against peroxidative damage (Frei, 1991).

1.2 STATEMENT OF THE PROBLEM

The widespread and extensive use of CPF raises the likelihood of inadvertent exposure to the pesticide in segments of the population either from short-term high level exposure or long-term low level exposure with consequent toxic effect (Cohn and Macphail, 1997; Tang *et al*., 2001). OP poisoning is a progressively worrying phenomenon as its production and consumption world-wide has doubled over the last three decades (Ali and Chia, 2008). Economic and health benefits are achieved with simultaneous increase in potential risk and possible adverse health outcome to humans, domestic animals and the environment (Rabideau, 2001).

Generally, poisoning due to pesticides is common in developing countries due to lack of enforced legislation, resulting in indiscriminate importation and use, and ignorance with regards to safety precautions while working with the chemicals (WHO/UNEP, 1990; Ambali*,* 2009). In Nigeria, pesticide withdrawal periods are not observed and the magnitude of pesticide residue in food resources of both plant and animal origin is largely unknown (Ambali *et al.,* 2009).

1.3 JUSTIFICATION OF THE STUDY

Chlorpyrifos is one of the largest selling insecticides in the world and is used in both agricultural and urban pest control (Cope *et al.*, 2004). Pesticide use in developing nations has increased dramatically in recent times with consequent adverse effects in humans and other non-target organisms. In most of these countries, safety equipments are rarely used, storage methods are unsafe and the instructions for pesticides use are not always understood as they are often written in unfamiliar languages (EJF, 2003; Ambali, 2009). In addition, many farmers or pesticide users are uneducated, and the instructions on the use of the pesticides are impossible to follow, hence, increasing the risk of exposure (Konradsen *et al*., 2003; Otitoju *et al.,* 2008). Therefore, the danger posed by exposure to pesticides will remain for a long time in many developing countries including Nigeria.

The sequelae arising from acute exposure to OPs are immediate and obvious. On the other hand, the hazards posed by low-level exposure are insidious and often damaging to the system, resulting in adverse health consequence (Ambali, 2009). Alterations in haematological and serum biochemical parameters are used as indicators of the health status of the individual.

Oxidative stress has been implicated in the molecular mechanism of CPF-induced toxicity (Gultekin *et al*., 2001; Ambali *et al*., 2007, 2010a-e; Mansour and Mossa, 2009). Earlier studies have shown the protective effect of vitamin C in CPF-induced toxicity (Gultekin *et al*., 2001; Ambali *et al.,* 2007, 2010c and d). Therefore, it is conceivable that the antioxidant, ALC may also ameliorate CPF-induced toxicity. Their synergistic or potentiative effect would also be exploited in this study. However, there is paucity of information on the protective role of ALC either alone or in combination with VC on CPF-induced haematological and biochemical changes. The present study, therefore, evaluated the role of ALC and/or VC in subacute CPF-induced haematological, biochemical and histopathological changes in Wistar rats.

1.4 GENERAL AIM OF THE STUDY

The aim of the present study was to evaluate the effects of ALC and/or vitamin C on haematological, biochemical and histopathological changes induced by subacute CPF exposure in Wistar rats.

1.5 SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of the study were to:

i Evaluate the haematological, biochemical, gross and histopathological changes, induced by subacute CPF exposure in Wistar rats.

ii Determine the role of oxidative stress induced by subacute CPF exposure on haematological, biochemical and histopathological changes in Wistar rats.

iii Evaluate the effect of ALC and/or VC on haematological, biochemical, lipoperoxidative and histopathological changes, induced by subacute CPF exposure in Wistar rats.

1.6 RESEARCH HYPOTHESES

1. Subacute exposure of Wistar rats to CPF does not induce haematological, biochemical and histopathological changes.
2. Oxidative stress induced by subacute CPF exposure does not play important role in haematological, biochemical and histopathological changes induced in Wistar rats.
3. Pre-treatment with ALC and/or VC does not ameliorate the haematological, biochemical, lipoperoxidative and histopathological changes, induced by subacute CPF exposure in Wistar rats.

CHAPTER 2

LITERATURE REVIEW

2.1 PESTICIDES

Pesticides are substances that are used to prevent, repel or destroy pest-organisms that compete for food supply, adversely affect comfort, or endanger human and animal health (Weiss *et al*., 2004). They are heterogenous groups of chemicals developed to control a variety of pests. The term can be applied to microorganisms with biocidal properties, and to common household products (Fenske and Day, 2005). The United States Environmental Protection Agency (USEPA) defines pesticide as "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest". In other words, pesticide is any chemical, physical or biological agent that would kill or destroy unwanted plants or animal pests (Ecobichon, 1995; Shah and Srivastava, 2002; Goel and Aggarwal, 2007). Several classes of compounds are used for this purpose. More than 20,000 pesticide products with nearly 900 active ingredients are registered for use as insecticides, miticides, herbicides, rodenticides, nematocides, fungicides, fumigants, wood preservatives and plant growth regulators (Weiss *et al*., 2004). Pesticides are used in every realm of the environment to control undesired pests. An estimated 85-90% of pesticides never reach their target organism. It is very likely that many non-target organisms are exposed to multiple pesticides throughout their lifetimes, either sequentially or concurrently (Rabideau, 2001).

2.1.1 Uses of Pesticides

Pesticides have played vital role in controlling agricultural, industrial, home/garden and public health pests globally (Rabideau, 2001; Bjørling-Poulsen *et al*., 2008). Pesticide usage helps to improve human nutrition through greater availability, longer storage life, and lower cost of food. They also reduce human labour requirements and attendant risks of injury. Pesticides also assist in the control of food-borne and vector-borne diseases (Ferreira *et al*., 2008) which affect humans and animals (Weiss *et al*., 2004). The use of pesticides has increased food production in parallel with the population growth in many parts of the world (Ather *et al*., 2008).World wide, about 3 billion kg of pesticides is applied each year (Pimentel, 2005), with developing countries accounting for around one fourth of world production (Miranda, 2003). Pesticides also pose animal and human health concerns because of their toxicity, wide use and release into the environment (Weiss *et al*., 2004; Calvert *et al*., 2008). Pesticide levels in the environment are likely to be higher in developing countries than in agricultural environments in developed countries, where pesticide usage is well regulated and monitored (Jaga and Dharmani, 2003; Otitoju *et al*., 2008; Peiris-john and Wickremasinghe, 2008). In developed countries, the safe use of pesticides causing minimum harm to humans and the environment is ensured by the availability of technology, infrastructure, regulation and economic support (Miranda, 2003; Peiris-john and Wickremasinghe, 2008).

2.1.2 Historical Uses of Pesticides

Pesticides have been used since ancient times. Over the centuries, man has developed many ingenious methods in attempts to control the invertebrates, vertebrates and microorganisms that constantly threaten the supply of food and fibre, as well as posing a threat to health. The Chinese used sulphur and arsenic to control insects (Ecobichon, 1995), while the early Romans used common salt to control weeds (Cope *et al.*, 2004). As early as 1690, water extracts of tobacco leaves (*Nicotiana tabacum*) were sprayed on plants as insecticides (Goel and Aggarwal, 2007), and *nux vomica* was introduced to kill rodents (Cope *et al*., 2004).

In the 1800s, the pulverized root of *Derris eliptica*, containing rotenone, was used as an insecticide, as was pyrethrum extracted from the flowers of chrysanthemums (*Chrysanthemum cinerriaefolium*) (Ecobichon, 1995; Cope *et al*., 2004; Goel and Aggarwal, 2007). Another material developed for insect control in 1800s was Paris Green, a mixture of copper and arsenic salt (Cope *et al*., 2004).

However, it was not until 1900s that the compounds identified today as having pesticidal properties came into existence. By the 1920s, the widespread use of arsenical pesticides caused considerable public health concern because some treated fruits and vegetables were found to have toxic residues. Although some of these early pesticides caused only minimal harm to humans exposed, other agents were exceedingly toxic (Ecobichon, 1995). The 1930s ushered in the era of modern synthetic chemistry, including the development of a variety of agents such as alkyl thiocyanate insecticides, dithiocarbamate fungicides, ethylene dibromide, methyl bromide, ethylene oxide, and carbon disulphide as fumigants (Cremlyn, 1978). The 1940s which coincided with the beginning of World War II saw the introduction of hydrocarbon insecticides like dichlorodiphenyltrichloroethane (DDT), dinitrocresol, 4-chloro-2-methyloxyacetic acid (MCPA), and 2,4-dichlorophenoxyacetic acid (2,4-D) (Ecobichon, 1995; Cope *et al*., 2004). In the post-war era, there was rapid development in the agrochemical field, with a plethora of insecticides, fungicides, herbicides and other chemical agents being introduced. Triazine herbicides, such as atrazine, introduced in the late 1950s, dominated the world herbicide market for years (Cope *et al*., 2004). But only during the last 50 years, with the discovery and manufacture of synthetic pesticides, has pesticide production become a successful industry (Miranda, 2003).

2.1.3 Classification of Pesticides

Depending on what a compound is designed to do, pesticides have been subclassified into a number of categories. The primary classes of pesticides in use today are fumigants, fungicides, herbicides and insecticides (Cope *et al*., 2004).

2.1.3.1 Insecticides

The development of insecticides has been based on specific structure-activity relationships, requiring the manipulation of a basic chemical structure to obtain an optimal shape and configuration for specificity towards a unique biochemical or physiological feature of the nervous system (Ecobichon, 1995). Most of the chemicals that are used as insecticides are not highly selective and they result in poisoning in many non-target species, including man and animals

(Fenske, 1997). They are commonly used to increase the production and quality of agricultural products, to minimize the damage caused by insects during storage of food grains, control ectoparasites of domestic animals, control certain vector borne disease, repel house-hold pests, and as anthelmintics (Shah and Srivastava, 2002). Most insecticides work by interfering with nervous system function (Weiss *et al*., 2004). The older insecticides of vegetable origin, including pyrethrins and rotenones, are practically non-toxic to warm blooded animals (Clarke and Clarke, 1975). The chlorinated hydrocarbons or organochlorine compounds as they are called now, such as DDT and lindane, interferes with membrane cation transport, resulting in neural irritability and excitation of the nervous system (Weiss *et al*., 2004). The OP compounds such as parathion and CPF are potentially dangerous. Other insecticidal groups include the carbamates such as carbaryl and adicarb (Clarke and Clarke, 1975).

2.1.3.2 Classes of insecticides

Insecticides have been classified on their chemical constituents into OPs, organochlorines, pyrethroids, carbamates, botanical and new insecticide class.

2.1.3.3 Classification of insecticides based on chemical constituent

Organochlorine insecticides: The chlorinated hydrocarbon insecticides were introduced in the 1940s and 1950s and they include insecticides like DDT, methoxyclor, chlordane, heptachlor, aldrin, dieldrin, toxaphene and lindane (Cope *et al*., 2004). They interfere with cation transport across nerve cell membrane, resulting in neural excitation of the central nervous system (Weiss *et al*., 2004; Goel and Aggarwal, 2007).

They have been extensively used in agriculture and forestry (Ecobichon, 1995), public health and household (Cope *et al*., 2004). The properties of the insecticides, which include low volatility, high chemical stability, lipid solubility, slow rate of biotransformation and degradation (Ecobichon, 1995), were initially considered a desirable attribute. However, these properties later became the basis for public concern (Cope *et al*., 2004) because they lead to increase in the environmental persistence, bioconcentration and biomagnification within various food chains (Ecobichon, 1995; Goel and Aggarwal, 2007) and potentiate their ability to act as a potent central nervous system stimulants, particularly in children (Rabideau, 2001). This eventually led to the ban of DDT and other chlorinated insecticides in many countries in the world (Cope *et al*., 2004). Some countries are, however, permitted to continue using DDT for indoor malaria control (Julvez and Grandjean, 2009).

Organophosphorous insecticides: Organophosphorous pesticides are among the most widely used insecticides for insect control (Cope *et al*., 2004) as they are widely used in homes and gardens. They inhibit the activity of AChE at nerve endings, resulting in excess of the neurotransmitter ACh in the nerve tissue and effector organs. OP insecticides include parathion, malathion, diazinon and CPF (Weiss *et al*., 2004).

Carbamate insecticides: The carbamate insecticides are esters of N-methyl (or occasionally N, N-dimethyl) carbamic acid (H_2NCOOH). The toxicity of the compound varies according to the phenol or alcohol group (Cope *et al*., 2004). Carbamates include carbaryl, aldicarb and maneb. Like the OP insecticides, the mode of action of the carbamates is AChE inhibition, but the inhibition is more readily reversible and of shorter duration than with OP compounds (Cope *et al*., 2004; Weiss *et al*., 2004).

Botanical insecticides: Extracts from plants have been used for centuries to control insects. Nicotine is an alkaloid occurring in a number of plants and was first used as an insecticide in 1763. Pyrethrin is an extract from several types of chrysanthemum, and is one of the oldest insecticides used by humans (Cope *et al*., 2004). Pyrethrins are rapidly metabolized by mammals and are less neurotoxic, but sometimes cause allergic reactions. They are commonly used as ingredients in anti-lice shampoos and as topical treatment for scabies (Weiss *et al*., 2004). They are applied at low doses and are considered to be non-persistent. Synthetic mimics of pyrethrins, known as the pyrethroids, were developed to overcome the lack of persistence of this botanical insecticide (Cope *et al*., 2004).

Pyrethroid insecticides: The lack of persistence by pyrethrins led pesticide chemists to develop compounds of similar structure having insecticidal activity, but being more persistent. The pyrethroids have greater insecticidal activity and are more photostable than the pyrethrins (Shah, 2002). Two broad classes of pyrethroids are known on the basis of their chemical nature; depending on whether the structure contains a cyclopropane ring (also known as Type-II pyrethroids; for example, cypermethrin, deltamethrin) or whether this ring is absent in the molecule (also known as Type-I pyrethroids; for example, permethrin, allethrin) (Shah, 2002; Cope *et al*., 2004). Pyrethroids affect nerve membranes by modifying or distrupting their membrane permeability to sodium and potassium channels, resulting in depolarization of the membranes (Weiss *et al*., 2004). The specific interaction of pyrethroids and the sodium channel slows down both the activation and inactivation properties of the channel, leading to a hyper excitable state (Bjørling-Poulsen *et al*., 2008).

New insecticide class: There are new classes of insecticides, which when applied at low dosages are extremely effective and are relatively non-toxic to humans. One such class is the fiproles, such as fipronil. Although it is used on corn, it is becoming a popular termiticide because of its low application rate and long-term effectiveness. Another class is the chloronicotinoids, represented by imidacloprid, which is also applied at low dose rate to soil and effectively control a number of insect species (Cope *et al*., 2004).

2.1.4 Classification of Pesticides Based on Target Species

2.1.4.1 Herbicides or Weedicides

They are agents which are used to destroy undesirable plants/weeds (Goel and Aggarwal, 2007). Examples include dinitro compounds, phenoxyacetic acid (2, 4-D, 2, 4, 5-T), chloroalipathic acid (dalapon), triazenes (atrazines, simazine), bipyridinum compounds (paraquat, diquat), substituted ureas (monouron, diuron, isoproturon) and substituted dinitroaniline compound (pendimethalin) (Shah and Srivastava, 2002). This class of pesticides is applied to crops for the purpose of eliminating or reducing weed population. Strategies used in their application include preplant incorporation and pre- and post-emergent applications (Cope *et al*., 2004). They may primarily cause irritation to the skin and respiratory tract during acute exposure. They all have different mechanisms of action. Some substances, such as paraquat, are highly corrosive and can cause multisystem injury and progressive pulmonary failure (Weiss *et al*., 2004).

2.1.4.2 Fungicides

Recent studies have shown that toxins and other air-borne organic compounds released from fungi inhabiting the interior of dwellings are probably responsible for a number of adverse health effects. Compound produced to combat these losses and adverse health effects are called fungicides (Cope *et al*., 2004). They include copper compound, sulphur preparations, inorganic and organic derivatives of mercury and miscellaneous compounds (Clarke and Clarke, 1975). All are effective fungicides and are used on a variety of crops, including grapes, sugar beets, and ornamental plants. One family of fungicide that is of concern is the dithiocarbamates, sulfur derivatives of dithiocarbamic acid, which include the metallic dimethyldithiocarbamates. Although relatively non-toxic, but when hydrolyzed they are known to produce known carcinogen such as ethylthiourea (Cope *et al*., 2004).

2.1.4.3 Rodenticides

Rodenticides are agents which are used to destroy the rodent pests. They include warfarin, zinc phosphide, fluoroacetate and thallium sulphate (Shah, 2002; Goel and Aggarwal, 2007). Most of the rodenticides are classified as restricted use and are applied only by licensed pest control operators (Cope *et al*., 2004). Human poisoning associated with rodenticides usually results from accidental or suicidal ingestion of the compound (Shah, 2002; Cope *et al*., 2004). Animal poisoning from rodenticides results from the ingestion of residues of the rodenticides or baits intended for target rodents, and from malicious poisoning, particularly to kill dogs and cats (Shah, 2002).

2.1.4.4 Acaricides

They are compounds used for the control of mites, ticks and lice. Acaricides can be grouped into two categories, those for agricultural use and those intended for veterinary use. Both are applied by external and internal applications. Organochlorines and OPs are among the pesticides used as acaricides. Others used include benzylbenzoate and monosulfram (Clarke and Clarke, 1975).

2.1.4.5 Fumigants

Fumigants are extremely toxic gases used to protect stored products, especially grains, and to kill soil nematodes (Cope *et al.,* 2004). They are applied to storage warehouses, freight cars, and houses infested with insects. They constitute a special hazard due to inhalation exposure and rapid diffusion into pulmonary blood; thus, extreme care must be taken when handling and applying this class of pesticides (Cope *et al*., 2004). The mechanisms of toxicity employed by various fumigants are poorly unknown. Among the metal phosphide fumigants, aluminium phosphide is one of the most extensively used. The phosphides are very toxic, because of their ability to liberate phosphine gas under moist condition (Bjørling-Poulsen *et al*., 2008). Another effective fumigant is methyl bromide that sterilizes soil when applied under a ground covering, it kills insects, nematodes and weed seeds, and it is also used to fumigate warehouses (Cope *et al*., 2004). Chloropicrin (trichloronitromethane) is another soil/space fumigant that has been used for many years (Cope *et al.*, 2004).

2.2 ORGANOPHOSPHATES

Organophosphates are chemicals which inhibit AChE and are employed widely as pesticides in residential setting and in agricultural practices (Saulbury *et al.*, 2009). OP pesticides are the major chemical class of insecticides used in the world today (Tuzmen *et al.*, 2008). They are usually esters, amides, or thiol derivatives of phosphoric, phosphonic, or phosphinic acids which have the general structural formula shown in Figures 2.1 and 2.2.

R_1

\

O (or S)

"

P ---X

/

R^2

Figure 2.1. General chemical structure of an organophosphate. R1 and R2 are alkyl-, alkoxy-, alkylthio-, or amido-groups. X is the acyl residue (labile fluorine-, cyano-, substituted- or branched aliphatic, aromatic, or heterocyclic groups). (Adapted from Balali-Mood and Balali-Mood, 2008).

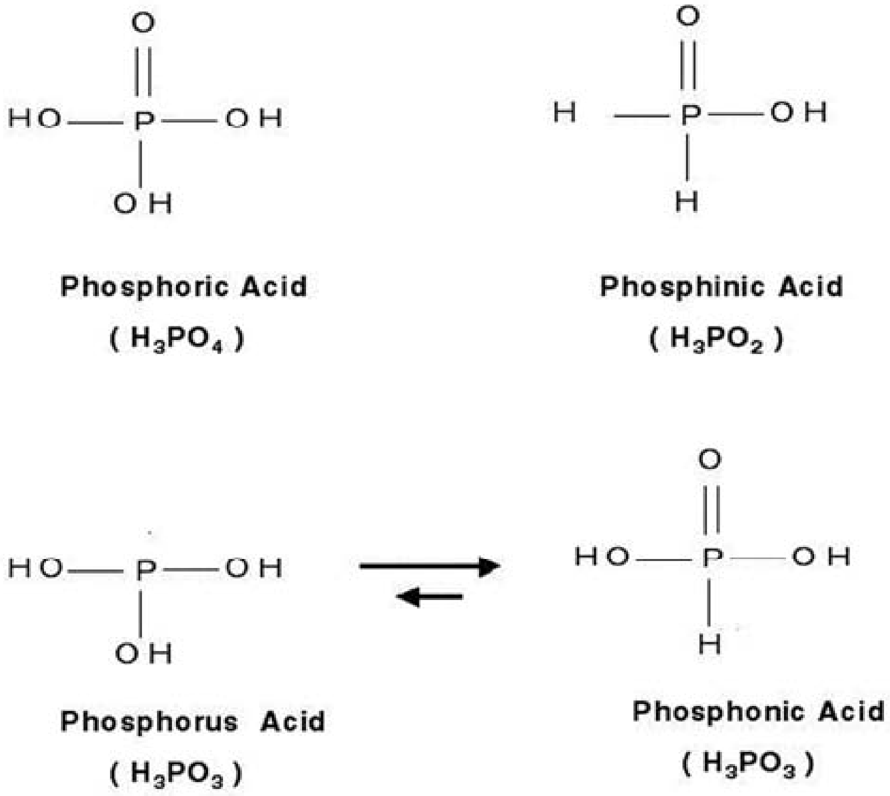


Figure 2.2 Chemical structures of phosphoric acid, phosphinic acid, phosphorus acid and phosphonic acid (Adapted from Abou-Donia, 2003).

2.2.1 Historical Perspectives

Organophosphorus compounds were known to exist since 1800s, when Lassaigne synthesized OP compounds. However, the earliest recorded description of their synthesis was by deClermont in 1854 (Singh and Khurana, 2009). An important step in the synthesis of OP compounds was made by Von Hofman, who synthesized methyl phosphorchloride in 1873 (Balali-Mood and Balali-Mood, 2008). Subsequently, Michelis in Germany and Arbusov in Russia described synthesis of a large number of OP compounds in early 1900s. However, the toxic effects of OP compounds were not recognized until 1932, when Lange and Von Kruger described the toxic effect of OP vapours (Balali-Mood and Balali-Mood, 2008; Singh and Khurana, 2009). They noted that the inhalation of these compounds, dimethyl and diethylphosphoroflumdate caused a persistent choking sensation and blurring of vision (Karalliedde and Senanayake, 1989). These observations led Schrader of I.G Farben Industries to develop OP compounds, first as agricultural pesticide, and later a potential chemical warfare agent. Consequently, during World War II, several highly toxic compounds were developed as nerve gases, and they include ethyl N-dimethylphosphoroamidocyanate (Tabun), isopropylmethylphosphoroflouridate (Sarin) and Pinacolylmethylphosphoroflouridate (Soman) (Karalliedde and Senanayake, 1989; Ecobichon, 1995; Abou-Donia, 2003; Wiener and Hoffmann, 2004). The allied countries in Europe also followed Lange and Krueger's lead and developed compound such as dissopropyl phosphoroflouridate (DFP) (Karalliedde and Senanayake, 1989). In 1944, Schrader synthesized parathion (0, 0- diethyl 0-p-nitrophenylphosphorothionate) and its oxygen analogue paraoxon (0, 0-diethyl-0-p-nitrophenylphosphate) (Karalliedde and Senanayake, 1989; Ecobichon, 1995). Although these two chemicals had the properties desired in an insecticide (low volatility, chemical stability in sunlight and in the presence of water, environmental persistence for efficacy), they both exhibited marked mammalian toxicity and were unselective with respect to target and non-target species (Ecobichon, 1995). Over the past five decades, numerous structurally different OPs have been synthesized for use as insecticides and pesticides world wide (Balali-Mood and Balali-Mood, 2008; Singh and Khurana, 2009).

2.2.2 Uses of Organophosphates

Organophosphates are widely used as insecticides globally and they are readily available commercially for domestic and industrial purposes. In addition, OPs are also utilized as therapeutic agents in the treatment of ophthalmic conditions (e.g., echothiophate, isofluorophate), as antihelminthics (e.g., trichlorfon), and agricultural pesticides as well as nerve agents in chemical warfare (e.g., sarin, soman) for military (Aardema *et al*., 2008; Leibson and Lifshitz, 2008) and terrorism purposes (Ali and Chia, 2003; Aardema *et al*., 2008; Balali-Mood and Balali-Mood, 2008; Pohanka *et al*., 2008).

2.2.3 Properties of Organophosphates

The biological action of OPs is related to their phosphorylating abilities. This is dependent on the electrophilicity (positive character) of the phosphorus atom, which is determined by its substituent groups. Steric factors of substituents also play a major role in determining the biological activity of these chemicals (Abou-Donia, 2003). Lipid solubility is important because it enhances the ability of the compounds to cross biological membranes and the blood-brain barrier, leading to increased biological activity (Vale, 1998; Abou-Donia, 2003). OPs do not persist in the environment as they break down quickly than the organochlorines (Fishel, 2005).

2.2.4 Toxicokinetic Aspects of Organophospates

2.2.4.1 Absorption

Majority of OP insecticides are lipophilic and not ionized, hence they are absorbed rapidly following inhalation or ingestion. Although dermal absorption of OP compounds tends to be slow, severe poisoning may ensue if exposure is prolonged. The degree of absorption depends on the contact time with the skin, the lipophilicity of the agent involved and the presence of solvents; for example, xylene and emulsifiers in formulations can facilitate absorption (Vale, 1998). Other important factors include the volatility of the pesticide, the permeability of the clothing, the extent of coverage of the body surface and personal hygiene (Vale, 1998).

2.2.4.2 Distribution and Storage

Following absorption, OP compounds accumulate rapidly in fat, liver, kidneys and salivary glands. The phosphorothioates (P=S), for example, diazinon, chlorpyrifos and parathion, are more lipophilic than the phosphates (P=O), such as dichlorvos. They are, therefore, stored extensively in fat, which may account for the prolonged intoxication (Vale, 1998; Balali-Mood and Balali-Mood, 2008).

2.2.4.3 Biotransformation

Phosphates (P=O) are biologically active as AChE inhibitor, whereas phosphorothioates (P=S) need bioactivation to their phosphate anologues (oxon) to become biologically active (Vale, 1998). As a consequence, the features of intoxication after exposure to phosphorothioates (P=S) are delayed unless aerial oxidation has occurred already to generate traces of oxon (Vale, 1998; Bjørling-Poulsen *et al.*, 2008).

OP compounds other than phosphates (P=O) are metabolically activated to their corresponding oxon by oxidative desulphuration, mediated by P_{450} isoforms (Ecobichon, 1995), flavin-containing mono-oxygenase enzymes, N-oxidation and S-oxidation (Vale, 1998). OP compounds also undergo other transformations mediated by cytochrome P_{450} that do not result in the production of an active metabolite, including oxidative dealkylation and dearylation, ring hydroxylation, thioether oxidation, deamination, alkyl and N-hydroxylation, N-oxide formation and N-dealkylation (Ecobichon, 1998; Balali-Mood and Balali-Mood, 2008).

2.2.4.4 Elimination

Elimination of metabolites occurs mostly in urine with lesser amounts in faeces and expired air. Some OP compounds such as dichlorvos which is not stored in fat to any great extent, may be eliminated in hours, whereas the inhibitory oxon of CPF may persist for days because of their extensive storage in fat (Vale, 1998; Balali-Mood and Balali-Mood, 2008).

2.2.5 Sources of Exposure to Organophosphorus Insecticides

Pesticide exposure is a global public health issue. OP exposure is a major public health issue in terms of health, morbidity, health care, and general safety from toxicity (Jaga and Dharmani, 2003). The World Health Organization has estimated that 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (Yang and Deng, 2007; Chheteri *et al*., 2008). OP exposure occurs among people who come into contact with the chemicals as a result of their occupation, and the general population is exposed by the spread of the chemicals into the environment (Jaga and Dharmani, 2003). Therefore, a simple classification of exposure to OP is; a) occupational exposure; and b) environmental or non-occupational exposure. Unintentional, accidental or suicidal poisoning can occur in both occupational and non-occupational exposure to OP (Jaga and Dharmani, 2003).

2.2.5.1 Occupational Exposure

Workers are exposed to pesticides in their work places as a result of their occupation. Irrespective of whether the job involves pesticide use, the presence of the chemical in the work environment constitutes an occupational exposure (Jaga and Dharmani, 2003). Most occupational exposure to OP pesticides are through inhalation or dermal exposure, and in some instances by ocular exposure (Vale, 1998; Jaga and Dharmani, 2003). Situations of occupational exposure are common among agricultural workers and pesticide applicators (Warren *et al*., 1985; Eskenazi, 1999; Ray and Richards, 2001; Jaga and Dharmani, 2003; Ali and Chia, 2008).

Exposure can come from mixing, loading, spray application, harvesting sprayed crops or transporting the chemicals (Ecobichon, 1995; Jaga and Dharmani, 2003; Fenske and Day, 2005; Rastogi *et al.*, 2009). In some situations, exposure can occur from accidental spills of the chemicals, leakage or faulty equipment (Jaga and Dharmani, 2003; Fenske and Day, 2005; Rastogi *et al*., 2009).

Industrial exposure to OP occurs in chemical plants that produce OP pesticides. Workers in these plants face risk situations that are similar to the ones that occur in workers exposed to pesticides on farm. Exposure is primarily from the packaging of chemicals in the hopper/bagging operation (Jaga and Dharmani, 2003; Fenske and Day, 2005).

Other occupational exposures occur in exterminators who use pesticides, green-house workers and florists, office workers, health-care worker exposure (nosocomial poisoning), veterinary exposure, autopsy of exposed patients, exposure in a retail store (Ecobichon, 1995; Jaga and Dharmani, 2003), and Gulf war veterans (Ali and Chia, 2008).

2.2.5.2 Environmental or Non-occupational Exposure

Environmental or non-occupational exposure to OPs occurs in any place where the exposure is not a result of the person's job or occupation (Jaga and Dharmani, 2003). There are several situations where exposure to OPs occurs other than work place and they include:

Residential exposure - OP insecticides are used for killing bugs in and around the house and in the garden. Accidental poisoning with OP in the home is a possibility from house or garden use of the pesticide. Exposure is likely to occur from spills, improper use, or poor storage (Jaga and Dharmani, 2003; Fenske and Day, 2005). OPs also settle on various objects and items in a home, thereby increasing the risk of exposure (Ecobichon, 1995; Eskenazi, 1999). Children constitute extremely vulnerable high-risk group for such exposure (Eskenazi, 1999; Jaga and Dharmani, 2003).

Para-occupational exposure - Agricultural workers who are exposed to pesticides in their work can also expose persons at home, if they return home with contaminated clothing and then have contact with their family members and house-hold items (Eskenazi, 1999; Jaga and Dharmani, 2003). This is known as para-occupational exposure (Jaga and Dharmani, 2003).

Environmental exposure may result from aerial spraying, exposure in public places, exposed organ donor (Jaga and Dharmani, 2003), suicidal (intentional) poisoning (Vale, 1998; Jaga and Dharmani, 2003; Nand *et al*., 2007; Ather *et al*., 2008) and chemical warfare (Jaga and Dharmani, 2003; Ali and Chia, 2008; Balali-Mood and Balali-Mood, 2008).

2.2.6 Mechanisms of Action of Organophosphates

Organophosphates exert their toxicity by inhibiting AChE in the nervous system. This enzyme catalyses the hydrolysis of AChE, a major neurotransmitter in the central and peripheral nervous system into choline and acetic acid (Ecobichon, 1995; Ali and Chia, 2008). Even though all OP compounds have a common mechanism of action, their effectiveness as inhibitors of AChE varies widely (Ecobichon, 1995; Balali-Mood and Balali-Mood, 2008). OP compounds can be classified as direct or indirect inhibitors of AChE (Srivastava and Dumka, 2002; Balali-Mood and Balali-Mood, 2008). Direct inhibitors are effective without any further metabolic modification after absorption into the body. Indirect inhibitors need to be transformed in the body to be effective. The practical importance of this classification is that direct inhibitors cause systems and signs quickly during or after exposure, whereas in the case of indirect inhibitors, symptoms and signs appear later and the effect last longer after ceasation of exposure (Balali-Mood and Balali-Mood, 2008).

When AChE is inactivated by phosphorylation of the serine hydroxyl group at its active site (Ather *et al*., 2008), ACh released from nerve terminals accumulates and overstimulate the muscarinic and nicotinic receptors (Aiub *et al*., 2002; Abou-Donia, 2003; Fishel, 2005; Ali and Chia, 2008). The phosphoric or phosphonic acid ester formed with the enzyme is extremely stable and is hydrolyzed very slowly. If the phosphorylated enzyme contains methyl or ethyl groups, the enzyme is regenerated in several hours of hydrolysis. On the other hand, virtually no hydrolysis occurs with an isopropyl group and

the return of AChE is dependent upon synthesis of a new enzyme (Abou-Donia, 2003). Phosphorylated AChE undergoes ageing, a process that involves the loss of an alkyl group resulting in a negatively charged monoalkyl enzyme (Ecobichon, 1995; Abou-Donia, 2003; Winrow *et al*., 2003; Leibson and Lifshitz, 2008). The rate of ageing depends upon the structure of the OP compound, factors like pH and temperature (Paudyal, 2008). Ageing of the phosphorylated enzyme leads to an inactive enzyme, after which reactivation is no longer possible. In this case, resynthesis of new enzyme in the liver is required, if AChE activity is to return normal (Vale, 1998).

2.2.7 Clinical Signs of Organophosphate Poisoning

2.2.7.1 Acute Organophosphate Poisoning

Acute organophosphate insecticide poisoning can manifest in three different phases namely; acute cholinergic crisis, intermediate syndrome (IMS) and delayed polyneuropathy (Ecobichon, 1995; Jaga and Dharmani, 2003; Yang and Deng, 2007). These three phases can occur individually in each case or occur sequentially in a poisoning episode (Nand *et al.*, 2007).

2.2.7.2 Acute Cholinergic Crisis

Acute cholinergic crisis develops within a few minutes to several hours after exposure, and cholinergic receptors in both the peripheral (muscarinic and nicotinic) and central nervous systems, through the inhibition of carboxylic esterase enzymes of which acetylcholinesterase is the most important (Yang and Deng, 2007). Typical manifestations of cholinergic crisis include those arising from stimulation of the muscarinic receptors of the parasympathetic nervous system resulting in salivation, lacrimation, bronchorrhoea, sweating, bronchoconstriction, miosis, nausea, vomiting, diarrhoea, gastrointestinal cramps, urination and bradycardia (Ecobichon, 1995; Rubin *et al.*, 2002; Yang and Deng, 2007; Balali-Mood and Balali-Mood, 2008). Nicotinic signs resulting from the initial stimulation and subsequent blockade of nicotinic receptors, including the ganglia of the sympathetic and parasympathetic division of the autonomic nervous system, the junctions between the nerves and muscles result in tachycardia, hypertension, muscle fasiculations, tremors, muscle weakness and/or flaccid paralysis (Ecobichon, 1995; Miranda *et al.*, 2004; Yang and Deng, 2007). Central nervous system symptoms are characterized by dizziness, headache, confusion, seizures, loss of memory, convulsion, coma and respiratory failure (Ecobichon, 1995; Jaga and Dharmani, 2003; Miranda *et al.*, 2004; Bjørling-Poulsen *et al.,* 2008).

2.2.7.3 The Intermediate Syndrome

The intermediate syndrome (IMS) is a paralytic condition (Ecobichon, 1995). The term was first coined by Senanayake and Karalliedde (1987), but was first described in 1975 by Wadia because it arose in the interval between the end of acute cholinergic crisis, and the onset of organophosphate-induced delayed neurotoxicity (OPIDN) (Rees, 1996; Yang and Deng, 2007). It consists of a sequence of neurological signs that appear between 24 and 96 hours after the acute cholinergic crisis, and its symptoms have been reported to last up to 18 days (Ecobichon, 1995; Miranda *et al.,* 2004). The pathogenesis of IMS has not been well characterized, but is suspected to involve a combination of pre- and post-synaptic dysfunction of neuromuscular transmission as a result of prolonged AChE inhibition (Miranda, 2003; Miranda *et al*., 2004; Yang and Deng, 2007). Other proposed mechanisms include myonecrosis mediated by calcium mobilization in the muscle fibres (Eyer, 1995), oxidative cellular damage to muscle membranes (Paudyal, 2008). An association between increased blood muscle enzymes and the development of IMS has been documented (Yang and Deng, 2007). It is primarily independent on the type of OP (Miranda, 2003). IMS is characterized by weakness of the proximal muscles, facial muscle, neck flexors, motor cranial nerves, and acute respiratory muscle paralysis (Ecobichon, 1995; Jaga Dharmani, 2003; Miranda *et al.,* 2004; Balali-Mood and Balali-Mood, 2008).

2.2.7.4 Organophosphate-induced Delayed Polyneuropathy

Organophosphate-induced delayed polyneuropathy (OPIDPN) also known as organophosphate-related delayed neurotoxic effect (Moretto and Lotti, 1998) or organophosphate-induced delayed neurotoxicity (OPIDN) (Yang and Deng, 2007). OPIDPN is a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia, and upper motor neurone spasticity as a result of single or repeated exposure to OP esters (Abou-Donia, 2003). It is caused by some phosphate, phosphonate, and phosphoramidate esters, only a few of which has been used as insecticides. Historically, this syndrome has been known for almost 100 years and has been associated with the chemical tri-o-tolylphosphate (Ecobichon, 1995). The neuropathological lesion is a central-peripheral distal axonopathy caused by a chemical transection of the axon (known as Wallerian-type degeneration), followed by myelin degeneration of the distal portions of the long and large-diameter tracts of the central nervous system and peripheral nervous systems.

Organophosphate-induced delayed polyneuropathy has been divided into 3 classes; Type I is caused by the pentavalent phosphates and phosphonates, as well as their sulphur analogues; Type II is produced by the trivalent phosphites; while type III is induced by phosphines. All three OPIDPN types are produced by OP compounds and are characterized by central-peripheral distal axonopathy (Abou-Donia, 2003). The OPIDPN occurs 2-3 weeks after acute exposure to certain insecticides. The clinical features are predominantly initial flaccidity, characterized by muscle weakness of the lower extremities, followed by progressive ascending weakness of limb muscles, hyperflexia, clonus and abnormal reflexes indicative of damage to the pyramidal tracts and a permanent upper motor neurone syndrome (Ecobichon, 1995; Kart, 2005; Yang and Deng, 2007; Paudyal, 2008). In addition to the damage to the peripheral nerves (Winrow *et al*., 2003), OPIDPN may also involve damage to the spinal cord (Davis *et al*., 1999), medulla oblongata. The symptoms may include foot drop, spasticity and hyperactive reflexes (Ecobichon, 1995; Miranda *et al*., 2004). A number of OP insecticides including dimethoate, trichloronate, trichlorfon, parathion, methamidophos, fenthion, and chlorpyrifos have been implicated in causing OPIDPN in humans (Ecobichon, 1995; Miranda *et al*., 2004; Nand *et al*., 2007) and animals (Ecobichon, 1995).

2.2.7.5 Organophosphorus Ester-induced Chronic Neurotoxicity

This disorder has been variously referred to as "chronic neurobehavioural effects" (Yokoyama *et al*., 1998), "chronic organophosphate-induced neuropsychiatric disorder (COPIND)" (Jamal, 1997; Jamal *et al*., 2002), "psychiatric sequelae of chronic exposure" (Gershon and Shaw, 1961), "psychological and neuropsychological alterations" (Metcalf and Holmes, 1969), "long-term effects" (Duffy *et al*., 1979), "neuropsychological abnormalities" (Pilkington *et al*., 2001), "neuropsychological effects of long-term exposure" (Stephen *et al*., 1995), "delayed neurological behavioural effects of subtoxic doses" (Scremin *et al*., 2003). However, Abou-Donia (2003) synthesized all the terminologies and named it organophorus ester-induced chronic neurotoxicity (OPICN). OPICN is produced by exposure to large, acutely toxic- or small subclinical doses of OP compounds. It could result from both long-term exposures to subclinical doses of OP and also after acute poisoning (Canâdas *et al*., 2005). Clinical signs which continue for a prolonged time, ranging from weeks to years after exposures, consist of neurological and behavioural abnormalities. Damage is present in both the peripheral and central nervous systems, with greater involvement in the latter (Abou-Donia, 2003). Neurological and neurobehavioural alterations are exacerbated by concurrent exposure to stress or other chemicals that cause neuronal cell death or oxidative stress. Since central nervous system injury predominates, improvement is slow and complete recovery is unlikely (Abou-Donia, 2003). Studies on the effects of exposure to OP compounds over the past half century have shown that chronic neurological and behavioural symptoms include headache, drowsiness, dizziness, emotional anxiety, apathy, mental confusion, restlessness, insomnia, lethargy, depression, irritability, generalized weakness and tremors (Gershon and Shaw, 1961; Dille and Smith, 1964). Respiratory, circulatory, and skin problems may be present, especially in chronic toxicity (Abou-Donia, 2003).

Recent studies have shown that large toxic doses of OP compounds cause early convulsive seizures and subsequent encephalopathy leading to the necrotic death of brain neuronal cells, whereas small doses produce delayed apoptotic death. Five phases that result in OP compound-induced cholinergic seizures have been proposed by Pazdernik *et al.* (2001); they include 1) initiation 2) limbic *status epilepticus*; 3) motor convulsion, 4) early excitotoxic damage, and 5) delayed oxidative stress. The occurrence and severity of OPICN is influenced by factors such as environmental exposure to other chemicals, stress and individual genetic differences (Abou-Donia, 2003). The exact mechanism for OPICN is not known, but several possible postulations have been put forward to explain chronic toxicity (Jamal *et al*., 2002).

2.2.8 Health Effects of Organophosphates

There are several reports on the adverse health impacts of low-level exposure to OPs (Peeples *et al*., 2005). There are toxicological evidences that repeated low-level exposure to OP may affect neurodevelopment in animals and humans (Gbaruko *et al*., 2009). Possible mechanism for these effects include inhibition of brain cholinesterase, down-regulation of muscarinic receptors, decreased brain DNA synthesis and reduced brain weight (Eskenazi, 1999). Neurological and neurobehavioural effects have been described in studies investigating chronic exposure to OPs in farmers, pesticide applicators (Wesseling *et al*., 2002; Jamil *et al*., 2007) and animals (Grue *et al*., 1997; Ambali *et al*., 2010f). The neurological effect include increased prevalence of self-reported symptoms such as sleep disturbance, fatigue, dizziness, loss of strength in the extremities, decreased sensory nerve function, decreased motor function and symptoms of Parkinsonism

(Jamil *et al.*, 2007). Neurobehavioural effects resulting from an acute episode or long-term exposure include increased depressive disorders and anxiety (Peeples *et al.*, 2005). Deficit in cognitive function, memory disturbance, vigilance, poor concentration and confusion were also observed in workers with varying levels of exposure (Weiss *et al.*, 2004; Peeples *et al.,* 2005). Effects on animals include vocalization, tremors and incoordination. Behavioural dysfunctions have been documented; and they include impact to learning in mammals, birds and fish, hyperactivity, behavioural "slump" and lethargy. Others include disruption of feeding, impacts to vision, learning and memory (Parsons *et al*., 2006; Jamil *et al*., 2007).

Genetoxic effects of exposure to OP compounds include increased aneuploidy in sperm genetic material, increased chromosomal aberrations and fragile sites in lymphocytes. Cytogenetic effects have been observed in workers with low exposure to OP (Jamil *et al*., 2007; Peiris-john and Wickremasinone, 2008). Studies on mammals, amphibians and fish showed DNA strand breakages (Parsons *et al.,* 2006).

Immunotoxicity or immune function impairment has been reported in agricultural workers and pesticide applicators. Decrease in immune system markers, changes in T-cell ratios and neutrophil dysfunction indicating humoral and cellular dysfunctions have been documented. Evidence of elevated autoantibodies suggests possible autoimmune effect in humans (Parsons *et al*., 2006; Li, 2007). Others include pathology of immune organs and decreased humoral and/or cell-mediated immunity. Altered non-specific immunity, decrease host resistance, hypersensitivity and autoimmunity are also features of immunotoxicity in OP poisoning (Galloway and Handy, 2003).

Some case studies have associated parental exposure to OP and OP use in the homes with some health conditions such as childhood brain tumour, leukaemia, lymphoma, testicular cancers and other cancers (Gbaruko *et al*., 2009). Increased risk for leukaemia has been reported in both adult and children after exposure to OPs. Increases in breast tissue lesions that may act as biomarkers for breast cancer were found in women who are green house workers, exposed to OPs (Parsons *et al*., 2006).

Some OPs have been implicated in the adverse reproductive outcomes in both male and females, changes in levels of hormone such as adrenocorticotrophic and follicle stimulating hormones and testosterone; impaired semen quality and concentration and increased risk of spontaneous abortion (Parsons *et al*., 2006; Peiris-john and Wickremasinghe, 2008). Parental exposure to pesticides or application of pesticides in the home is associated with certain birth defect, including neural tube defects (Eskenazi, 1999) and foetal death (Parsons *et al*., 2006). Altered birth parameters such as low birth weight and birth length have been reported in humans and animals exposed to OPs (Eskenazi, 1999; Parsons *et al*., 2006; Ambali *et al*., 2009).

Hyperthermia is a common effect in humans exposed to OP (Aaredema *et al*., 2008). With lower dose exposures, the interaction of OP with thermoregulatory system functions may affect the ability of the affected individuals to dissipate heat while working or exercising (Parsons *et al*., 2006). Impact on thermoregulation is one of the most important outcomes of OP exposure in homoiothermic mammals and birds (Parsons *et al*., 2006).

Hypothernia may reduce metabolic rate and, therefore, reduce the activation of toxic compounds and metabolites (Grue *et al.*, 1997; Parsons *et al.*, 2006). Extrapolation from animal studies suggests that a lowering of body temperature can be observed following acute exposure to high doses of OP (Hantson *et al.*, 1996). Other health effects include decreased pulmonary function, increased incidence of asthma, allergic dermatitis or erythema have been reported among workers with high and frequent exposure to OPs (Parsons *et al.*, 2006).

2.3 CHLORPYRIFOS

Chlorpyrifos *(0, 0-diethyl-0-[3, 5, 6-trichloro-2-pyridyl*]) phosphorothiate, is a broad spectrum chlorinated OP insecticide, utilized extensively in agriculture and residential pest control throughout the world (Cox, 1995; Gotoh *et al.*, 2001; Mitra *et al.*, 2008; Mehta *et al.*, 2009). CPF which was first manufactured by Dow Elanco Company in USA was introduced into American market in 1965 (Cox, 1994).

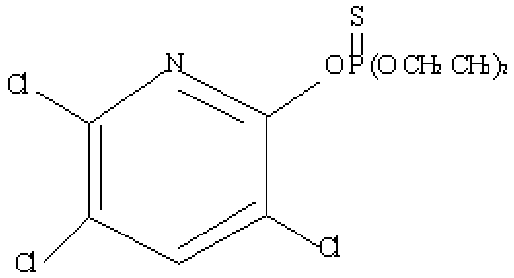


Figure 2.3: Chemical structure of chlorpyrifos (Adapted from Eaton *et al.*, 2008)

2.3.1 Uses of Chlorpyrifos

It is used in both agricultural and non-agricultural purposes throughout the world (Verma *et al*., 2007). In the homes, it is applied to destroy cockroaches, fleas, spiders and flies indoors as well as mosquitoes, ticks and bees outdoors (Mitra *et al*., 2008).

On the farm, CPF is occasionally used for the control of ticks on cattle, but its major use is in crop protection (Eaton *et al*., 2009). Other uses are on fire ant mounds, as a mosquito adulticide, on golf courses, shipholds, boxcars, industrial plants, and processed wood products. Until 2000, CPF was commonly used as residential pesticide. The U.S. Environmental Protection Agency cancelled most home, lawn and garden use based on human health risk (Iyer *et al*., 2008).

2.3.2 Properties of Chlorpyrifos

Technical CPF is a white crystalline solid with a melting point of 41.5-42.5°C. It is stable in neutral and acidic aqueous solutions; however, stability decreases with increasing pH. CPF is practically insoluble in water, but is soluble in most organic solvents (Smegal, 2000; Iyer *et al*., 2008). The physico-chemical properties of CPF are shown in Table 2.1;

Table 2.1: The Physical and Chemical Properties of Chlorpyrifos

Chemical formula	$C_9H_{11}Cl_3NO_3PS$
Molecular weight	350.57
Melting point	41-42°C

Boiling point	Decomposes at about 160˚C
Density at 43.5˚C	1.398 g/cm^3
Water solubility at 20˚C	0.7 mg/L
Water solubility at 25˚C	2.0 mg/L
Organic solvent solubility	79% w/w in isooctane 43% w/w in methanol Readily in others
Partition coefficients	Log K_{ow}4.82 Log K_{oc}3.73
Vapour pressure at 20˚C and 25˚C	1.87 x 10^{-5} mm Hg
Henry's law constant at 25C	1.23 x 10^{-5} atm-m^3/mol
Conversion factor at 25˚C	1ppm = 14.3 mg/ m^3 1 mg/m^3 = 0.07 ppm

Table 2. 1 (Adapted from Eaton *et al*., 2008).

2.3.3 Environmental Distribution and Fate

Following application to crops, CPF quickly binds to soil and plants. It typically degrades rapidly in the environment, but its residual level can last for long period of time (Eaton *et al*., 2008). CPF persistence in soil varies depending on the soil type and environmental conditions. The typical aerobic soil metabolism half-life ($t^{1/2}$) ranges from 11 to 180 days, with a mean of 28.7 days. Much longer soil half-lives of 175 to 1576 days have been reported for termiticide application rate. The soil/water partition coefficient (K_{oc}) values range from 360 to 31000, indicating that it is not very mobile in soil (Smegal, 2000). It undergoes a number of degradation pathways while in the environment. However in indoor environment, CPF can persist for several months because of relative lack of sunlight, water, and/or soil microorganisms that contribute to its rapid degradation in the outdoor environment (Eaton *et al*., 2008).

2.3.4 Toxicokinetics of chlorpyrifos

2.3.4.1 Absorption

Absorption of CPF varies with species. It is rapidly absorbed and transported to the brain with oral dosing (Iyer *et al*., 2008). CPF appears to be relatively well absorbed from the intestine (Eaton *et al*., 2008; Mitra *et al*., 2008). In humans, about 70% was absorbed after oral exposure of volunteers (Iyer *et al.,* 2008). Studies have shown that formulation has a significant influence on dermal penetration rate, at least *in vitro*. It is generally assumed to be well absorbed through the lungs following air-borne exposure (Eaton *et al*., 2008).

2.3.4.2 Distribution

The highest tissue concentrations were found in the liver and kidneys, but CPF does not bioconcentrate in these tissues (Iyer *et al*., 2008). The highest concentration of CPF has been found in the fat and fatty tissues. It is known to bind to various proteins, especially albumin and it also readily passes through the placenta (Eaton *et al.*, 2008).

2.3.4.3 Metabolism of Chlorpyrifos

CPF is bioactivated to CPF-oxon in the liver through cytochrome P_{450} mixed function oxidase-mediated desulphuration (Timchalk *et al.*, 2002; Kousba *et al.*, 2004). However, extrahepatic metabolism has been reported in other tissues, including the brain (Timchalk *et al.*, 2002). In addition, oxidative dearylation of CPF by both A- and B- esterases leads to the formation of 3, 5, 6-trichloro-2-pyridinol (TCP), which is the major biological metabolite and environmental break-down product of CPF, and diethylphosphate (DEP) (Sams *et al.*, 2004; Iyer *et al.*, 2008; Tripathi and Srivastav, 2010) (Figure 2.4). TCP undergoes phase II sulphation and glucoronidation (Timchalk *et al.*, 2007). A-esterase (e.g., oxonase and paraoxonase) and carboxylesterase play an important role in the detoxification of CPF (Poet *et al.*, 2003; Iyer *et al.*, 2008). A-esterase can effectively metabolize CPF-oxon to TCP and DEP without inactivating the enzyme. B-esterase such as carboxylesterase, and butyrylcholinesterase (BUChE) can likewise detoxify CPF-oxon. However, they become irreversibly bound to the CPF-oxon and thereby become inactivated (Timchalk *et al.*, 2002). Detoxification of CPF is not limited to carboxylesterase or A-esterase; bovine serum albumin has been shown to prohibit the effects of CPF on DNA synthesis *in vitro* (Iyer *et al.*, 2008).

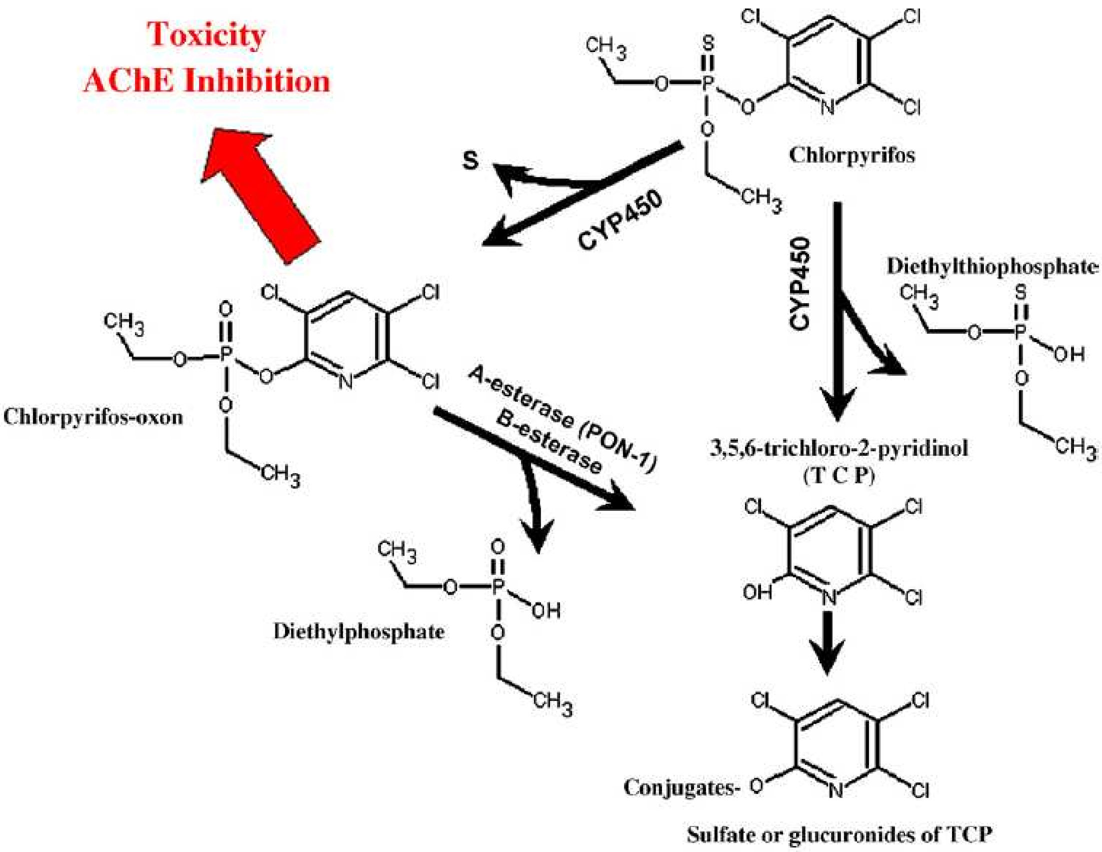


Figure 2.4: Metabolic Scheme for Chlorpyrifos (Adapted from Timchalk *et al*., 2007).

2.3.4.4 Excretion

Chlorpyrifos is excreted primarily through the kidneys in the urine (Iyer *et al.,* 2008; Tripathi and Srivastav, 2010). The mean pharmacokinetic half-life for 3, 5, 6-TCP in the urine was approximately 27 hours following both oral and dermal exposure (Smegal, 2000).

2.3.5 Mechanism of Toxicity of Chlorpyrifos

Chlorpyrifos is directly toxic to the nervous system (Cox, 1994; Okechukwu *et al*., 2007). Like all OPs, CPF kills insects and other animals, including humans because of its toxicity to the nervous system (Cox, 1994). CPF elicits toxicity by inhibiting an enzyme, AChE (that breaks down acetylcholine) in the central and peripheral nervous systems, allowing accumulation of ACh resulting in excessive stimulation of postsynaptic receptors and consequent signs of neurotoxicity (Cox, 1994; Zheng *et al*., 2000; Timchalk *et al*., 2002; Albers *et al*., 2005; Al-Badrany and Mohammad, 2007; Mehta *et al*., 2009). However, phosphorothionates, such as CPF do not directly inhibit AChE, but is first metabolized to the corresponding oxygen analogue (CPF-oxon), which is a more potent inhibitor of AChE. The activation of CPF to CPF-oxon is mediated by cytochrome P_{450} mixed-function oxidase (CYP450), primarily within the liver. However, extrahepatic metabolism has been reported in other tissues, including the brain (Tang *et al*., 2001; Timchalk *et al*., 2002; Sams *et al*., 2004). The inhibition of AChE activity caused by CPF is more persistent than that caused by other OPs. It is believed that this is because CPF is lipophilic (attracted to the non-water soluble, fatty parts of the body tissues). This means that the CPF is stored, but then released gradually and transformed to CPF-oxon, so that its effects occur over a long interval (Cox, 1994).

2.3.6 Acute Toxicity of Chlorpyrifos

Different kinds of exposure to CPF can cause acute toxicity. Direct skin contact with the insecticide, either as a solid or in water can be toxic. Ingestion, breathing of vapours, or contact with CPF-treated soil is also toxic (Cox, 1994). Symptoms of acute CPF poisoning in human and animals include headache, nausea, dizziness, muscle twitching, weakness, increased sweating and salivation (Cox, 1994; Akhtar *et al*., 2009). These symptoms are common to all OP insecticides. Unconsciousness, convulsions and death can result with sufficient exposure (Cox, 1994; Zheng *et al*., 2000). The oral acute LD_{50} of CPF is 82-270 mg. kg^{-1} for rats (Cox, 1994), 1000 mg. kg^{-1} for rabbits and 504 mg. kg^{-1} for guinea pigs (Akhtar *et al*., 2009).

2.3.7 Delayed Neuropathy

In addition to inhibition of AChE activity, some OPs cause a less common but potentially more devastating syndrome called delayed neuropathy. The syndrome typically occurs one to three weeks after exposure to an OP (Cox, 1994; Albers *et al*., 2005). CPF has been shown to cause delayed neuropathy only after a high dose of exposure in chickens (Smegal, 2000), cats (Cox, 1994; Dewey, 2005) and humans (Richardson *et al*., 1993; Nand *et al*., 2007). Symptoms of this nervous system disorder include cramps, weakness, tingling and numbing of the extremities, a high stepping gait, paralysis of the lower limbs and (in some severe cases) quadriplegia (Cox, 1994).

2.3.8 Adverse Health Effects of Chlorpyrifos

2.3.8.1 Effects on Reproduction

The CPF-related problems with associated with in uteroexposure include cerebral palsy, cataracts and seizure (Cox, 1994). Other studies found increase in skull defects that allowed the brain to be exposed, reduced pup weight and survival and increased fetal mortality (Smegal, 2000; Ambali *et al*., 2009), death of all the cells that make up the semen-producing tubules (small tubes) in the testes, decreased litter size and reduced sperm production (Cox, 1994). Decreased in birth weight (Breslin *et al*., 1996; Smegal, 2000; Zhao *et al*., 2004) and birth length in humans (Smegal, 2000; Saulbury *et al*., 2009) and animals (Ambali *et al*., 2009) has been documented. Shortening of gestation period also in humans has been reported (Akhtar *et al*., 2006).

2.3.8.2 Effects on the Immune System

Studies have identified immune system abnormalities in individual following CPF exposue. Higher than usual frequencies of allergies and sensitivities to antibiotics together with atypical abundance of certain types of lymphocyte (decrease in T cells and increase in CD26 cells) have been observed in patients exposed to CPF (Thrasher *et al.*, 1993; 2002). Increased expression of CD26 cells is associated with autoimmunity, where an individual's immune system acts against itself rather than against infection (Cox, 1994; Li, 2007).

2.3.8.3 Carcinogenic and Genotoxic Effect

Chlorpyrifos has been evaluated and reported as non-carcinogenic (Akhtar *et al*., 2006). However, it can cause genetic damage, increase in the frequency of sister chromatid exchanges (Cox, 1994; Mehta *et al*., 2009). It can also cause genetic damage in organisms other than mammals. In fruit flies, it causes an increase in mutations of wing primordial cells, and increase in the frequency of recessive sex-linked lethal mutations and DNA damage in bacteria (Cox, 1994; Smegal, 2000).

2.4 OXIDATIVE STRESS

Oxidative stress is defined as a distruption of the prooxidant antioxidant balance in favour of the former, leading to potential damage (Sies, 1997; Schneider and de Oliveira, 2004; Halliwell, 2007; Packer and Cadenas, 2007; Aly *et al*., 2010; Costantini and Verhulst, 2009). It can be classically defined as a redox imbalance with an excess of oxidants or a deficiency in antioxidants (Miguel *et al*., 2009). It leads to a disruption of redox signaling and control that recognizes the occurrence of compartimentalized cellular redox circuit (Parker and Cadenas, 2007). Costantini and Verhulst (2009) defined oxidative stress as the rate at which oxidative damage is generated. Damage induced by oxidative stress includes alteration of cellular macromolecules such as membrane lipids, DNA, and/or protein (Vandana *et al*., 2006; Verma *et al*., 2007; Aly *et al.,* 2010). One of the main lesion mechanisms is lipoperoxidation, the oxidation of the lipid layer of cellular membrane (Schneider and de Oliveira, 2004). Malondialdehyde (MDA) is the end-product of lipid peroxidation and can be measured by TBARS test (Jusman and Halim, 2009; Ahmad *et al*., 2010).

2.4.1 Chlorpyrifos and Oxidative Stress

Although cholinesterase inhibition is the main mechanism implicated in OP toxicity (Li, 2007), recent evidences have implicated other mechanisms (Slotkin *et al*., 2006). One of the mechanisms implicated in both acute and chronic OP poisoning is oxidative stress. Several studies have demonstrated the role of oxidative stress in OP-induced poisoning (Gultekin *et al*., 2001; Ambali *et al.,* 2007, 2010b-c; Durak *et al*., 2008). Oxidative stress occurs when the production ROS exceeds the body's natural antioxidant defence mechanism, causing damage to macromolecules such as DNA, proteins and lipids (Vandana *et al*., 2006). It results from an increase in ROS, an impairment of antioxidant defence system or an insufficient capacity to repair oxidative damage (Aly *et al*., 2010). Cells succumb to oxidative damage when the indigenous store of antioxidant is used up by the oxidant exposure. This leads to increased lipoperoxidative damage to cell membrane, in addition to alteration in the levels of both antioxidant enzymes and molecules (Verma *et al*., 2007).

The antioxidant machinery is composed of enzymes and non-enzymatic components (Gultekin *et al*., 2006; Kahn and Kour, 2007; Aly *et al*., 2010). Enzymatic and non-enzymatic antioxidants have been shown to scavenge free radicals and ROS (Gultekin *et al*., 2006). The antioxidant enzyme defence system is made up of free radical scavengers like superoxide dismutase (SOD) and catalase (CAT) as well as glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione-s-transferase (GST) (Banerjee *et al*., 1999; Kahn and Kour, 2007; Tuzmen *et al*., 2007; Aly *et al*., 2010). The non-enzymatic component is primarily composed of thiols, glutathione (GSH) (Khan and Kour, 2007; Aly *et al*., 2010), vitamins C and E (Costantini and Verhulst, 2009), uric acid, ceruloplasmin, β-carotene and ubiquinone (Davis, 2000). Oxidative stress in pesticide exposure is evidenced by increased concentration of blood and tissue thiobarbituric acid reactive substances (TBARS), changes in antioxidant status, and altered activities of cellular enzymes (Lopez *et al*., 2007; Ambali *et al*., 2010).

2.4.2 Free Radicals

Free radicals are chemical species possessing one or more unpaired electron in their outer orbit that can be considered as fragments of molecules and which are generally very reactive (Cheeseman and Slater, 1993; Menvielle-Bourg, 2005; Tkaczyk and Vizek, 2007; Hammadeh *et al.*, 2009). They are produced continuously in cells either as accidental by-products of metabolism or deliberately as seen during phagocytosis. The most important reactants in free radical biochemistry in aerobic cells are oxygen and its radical derivatives (superoxide (O_2^-) and hydroxyl radical (OH^-)), hydrogen peroxide (H_2O_2) and transition metals (Cheeseman and Slater, 1993). Reactive free radicals formed within cells can oxidize biomolecules and lead to cell death and tissue injury (Cheeseman and Slater, 1993). Free radicals play an important role in the toxicity of pesticides, environmental chemicals (Banerjee *et al.*, 1999) and the development and progression of many human diseases (Ahmad *et al.*, 2010). Pesticide chemicals induce oxidative stress leading to generation of free radicals and alteration in antioxidants or oxygen free-radical (OFR) scavenging enzyme systems (Banerjee *et al.*, 1999; Abdollahi *et al.*, 2004). These oxidants are widely known as ROS and, due to their unpaired electron(s) they tend to strongly react with other chemical compounds. More specifically, they seek stability by “stealing” electrons from nucleic acids, lipids and protein, leading to the damage of cells and consequently disease phenomena (Hammadeh *et al.*, 2009).

2.4.3 Formation and Mechanism of Toxicity

Reactive oxygen species are produced in the body by mitochondria, phagocytes, arachidonate pathways and other physiological processes in which they act as vital signaling molecules (Dundar and Aslan, 2000; Leeuwenbourgh and Heinecke, 2001; Singh *et al.*, 2004; Hammadeh *et al.*, 2009).

They are products of natural oxygen metabolism and represent approximately 1 to 2 % of metabolized oxygen (Aquil *et al*., 2006; Hammadeh *et al*., 2009). They are also produced by cytochrome p_{450} in phase I of drug and toxin metabolism, mostly occurring in the liver (Wall, 2000). Their production, however, multiplies several folds during pathological conditions (Singh *et al*., 2004). The balance between production and disposal of oxidant molecule is essential for tissue homeostasis. Increased rate of free radical production or decreased rate of removal leads to free radical accumulation and cellular damage (Hammadeh *et al*., 2009). Additionally, their production is induced by external factors such as cigarate smoke, ultraviolet light radiation, among others (Rice-Evans and Diplock, 1993; Menvielle-Bourg, 2005).

Due to the electronic configuration, the oxygen presents strong tendency of receiving one electron at a time. The univalent conversion of oxygen into water is given as follows:

a) The addition of one electron to one oxygen molecule in its fundamental state generates the formation of O_2^- (Equation 1):

$O_2 + e^- \rightarrow O_2^-$ ……………………………………………………………………….1

b) The superoxide receives more than one electron and two hydrogen ions thereby forming H_2O_2 through the process called dismutation. This reaction is catalyzed by the enzyme SOD (Equation 2) (Schneider and deOliveira, 2004).

$2\ O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2$……………………………………………2

c) When H_2O_2 receives more than one electron and one hydrogen ion, OH^- is formed, which is the most reactive of the intermediate ones, as it reacts and changes any nearby cellular structure, thus influencing enzymes, membranes and nucleic acid. The hydroxyl radical may be formed when H_2O_2 reacts with iron or copper ions (Equation 3). This reaction is known as Fenton reaction (Tkaczyk and Vizek, 2007).

$Fe^{2+}/CU^+ + H_2O_2 \rightarrow OH^+ + OH^- + Fe^{3+}/\ CU^{2+}$ ……………………………………3

The ions of transition metals may as well catalyze the reaction between H_2O_2 and O_2^-, leading to the production of OH^- (Equation 4) called Haber-Weiss reaction (Halliwell, 1990).

$H_2O_2 + O_2 \xrightarrow{\text{Fe3+/Cu2+}} OH^+ \rightarrow OH^{-\circ} + O_2$………………………… 4

The O_2^- and OH^- present unpaired electrons in its outer orbit and are therefore, called free radicals. The hydrogen peroxide is not a free radical. However, it represents a partly reduced metabolite and if considered as a whole, are called oxygen reactive species due to their increased reactivity with regards to biomolecules. H_2O_2 is relatively stable and can migrate from its site of origin; therefore, it is capable of affecting a large scale of important cellular molecule (Tkaczyk and Vizek, 2007). Besides, the superoxide radical may react directly with nitric oxide (NO) to generate peroxynitrite ($ONOO^-$). This compound may lead to the formation of an oxidant agent with OH^- features (Equation 5) (Leeuwenburgh and Heinecke, 2001; Schneider and deOliveira, 2004).

$O_2^- + NO \rightarrow ONOO^- \rightarrow ONOO^- + H^+ \rightarrow OH^-$……………………………………….5

Most free radicals in biological system fit within the broader category of ROS, which include not only oxygen-centered radicals such as the O_2^-, OH^- or NO^-, but also some potentially dangerous non-radical derivatives of oxygen, such as H_2O_2, peroxynitrite anion ($ONOO^-$) (formed from the reaction of NO^- and O_2^-) (Singh *et al*., 2004) and hypochlorous acid (HOCl) (Guetens *et al*., 2002; Hammadeh *et al*., 2009). The hydroxyl and alkoxy free radicals are very reactive species and rapidly attack the macromolecules in cells. The O_2^- anion, lipid hydroperoxide, and NO^- are comparatively less reactive (Singh *et al*., 2004).

2.4.4 The Role of Reactive Oxygen Species in Diseases

Reactive oxygen species have been associated with the pathology of numerous diseases such as neurodegenerative diseases, vascular diseases, cancer, diabetes, periodontal diseases and human infertility (Adams and Odunze, 1991; McCall and Frei, 1999; Halliwell, 2009; Oyagbemi *et al*., 2009; Sunil and Dinesh, 2009). Damages due to free radicals caused by ROS, leads to several damaging effects as they attack lipids, protein/enzymes, carbohydrates and DNA in cells and tissues (Abdollahi *et al*., 2004; Verma *et al*., 2007; Oyagbemi *et al*., 2009). They induce undesirable oxidation causing membrane damage and cell death induced by DNA fragmentation and LPO (Tkaczyk and Vizek, 2007; Satyanarayana *et al*., 2009). This oxidative damage/stress associated with ROS is believed to be involved not only in the toxicity of xenobiotics, but also in the pathophysiological role in ageing (Costantini and Verhulst, 2009), and several diseases like heart disease (atherosclerosis), cataract, cognitive dysfunction, cancer, diabetic retinopathy, shock, chronic inflammatory disease of the gastrointestinal tract, organ dysfunction and ischaemia (Singh *et al*., 2004).

2.5 ANTIOXIDANT

An antioxidant is any substance capable of preventing oxidation (Valko *et al*., 2005). They are the exogenous or endogenous compounds, which either prevent the generation of toxic oxidants or intercept those that are generated and inactivate them, thereby blocking the chain propagation induced by the oxidants (Rangan and Bulkley, 1993). The term “oxidisable substrate” includes almost everything found in living cells, including proteins, lipids, carbohydrates and DNA (Halliwell, 1990). Halliwell (2007) further defined antioxidants as any substance that delays, prevents or removes oxidative damage to a target molecule.

It is worthy emphasizing that the composition of the antioxidant molecules is distinguished from tissue to tissue, from type of cell to type of cell and possibly from cell to cell of the same type in a given tissue (Schneider and deOliveira, 2004). The antioxidant defence system is divided into enzymatic and non enzymatic (Schneider and deOliveira, 2004; Tkaczyk and Vizek, 2007). The enzymatic antioxidants include SOD, CAT and GPx.

The CAT plays important role in the elimination of H_2O_2, furthering its catalysis into water (Equation 6).

$H_2O_2 + H_2O_2 \xrightarrow{CAT} O_2 + 2\,H_2O$………………………………………….. 6.

The GPx also acts as mechanism of protection against the oxidative stress, converting the GSH into GSSG, removing H_2O_2 and forming water (Equation 7).

$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$ ………………………………….7

Thus, both the CAT and the GPx avoid the accumulation of the O_2^- and H_2O_2 so that the OH^- is not produced, against which there is no defence enzymatic system (Schneider and deOliveira, 2004).

The non-enzymatic system includes compounds synthesized by the human organism such as bilirubin, albumin, ceruloplasmin, sexual hormones, melatonin, co-enzyme Q, uric acid and others ingested through regular diet or through supplementation such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene (precursor of vitamin A) and phenol groups of plants (flavonoids) (Schneider and deOliveira, 2004; Verma *et al*., 2007). They are mostly “scavengers” of free radicals and could also prevent ROS formation like allopurinol, which is a potent inhibitor of xanthine oxidoreductase (Tkaczyk and Vizek, 2007).

2.5.1 Types of Antioxidant Defences Based on Function

1) Primary or chain breaking antioxidants (scavenger antioxidants). These antioxidants can neutralize free radicals by donating one of their own electrons, ending the electron “stealing” reaction. The resultant antioxidants which become free radicals because of one electron left in their outer shell are relatively safe, stable and in normal circumstance insufficiently reactive to initiate any toxic effect (Cheeseman and Slater, 1993; Noguchi *et al*., 2000; Tkaczyk and Vizek, 2007). For example, α-tocopherol after scavenging free radical becomes tocopheroxyl radical, which is relatively safe (Bender and Mayes, 2003).

2) Secondary or preventive antioxidants. They act through numerous possible mechanisms. They include sequestration of transition metal ions which are not allowed to participate in metal catalyzed reactions, removal of peroxides by CAT and GPx that can react with transition metal ions to produce ROS or the outright removal of ROS (Cheeseman and Slater, 1993; Noguchi *et al*., 2000).

3) Tertiary antioxidant defences. They are the repair processes, which remove damaged biomolecules before they can accumulate and before their presence results in altered cell metabolism and viability. Damaged DNA repaired by enzyme methionine sulphoxide reductase, oxidized proteins removed by proteolytic enzyme system, and oxidized lipids acted upon by lipase, peroxidases and acyl transferases are some of the examples (Cheeseman and Slater, 1993; Noguchi *et al*., 2000).

4) The fourth line is an adaptation where the signal for the production and reaction of free radicals induces formation and transport of the appropriate antioxidant to the right site (Noguchi *et al*., 2000).

2.5.2 Vitamin C

Vitamin C or ascorbic acid (VC) is a water-soluble micronutrient required for multiple biological functions (Naidu, 2003; Duarte and Lunec, 2005). It is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not by non-human primates and guinea pigs (Aguirre and May, 2008). These species do not have the enzyme gulonolactone oxidase, which is essential for synthesis of the ascorbic acid immediate precursor 2-keto-L-gulonolactone (Padayatty *et al*., 2003; Duarte and Lunec, 2005; Wilson, 2005). VC is currently the most widely used vitamin supplement throughout the world (Naidu, 2003).

2.5.2.1 Properties of Vitamin C

L-ascorbic acid ($C_6H_8O_6$) is the trivial name of VC. The chemical name is 2-oxo-L-theo-hexo-1, 4-lactone-2, 3-enediol. Ascorbic acid is sensitive to air, light, heat, and is easily destroyed by prolonged storage and over processing of food (Naidu, 2003). It is well absorbed from the gastrointestinal tract (Duarte and Lunec, 2005). Absorption is primarily in the distal small intestine via active transport through an ascorbate transporter termed the sodium-dependent VC transporter-type 1 (SVC T1) (Aguirre and May, 2008). Ascorbic acid, being a water-soluble compound is easily absorbed but it is not stored in the body. The average half-life of ascorbic acid in adult human is about 10-20 days. Hence, ascorbic acid has to be regularly supplemented through diet or tablets to maintain ascorbic acid pool in the body (Naidu, 2003). The main route of elimination of ascorbic acid and its metabolites is through urine. It is excreted unchanged, when high doses of ascorbic acid are consumed (Naidu, 2003; Aguirre and May, 2008). Intake of 250 mg/day and higher are required to saturate ascorbate concentration in plasma and content of

WBC (Aguirre and May, 2008). Urinary loss of ascorbate decreases ascorbate concentrations in plasma and in most tissues, but not the brain (Harrision and May, 2009).

2.5.2.2 Antioxidant Properties of Vitamin C

Vitamin C is a potent reducing agent and scavenger of free radicals in biological system (Duarte and Lunec, 2005). It is probably the most effective, least toxic antioxidant identified in mammalian system (Sauberlich, 1994). It is water-soluble, chain-breaking antioxidant that reacts directly with O_2^-, OH^-, and singlet oxygen at very low concentration (Sauberlich, 1994; Naidu, 2003).

Vitamin C is an electron donor and, therefore, a reducing agent. It donates two electrons from a double bond between the second and third carbon of the 6-carbon molecule. It is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (Padayatty *et al*., 2003). In addition, ascorbic acid can regenerate other antioxidants such as α-tocopheroxyl, urate and β-carotene radical cation from their radical species (Sauberlich, 1994; Naidu, 2003). Thus, it acts as a co-antioxidant for α-tocopherol by converting α-tocopheroxyl radical to α-tocopherol and helps to prevent the α-tocopheroxyl radical-mediated peroxidation reaction (Sauberlich, 1994; Naidu, 2003; Harrison and May, 2009). Ascorbate has been shown to spare/recycle α-tocopherol in lipid bilayer and in erythrocytes (Harrison and May, 2009). The importance of this recycling of α-tocopherol stems from properties of the latter as a lipophilic antioxidant, capable of interfering with atherogenic oxidative modification of low-density lipoprotein (LDL). Although the tocopheroxyl radical is embedded in the LDL micelle or membrane bilayer, it is accessible to ascorbate in the aqueous phase for reduction. This cooperative relationship has been shown to significantly decrease copper-mediated LDL oxidation *in vitro* (Aguirre and May, 2008).

Ascobate oxidation is reversible, which allows for recycling from its oxidized forms (Aguirre and May, 2008). Ascorbate readily undergoes two consecutive, yet reversible, one-electron oxidations to generate dehydroascorbate and an intermediate, the ascorbate free radical (Figure 2.5). AFR is however, a relatively unreactive free radical, with a reduction potential considerably low compared to α-tocopherol radical, the glutathione radical and virtually all reactive oxygen and nitrogen species that are thought to be involved in diseases (Naidu, 2003; Mahan *et al.*, 2004). These properties make ascorbate an efficient electron donor in many biological redox reactions, capable of replacing potentially damaging radicals by the poorly reactive ascorbate radical; according to Equation 8, where $AScH^-$ represents ascorbate, $ASc^{\circ -}$ represents the ascorbate free radical; and X° represents the oxidizing species:

$AScH^- + X^\circ \rightarrow ASc^{\circ -} + XH$ ……………………………………………………………8

Ascorbate is the reduced and biologically active form; dehydroascorbate is the oxidized form of vitamin C. In most body tissues dehydroascorbate is rapidly converted to ascorbate because of its potential oxidizing effect (Mahan *et al.*, 2004; Daurte and Lunec, 2005). Since ascorbate provides electron rapidly to electron receptors, it produces oxidized compound that are essential for body development, or its electrons are involved in activating various enzymes (Mahan *et al.*, 2004).

Ascorbic acid is known to protect completely lipids in plasma and low density LDL against peroxidative damage (Sauberlich, 1994). It may be important in protecting against oxidative stress-related diseases and degeneration associated with ageing, including coronary heart disease, cataract formation, and cancer (Sauberlich, 1994; Naidu, 2003). Thus, vitamin C is one of the body's most effective and rapidly-acting water soluble antioxidant (Mahan *et al.*, 2004).

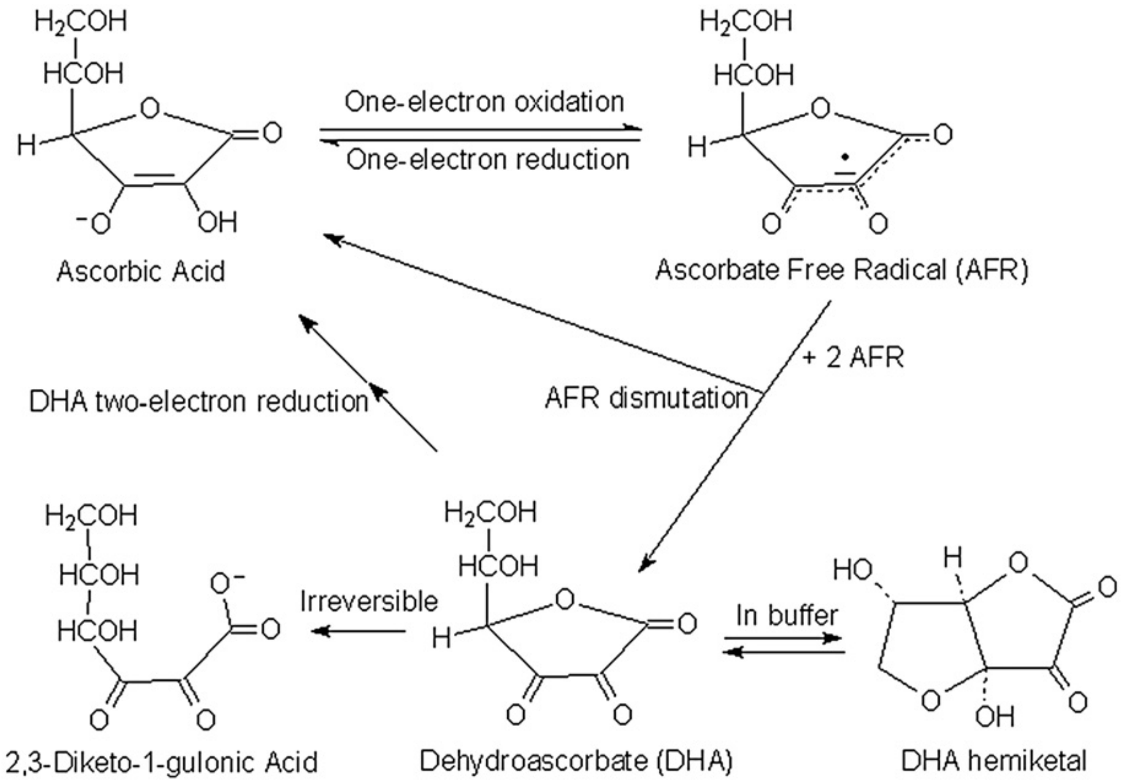


Figure.2.5. Chemical structures and reactions involving ascorbate metabolism (Adapted from Harrison and May, 2009).

2.5.2.3 Pro-oxidant Effect of Vitamin C

Paradoxically, vitamin C is also known to act as a pro-oxidant *in vitro*. VC may contribute to oxidative damage formation by reducing ferric Fe^{3+} to ferrous Fe^{2+}ions (and Cu^{+} to Cu^{2+}), which in turn can reduce H_2O_2 to OH^- (Duarte and Lunec, 2005; Harrison and May, 2009). The reactions are shown in equations 9-15 (Carr and Frei, 1999):

$AH^- + Fe^{3+}$.....→ $A^{\circ -} + Fe^{2+} + H^+$ 9

$AH^- + Cu^{2+}$.....→ $A^{\circ -} + Cu^+ + H^+$ 10

$H_2O_2 + Fe^{2+}$ .....→ $HO^\circ + Fe^{3+} + {}^-OH$ 11

$H_2O_2 + Cu^+$.....→ $HO^\circ + Cu^{2+} + {}^-OH$ 12

$LOOH + Fe^{2+}$.....→ $LO^\circ + Fe^{3+} + {}^-OH$ 13

$LOOH + Cu^+$.....→ $LO + Cu^{2+} + {}^-OH$ 14

HO°, LO° .....→ Lipid peroxidation 15

These radical species are highly reactive and can trigger LPO reaction (Naidu, 2003). Another factor that may affect pro-oxidant versus antioxidant property of ascorbic acid is its concentration. The *in vitro* data suggests that at low concentrations ascorbic acid acts as a pro-oxidant, but as an antioxidant at higher levels (Naidu, 2003). However, when provided in excess it may serve as a pro-oxidant (Mahan *et al*., 2005; Aguirre and May, 2008).

2.5.2.4 Non-antioxidant Function of Vitamin C

Vitamin C has numerous biologic functions. It helps maintain various enzymes in their required reduced forms (Sauberlich, 1994). Eight known enzymes require ascorbate for their activation (Padayatty *et al*., 2003; Mahan *et al*., 2005). These reactions involve hydroxylation of proline, hydroxyproline or lysine in collagen synthesis (Mahan *et al*., 2005). Therefore, ascorbic acid plays an important role in the maintenance of collagen, which represents about one third of the body proteins and also plays a critical role in wound repair and healing/regeneration process as it stimulates collagen synthesis (Naidu, 2003). Ascorbic acid also serves as a co-factor for the enzyme, dopamine-β-hydroxylase and peptidyl glycine α-amidating monooxygenase system, which participate in the biosynthesis or conversion of neurotransmitter, dopamine to norepinephrine (Aguirre and May, 2008) and various α-amidated peptides, (Sauberlich, 1994; Naidu, 2003; Harrison and May, 2009) respectively. These properties are necessary for maximal activity of the hormones, oxytocin, vasopressin, cholecystokinin and alpha-melanotropin (Naidu, 2003; Padayatty *et al*., 2003; Mahan *et al*., 2005).

Ascorbic acid participates in the biosynthesis of carnitine and neurone-endocrine peptides (Eipper and Mains, 1991). Carnitine biosynthesis requires the participation of ε-N-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase, both of which require ascorbic acid as a co-factor (Sauberlich, 1994; Padayatty *et al*., 2003). Carnitine is required for transport and transfer of fatty acids into mitochondria, where it can be used for energy production (Naidu, 2003).

Ascorbic acid is involved in the transformation of cholesterol to bile acids as it modulates the microsomal 7α-hydroxylation, the rate limiting reaction of cholesterol catabolism in the liver (Sauberlich, 1994; Naidu, 2003). In vitamin C deficiency, this reaction becomes slowed down, thus, resulting in an accumulation of cholesterol in the liver, hypercholesterolaemia and formation of cholesterol gall stones (Naidu, 2003). There is also evidence that ascorbate is involved in the regulation of both ACh and catecholamine release from synaptic vesicles (Harrison and May, 2009).

2.5.3 Acetyl-L-carnitine

Acetyl-L-carnitine (ALC) (γ-trimethyl-β-acetylbutyrobetaine) is the acetyl ester of carnitine and the primary acyl carnitine in human tissues (Flatter *et al*., 2006). It is synthesized in the human brain, liver, and kidneys by the enzyme carnitine acetyl transferase (Hao *et al*., 2004); with lysine, methionine and vitamin C among the substrate and co-factors (Furlong, 1996). It is the predominant ester of carnitine (Illias *et al*., 2004) and is present throughout the central and peripheral nervous system, and play essential role in the oxidation of free fatty acid (Flatter *et al*., 2006). ALC, which is similar in structure to ACh, exerts a cholinomimetic effect (Hao *et al*., 2004).

Its treatment increased the activity of AChE in synaptic plasma membranes from rat frontal cerebral cortex (Pettegrew *et al*., 2000).

2.5.3.1 Properties of Acetyl-L-carnitine

Acetyl-l-carnitine is synthesized by carnitine acyltransferase from acylCoA and carnitine (Pettegrew *et al*., 2000). It is a water-soluble organic solute and forms a component of endogenous carnitine pool in humans and most animal species (Calo *et al*., 2005). ALC transport occurs more easily through the lipid component of the intestinal barrier, suggesting possible better bioavailability (Mansour, 2006). ALC is absorbed by simple diffusion in the jejunum after oral administration, and excreted by the kidneys in an extent that is proportional to their plasma concentration (Furlong, 1996; Illias *et al*., 2004). ALC penetrates cells and crosses the blood-brain barrier more efficiently than LC (Furlong, 1996; Illias *et al*., 2004; Liu *et al*., 2004; Picconi *et al*., 2006), and accumulates in the brain (Picconi *et al*., 2006).

ALC levels increase rapidly after intraveinous administration to healthy volunteers, declining to base-line after 12 hours. ALC does not interact with either albumin or plasma protein in rats, dogs and humans (Pettegrew *et al*., 2000).

2.5.3.2 Metabolic effect of acetyl-L-carnitine

Acetyl-L-carnitine influences the maintenance of key mitochondrial protein for maximal energy production. In the brain, ALC is involved in the regulation of carbohydrate, lipid and protein metabolism (Picconi *et al*., 2006). Studies in humans and animals suggest ALC has a favourable role in restoring cerebral energy metabolism. Firstly, it modulate glucose metabolism and stimulates glycogen synthesis in rats. Secondly, ALC increases plasma level of adenosine and ATP, with the rise in adenosine levels preceding that of ATP. The increase in adenosine levels before ATP after ALC administration suggests a role for ALC in the turn-over of adenosine. Adenosine acts in a paracrine/autocrine manner to modulate cellular activity via specific membrane receptors. ALCs ability to increase adenosine levels could result in decreased membrane excitability, which could have protective effect (Pettegrew *et al*., 2000).

2.5.3.3 Antioxidant Properties of Acetyl-L-carnitine

Acetyl-L-carnitine may act as an antioxidant either by having a primary antioxidant activity (inhibiting free-radical propagation reaction) or, more likely, functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acids esterified in membrane phospholipids) (Liu *et al*., 2004). They are also scavengers of oxygen free radicals in mammalian tissues. Although the level of beta-oxidation in the normal adult brain is very low, ALC is transported through the blood-brain barrier and accumulates in neural cells offering protection against oxidative damage (Al-Majed *et al*., 2006).

2.5.3.4 Mechanism of Antioxidant Effect of Acetyl-L-carnitine

Acetyl-L-carnitine prevents the accumulation of end-products of lipid peroxidation (Cetinkaya *et al*., 2006). It plays a major role, as a co-factor, in the transport of free fatty acid from the cytosol to the mitochondria. Free fatty acid is degraded to acyl-CoA by β-oxidation and these substances enter the tricarboxylic acid cycle. A large amount of O_2 is consumed in this reaction and ATP is synthesized in the steps of the electron transport chain and oxidative phosphorylation. O_2 is reduced to H_2O at the end of the TCA cycle and this process reduces the O_2 concentration. Thus, toxic products such as ROS and products of free fatty acid are cleared from cells (Illias *et al.*, 2004; Cetinkaya *et al.*, 2006; G�lcin, 2006).

Acetyl-L-carnitine is associated with a reduction in xanthine oxidase activity (Liu *et al.*, 2004; Bloomer *et al.*, 2009) and is also known to posses free radical scavenging activity (Bloomer *et al.*, 2009). ALC has been shown to have neuroprotective benefits in the hippocampus, prefrontal cortex, *substantia nigra* and muscarinic receptor portion of the brain probably due to its antioxidant activity and its ability to improve mitochondrial energetics stabilizes the intracellular membranes and enhances cholinergic neurotransmission (Furlong, 1996). ALC plays neuroprotective role in some pathological conditions of the brain (Picconi *et al.*, 2006) and also offers protection against toxicity induced by some neurotoxic agents (Pettegrew *et al.*, 2000; Illias *et al.*, 2004). It has also been shown to ameliorate oxidative organ injury in animal models (Calo *et al.*, 2005).

2.5.3.5 Non-antioxidant Function of Acetyl-L-carnitine

Acetyl-L-carnitine induces many of its biological actions through the metabolic effects of its carnitine and acetyl moieties (Pettegrew *et al.*, 2000). It has the potential to act as a molecular chaperone and interacts with large molecules such as protein and membrane lipids (Pettegrew *et* *al*., 2000; Calabrese *et al*., 2006). It changes the conformation of the larger molecules and possibly alters their functional activity (Pettegrew *et al*., 2000). ALC profound effects on some depressed patients with high cortisol levels and participates in immunomodulatory mechanism, a process that holds promise in the treatment of HIV infection (Furlong, 1996; Illias *et al*., 2004). It also induced improvement in the short-term memory deficit and cognitive function in elderly human and animal subjects given ALC and in old rats (Hagen *et al*., 2002; Liu *et al*., 2002). ALC appears to have a neuromodulatory effect on synaptic morphology as well as on synaptic transmission in multiple neurotransmitter system (Pettegrew *et al*., 2000). It modulates melatonin secretion in both adult and old rats, suggesting that ALC can modify noradrenergic neurotransmission and signal transduction in the pineal gland (Pettegrew *et al*., 2000). ALC facilitates the uptake of acetyl CoA into mitochondria during fatty acid oxidation, enhances ACh production, and stimulates protein and membrane phospholipid synthesis (Hao *et al*., 2004; Gülcin, 2006). ALC enzymatic formation in the mitochondrial matrix is reversible, providing free coenzyme A and acetyl CoA, which can readily be exchanged across membranes, thus providing metabolic energy to intracellular organelles (Furlong, 1996).

CHAPTER 3

RESEARCH PROCEDURES

3.1 EXPERIMENTAL ANIMALS

Sixty eight male Wistar rats (10-12 weeks old) weighing 110-150g used for this experiment were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were housed in the General laboratory of the Department. The rats were fed using standard rat chow, and water was provided *ad libitum.*

3.2 CHEMICALS AND PREPARATIONS

Commercial grade Chlorpyrifos (Termicot®, Sabero Organic, Gujarat, India) was obtained from a chemical store in Zaria. Acetyl-L-carnitine (L-carnipure®, Ideasphere Inc. America Fork, UT 84003 U.S.A.) and vitamin C (Mopson Pharmaceutical Limited, Lagos, Nigeria) were obtained from a reputable Pharmaceutical sales' outlet in Zaria. Analytical grade thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were obtained from Sigma Aldrich Inc., U.S.A.

Soya oil (Grand®, Grand Cereals and Oil Mills Ltd., Jos, Nigeria) was used to reconstitute the CPF and ALC, while distilled water was used to reconstitute vitamin C to the appropriate working concentration.

3.3 EXPERIMENTAL PROTOCOLS

3.3.1 Median Lethal Dose Determination

The median lethal dose (LD_{50}) was determined via a two phase approach as described by Lorke (1983). The first phase involved nine animals divided into three groups of three animals each. Groups I, II and III were administered with CPF at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight *per os*, respectively. Death was observed over a period of 48 hours. The result showed that all the rats in the groups dosed with 100 mg/kg and 1000 mg/kg died. The protocol for phase II, which depends on the results obtained from phase 1 consisted of three animals divided into three groups of one animal each were administered with CPF at 80 mg/kg, 90 mg/kg and 100 mg/kg, respectively. The LD_{50} was then calculated by finding the average of the value obtained when the least dose that killed the animal was added to the lowest dose that did not kill the animal.

3.3.2 Experimental Treatments and Groups.

Experimental procedures involved 56 rats divided into eight groups of seven animals per group. The dosage schedules used for the study were as follows: Group I was administered soya oil (S/oil) only at 2 ml/kg, while rats in groups II (VC) were administered vitamin C (100 mg/kg). Group III (ALC) was administered with acetyl-L- carnitine only (300 mg/kg) while group IV (VC+ALC) was co-administered with acetyl-L-carnitine (300 mg/kg) and vitamin C (100 mg/kg). Group V (CPF) received CPF (8.5 mg/kg ~10 % of LD_{50}) only while group VI (VC + CPF) was pre-treated with vitamin C (100 mg/kg) and then followed by CPF (8.5 mg/kg), 30 minutes later. Rats in group VII (ALC + CPF) were pre-treated with ALC (300 mg/kg) and then exposed to CPF (10% LD_{50}) 30 minutes later, while rats in group VIII (VC + ALC + CPF) were pre-treated with ALC (300 mg/kg) and vitamin C (100 mg/kg) and then administered with CPF (8.5 mg/kg), 30 minutes later. The regimens were administered orally by gavage once daily for a period of 28 days. During this period, weight changes and signs of toxicity were recorded.

3.3.3 Evaluation of the Effect of Treatments on Haematological Profile

The animals were sacrificed after light chloroform anaesthesia at the end of 28 days of treatment by severing the jugular vein; two mililitre of blood was collected into heparinized test tubes. The blood was analyzed for packed cell volume (PCV), haemoglobin (Hb) concentration, total erythrocyte (RBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte counts, differential leucocyte count and platelet counts, using Abbott Haematological Analyser Cell-Dyn 1700 (Abbott Laboratories, Abbott Park, Illinois, U.S.A.).

3.3.4 Evaluation of the Effect of Treatments on Serum Biochemical Profile

From each animal, another 3 ml of blood was collected into a centrifuge tube, allowed to clot and then incubated for 30 minutes, then followed by centrifugation at 1000 x g for 10 minutes. The serum collected was used for the determination of total protein (TP), albumin, glucose, urea and creatinine concentrations. The concentration of some electrolytes (Na^+, K^+, Cl^-, and HCO_3^-) and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine kinase (CK) were determined. The biochemical assay was carried out using an auto-analyzer (Bayer Express Plus®, Bayer, Germany).

3.3.5 Evaluation of the Effect of Treatments on Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility was determined according to the method of Fraulkner and King (1970) as modified by Oyewale (1993). Sodium chloride stock solution was prepared in volumes of 500 ml for each test in concentrations of 0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%. A set of 6 test tubes was used and each tube contained 10 ml of the corresponding NaCl concentration from the stock solution. The test tubes were then labeled with the corresponding concentrations and arranged serially in a rack of 6 test tubes. One ml pipette was used to transfer 0.02 ml of each blood sample into each of the 6 test tubes in a set, after which the contents of the tubes were mixed by gentle inversion and allowed to stand at room temperature for 30 minutes. Thereafter, the contents of the test tubes were centrifuged at 800 x g for 15 minutes. The supernatant was transferred into a glass cuvette. The concentration of haemoglobin was then measured at a wavelength of 540 nm using a spectrophotometer (Jenway, 645, Japan) by reading the absorbance. The percent haemolysis was calculated based on the formula of Fraulkner and King (1970):

$$\text{Percent Haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard (distilled water)}} \times 100$$

3.3.6 Effect of Treatments on Oxidative Stress Parameters.

Freshly obtained liver samples were homogenized, suspended in ice-cold phosphate-buffered saline (PBS) and then vortexed for 5-10 seconds. The mixture was centrifuged at 1000 x g for 15 minutes and the supernatant decanted into sterile test tubes, stored on ice and immediately used for the analysis of the activities of antioxidant enzymes SOD and CAT and the concentrations of MDA and tissue proteins. The tissue proteins were determined using the method described by Lowry *et al*. (1951).

3.3.7 Evaluation of the Effect of Treatments on Liver Malonaldehyde Concentration

The level of thiobarbituric acid reactive substances, malonaldehyde (MDA) concentration as an index of LPO was evaluated in each liver sample using the double heating method of Draper and Hadley (1990) as modified by Yavuz *et al*. (2004). The principle of the method was based on spectophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with MDA. MDA concentration in the supernatant of the liver homogenate was evaluated, thus; 2.5 ml of 100 g/L TCA solution was added to 0.5 ml of the supernatant of the liver homogenate in a centrifuge tube and placed in a boiling waterbath for 15 minutes. After cooling under running water for 5 minutes, the mixture was centrifuged at 1000 x g for 10 minutes. Two mililitre of the supernatant was then added to 1 ml of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 minutes. The solution was cooled under running water and the absorbance measured using a UV spectrophotometer ($T80^{+}$ UV/VIS spectrometer, PG instruments Ltd., Alma Park, Wibtoft, Leicestershire, LE 175 BE, United Kingdom) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 10^5 /cm, and expressed in nmol/g of protein.

3.3.8 Evaluation of the Effect of Treatments on Liver Superoxide Dismutase Specific Activity

The specific activity of the SOD was measured using the method described by Misra and Fridovich (1972) as modified by Rabideau (2001). It is an indirect method of inhibiting auto-oxidation of adrenaline to its adrenochrome using SOD activity in the liver sample. By measuring the rate at which SOD decreased this O_2^--dependent auto-oxidation reaction, the specific activity of SOD in each sample was calculated. Briefly, in a 0.5 ml quartz cuvette, 485 µl of 30ºC, 0.05 M $NaHCO_3$ buffer (pH 10.2, 10^{-4} M EDTA) and 15 µl of adrenaline 10^{-2} M (DL-adrenaline; store ≤ 5 days) were mixed and a kinetic rate (ΔA/min: 480 nm; 30ºC) was obtained on an automated Shimadzu UV spectrophotometer with CPS Temperature controller (Model UV 160; Kyoto, Japan). The amount of adrenaline was adjusted until a rate of 0.025 ± 0.002 was obtained. Thereafter about 25 µl of the sample was added and the reaction rate recorded. The change in rate, or ΔA/min, for each sample was compared to the adrenaline control. The percent of the sample that inhibited adrenaline auto-oxidation was calculated as percent inhibition. Specific activity (Units/mg protein) was calculated using the ΔA/min as follows:

$$\text{Percent inhibition (PI)} = (1-[B/A] \times 100$$

A = Adrenaline ΔA/min at 480 nm

B = Sample ΔA/min

Where

VS50 = Volume (µl) of sample needed for 50% inhibition; contained 1 Unit of SOD

Unit of SOD/ml = 1000/VS50

$$\text{Specific activity} = \frac{\text{Unit of SOD/ml}}{\text{mg protein/ml}} = \text{Unit of SOD/mg protein}$$

3.3.9 Determination of the Effect of Treatments on Liver Catalase Specific Activity

The specific activity of CAT in the liver was determined using the method adapted from Beers and Sizer (1952) and the *Worthington Enzyme Manual (*1972*)*. CAT is an enzyme that scavenges H_2O_2 and converts it to water and molecular oxygen. By monitoring the rate of breakdown of H_2O_2 spectrophotometrically the specific activity of CAT in each sample was measured. Briefly, 167 µl of substrate (0.053 M H_2O_2) in 0.05 M potassium phosphate buffer (pH 7.0, 25ºC) and approximately 25 µl of sample were added to a 0.5 ml quartz cuvette and brought to a final volume of 0.5 ml with 25ºC, 0.05 M potassium phosphate buffer, pH 7.0. The rate of decrease in absorbance at 240 nm was monitored on Shimadzu UV spectrophotometer at 25ºC. The specific activity was calculated using the coefficient of H_2O_2 43.6 M^{-1} cm^{-1} as follows (Beers and Sizer, 1952; Kukueka and Misra, 1993):

$$\text{Unit/ml} = \frac{\Delta A/\text{min at 240 nm x cuvette volume (ml) x dilution factor x 1cm lightpath}}{43.6\ M^{-1}\ cm^{-1}\ \text{x sample volume used (ml)}}$$

$$\text{Specific activity} = \frac{\text{Unit/ml}}{\text{mg/ml protein}}$$

3.3.10 Evaluation of the Effect of Treatments on Gross and Histopathological Changes

The animals were examined for gross lesions. The brain, liver, thyroid, adrenal glands and pancreas were obtained from each group, fixed and immersed in 10% phosphate-buffered formalin for one week before embedding. After one week, the samples were washed in water and kept there-in for 24 hours. Thereafter, they were dehydrated in a series of 70%, 80%, 90%, 95% and 100% alcohol in that order, leaving them in each solution of alcohol for one hour. The tissues were cleared in xylene, infiltrated with molten paraffin wax at 50˚C, blocked in paraffin according to standard procedures and labeled. Sections were cut at a nominal thickness of 10µm through the entire extent of the organs using Jung Rotary Microtome (Model 42339, Berlin, Germany). The sections mounted on glass slides were dried, deparaffinized, stained with haematoxylin and eosin stain using cytoseal 60 as the mounting medium (Luna, 1960). The slides prepared were viewed under light microscope at different magnifications for any tissue/cellular morphological changes.

3.3.11 Statistical Analysis

Data obtained were expressed as mean ± standard error of the mean (mean ± SEM) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post-hoc test, using GraphPad Prism version 4.0 for windows (from GraphPad software, San Diego, California, U.S.A.) to compare the level of significance between the groups. Values of $P < 0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50}) FOR CHLORPYRIFOS

Within 24 hours post administration, all the three rats in each of the groups dosed during phase I of the LD_{50} with CPF at 100 mg/kg and 1000 mg/kg died, while those dosed with 10 mg/kg survived. The clinical signs observed prior to death included depression, huddling, piloerection, tremor, intermittent convulsion, respiratory depression, ataxia and death. Gross findings observed included haemorrhagic gastro-enteritis, filling of the stomach with gas bubbles, congestion and petechial haemorrhages of the liver, congestion of the lungs, heart, kidneys, testes and brain blood vessels, and flabby thigh muscle. In the phase II, out of the individual rats dosed, only the one that was administered 80 mg/ kg survived.

The result obtained in phase II from the three groups comprising one animal each, is represented in Table 4.1.

Table 4.1: Summary of the data from phase II median lethal dose (LD_{50}) evaluation.

Group	Dose (mg / kg)	No.dead / dosed
1	100	1/1
2	90	1/1
3	80	0/1

Thus, based on the method of Lorke (1983), the result of the $LD_{50} = \frac{90 + 80}{2} = 85$ mg/kg

4.2 SUBACUTE TOXICITY STUDY

4.2.1 Clinical Signs

No death was recorded in any of the experimental groups, including the control group. Rats in the soya oil, VC, ALC, VC + ALC groups did not show any apparent sign of toxicity, while those administered CPF showed weakness, lacrimation and congested ocular mucous membranes. The group pretreated with VC, ALC and the combination of VC and ALC before CPF exposure also did not show any apparent sign of toxicity.

4.3 EFFECT OF TREATMENTS ON HAEMATOLOGICAL PARAMETERS

4.3.1 Effect of Treatments on Red Blood Cell Count

There was no significant difference ($P > 0.05$) between the groups. The lowest mean RBC count was observed in the CPF group ($8.073 \pm 0.3 \times 10^{12}$/L), which decreased by 5% compared to the S/oil group (Figure 4.1). However, there were 5.3%, 8.3% and 1.4% increase in RBC concentration in VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, when compared to the CPF group.

4.3.2 Effect of Treatments on Packed Cell Volume Level

There was also no significant difference (P > 0.05) between the groups (Figure 4.2). The CPF group had the lowest PCV (41.53 ± 20%) as it slightly decreased by 14.7% compared to the S/oil group. There was a comparative increase in PCV in VC + CPF (3%), ALC + CPF (8%) and VC+ALC+CPF (2%) groups, respectively, when compared to the CPF group. However, the PCV in the S/oil group was comparatively higher than either VC+CPF, ALC+CPF or VC+ALC+CPF groups.

4.3.3 Effect of Treatments on Haemoglobin Concentration

No significant difference (P > 0.05) in the Hb concentration was observed between the groups. The lowest mean Hb value was, however, observed in the CPF group (13.70 ± 0.7 g/dL) as it slightly decreased by 7.9% compared to the S/oil group (Figure 4.3). The pretreatment groups, VC+CPF, ALC+CPF and VC+ALC+CPF groups increased by 0.7%, 5.3% and 4.2%, respectively, compared to the CPF group.

4.3.4 Effect of Treatments on Red Blood Cell Indices

4.3.4.1 Effect of Treatments on Mean Corpuscular Haemoglobin Concentration

No significant difference (P > 0.05) was observed in MCHC between the CPF group compared to the S/oil group. The MCHC value in the CPF group (32.50 ± 0.8 g/dL) slightly increased by 6.1% compared to the S/oil group (30.63 ± 0.58 g/dL). Conversely, there was a significant increase (P < 0.05) in MCHC between the S/oil and the VC + CPF groups (Figure 4.4). The MCHC value slightly increased in the VC+CPF (5%), ALC+CPF (2%) and VC+ALC+CPF (2%) groups, respectively, relative to the CPF group.

4.3.4.2 Effect of Treatments on Mean Corpuscular Haemoglobin

No significant change (P > 0.05) was observed between the CPF group and the other groups. The highest mean MCH value was observed in the VC+CPF+CPF group (17.60 ± 1.5 picogram/cell) as it slightly increased by 0.6% compared to S/oil group (Figure 4.4). However, the MCH value decreased in the CPF (3%) compared to S/oil group. The MCH value slightly decreased by 4% and 2%, respectively in the VC+CPF and ALC+CPF groups, but increased by 4.0% in the VC+ALC+CPF group, relative to the CPF group.

4.3.4.3 Effect of Treatments on Mean Cell Volume

The differences observed between the treatment groups did not differ (P > 0.05) (Figure 4.4). However, the VC+CPF group had the lowest MCV as it decreased by 16% compared to the S/oil group. The MCV value also slightly decreased in the CPF (10%) relative to S/oil group. There was a comparative decrease in MCV value in VC+CPF (6.8%) and ALC+CPF (0.7%) groups, and a marginal increase in the VC+ALC+CPF (2%) group when respectively compared to CPF group.

4.3.5 Effect of Treatments on Platelet Count

There was no significant change (P > 0.05) observed in the platelet count between the treatment groups (Figure 4.5). The platelet count slightly decreased in the CPF (13.4%), compared to the S/oil group. However, there was a decrease in platelet count in the VC+CPF (1.5%) and VC+ALC+CPF (16.6%) groups, and a marginal increase in the ALC+CPF (1.2%) group when respectively compared to the CPF group.

4.3.6 Effect of Treatments on Total White Blood Cell Count

There was no significant change (P > 0.05) in total WBC count between the groups (Figure 4.6). The WBC slightly increased in the CPF group by 7.5% compared to the S/oil group. However, there was a decrease of 31%, 10% and 24% in WBC in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively when compared to the CPF group.

4.3.7 Effect of Treatments on Absolute Neutrophil Count

No significant difference (P > 0.05) was observed between the treatment groups. There was an increase by 7% in the CPF group compared to the S/oil group. However, in the pretreatment groups, there was a decrease in the VC+CPF (10%) group and a slight increase in the ALC+CPF (30%) and VC+ALC+CPF (24%) groups, respectively, relative to the CPF group (Figure 4.7).

4.3.8 Effect of Treatments on Absolute Lymphocyte Count

There was no significant change (P > 0.05) in lymphocyte count between the treatment groups. The highest mean lymphocyte count was observed in the CPF group (12.80 ± 1.2 x 10^9/L) as it increased by 8%, compared to the S/oil group. However, the pretreatment groups had slightly decreased lymphocyte counts in the VC+CPF (35%), ALC+CPF (17%) and VC+ALC+CPF (33%) groups, respectively, relative to the CPF group (Figure 4.7).

4.3.9 Effect of Treatments on Neutrophil/Lymphocyte Ratio

There was no significant difference (P > 0.05) in the neutrophil/lymphocyte ratio between the groups (Figure 4.8). The neutrophil/lymphocyte ratio was lowest in the CPF group compared to the other groups as it decreased by 3% compared to the S/oil group. However, there were 45%, 53% and 68% increases in neutrophil/lymphocyte ratio in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, relative to the CPF group.

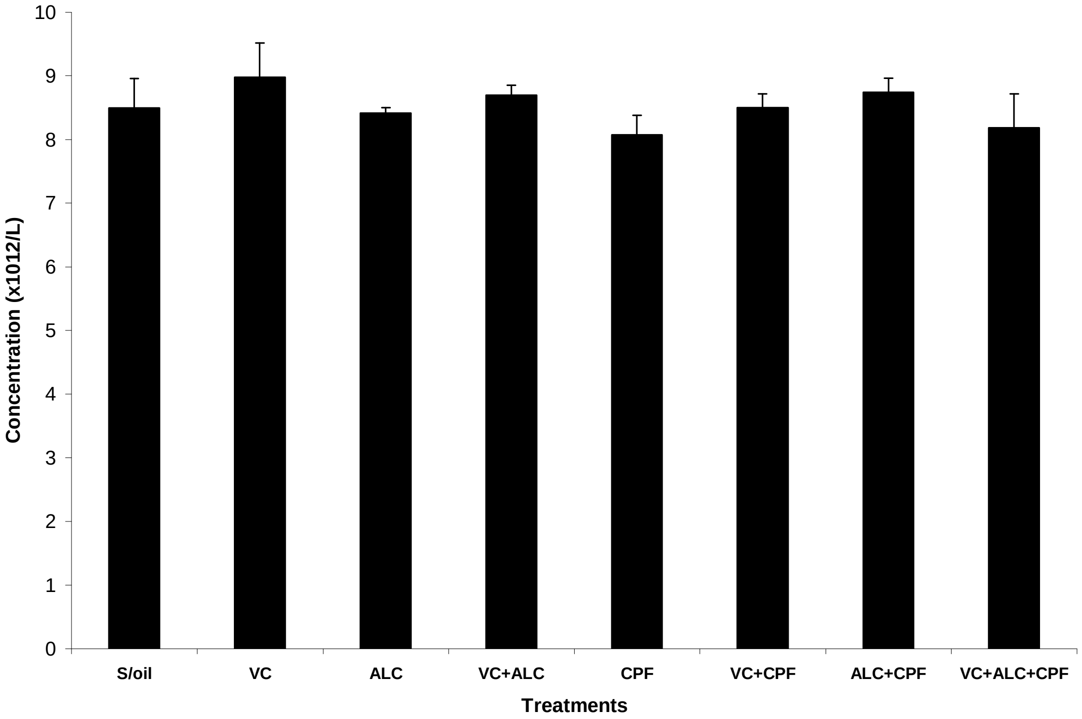


Figure 4.1: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on Erythrocyte count in male Wistar rats (n = 7 per group).

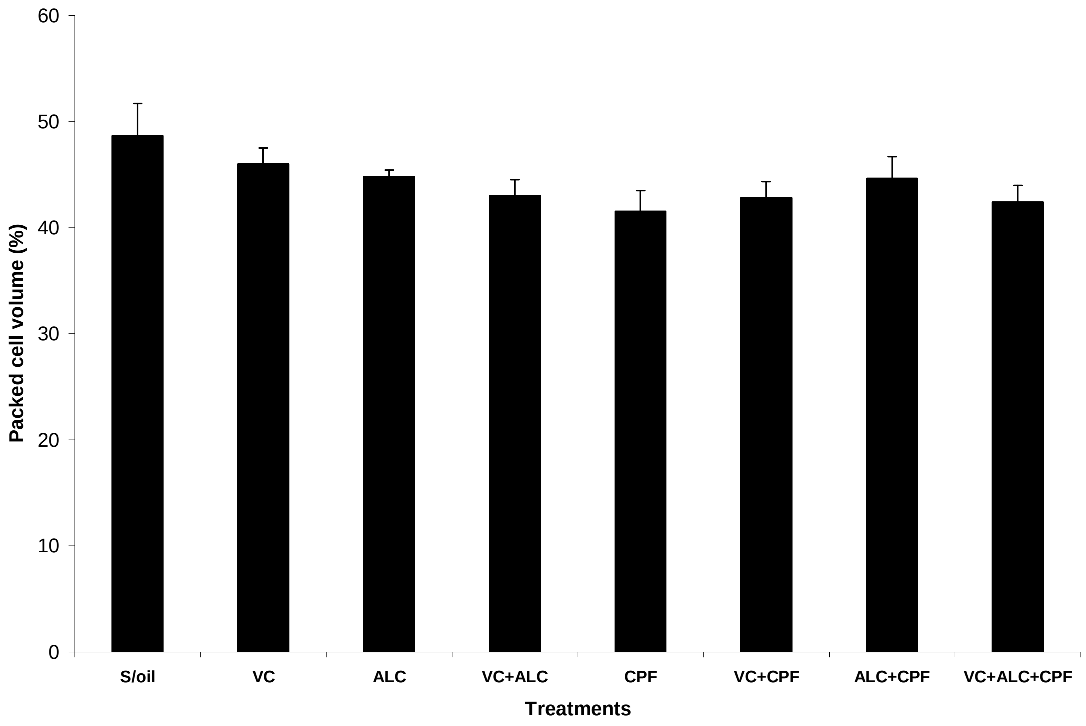


Figure 4.2: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on PCV in male Wistar rats (n = 7 per group).

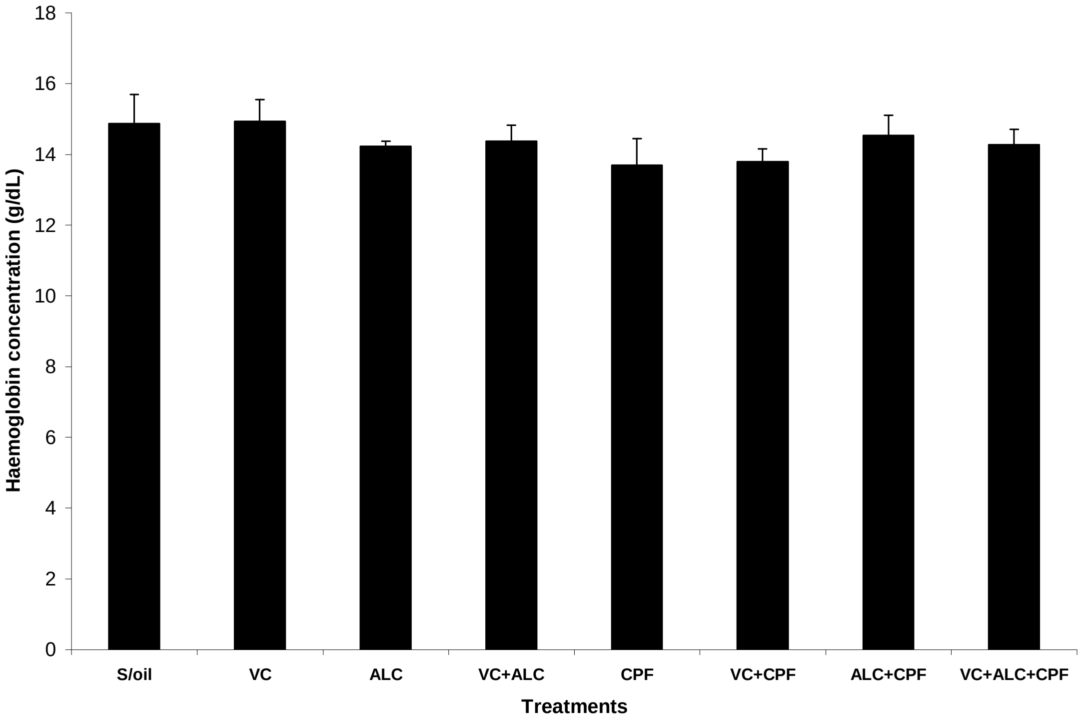


Figure 4.3: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on Hb concentration in male Wistar rats (n = 7 per group).

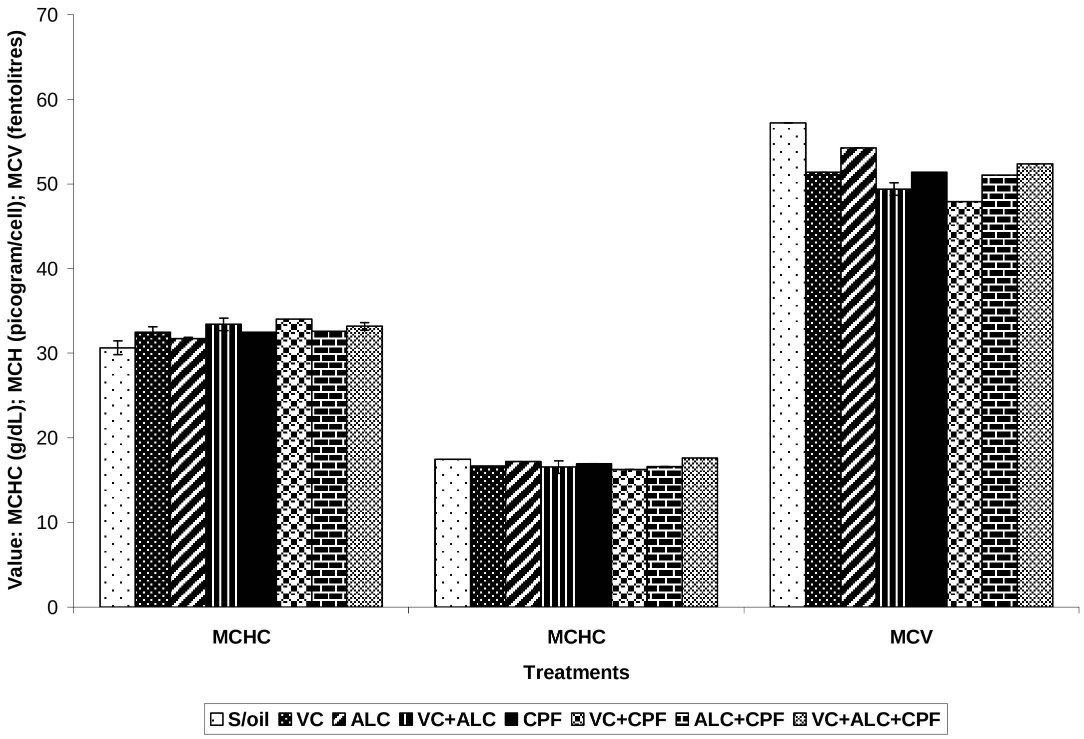


Figure 4.4: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on red blood cell indices in male Wistar rats (n = 7 per group).

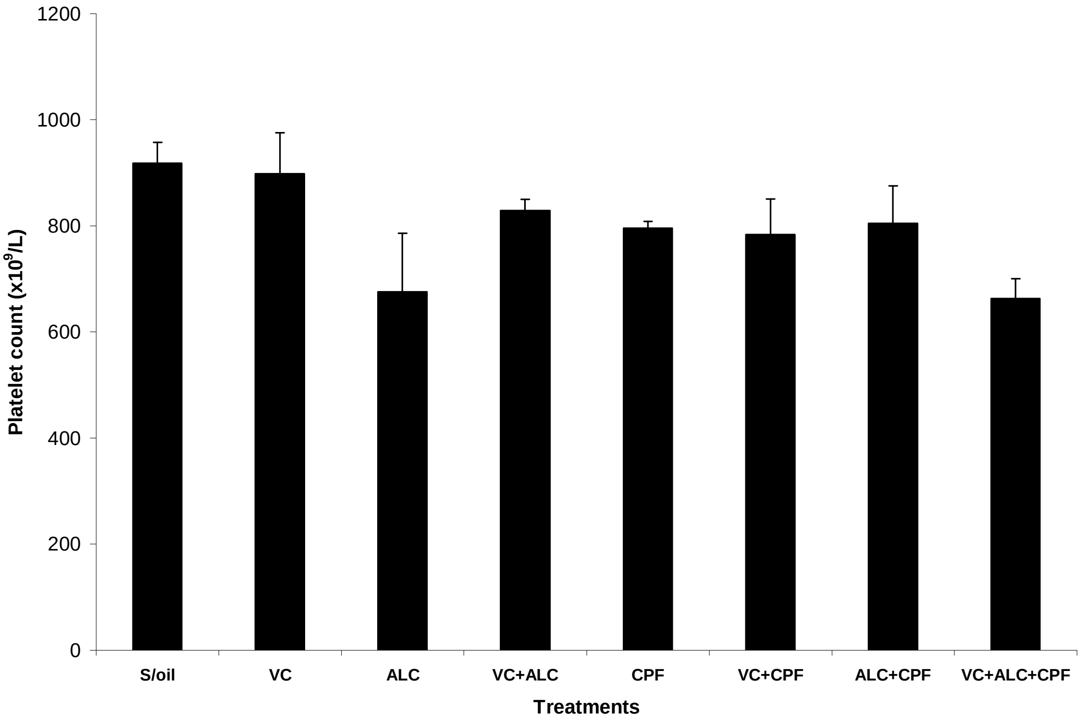


Figure 4.5 Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on platelet count in male Wistar rats (n = 7 per group).

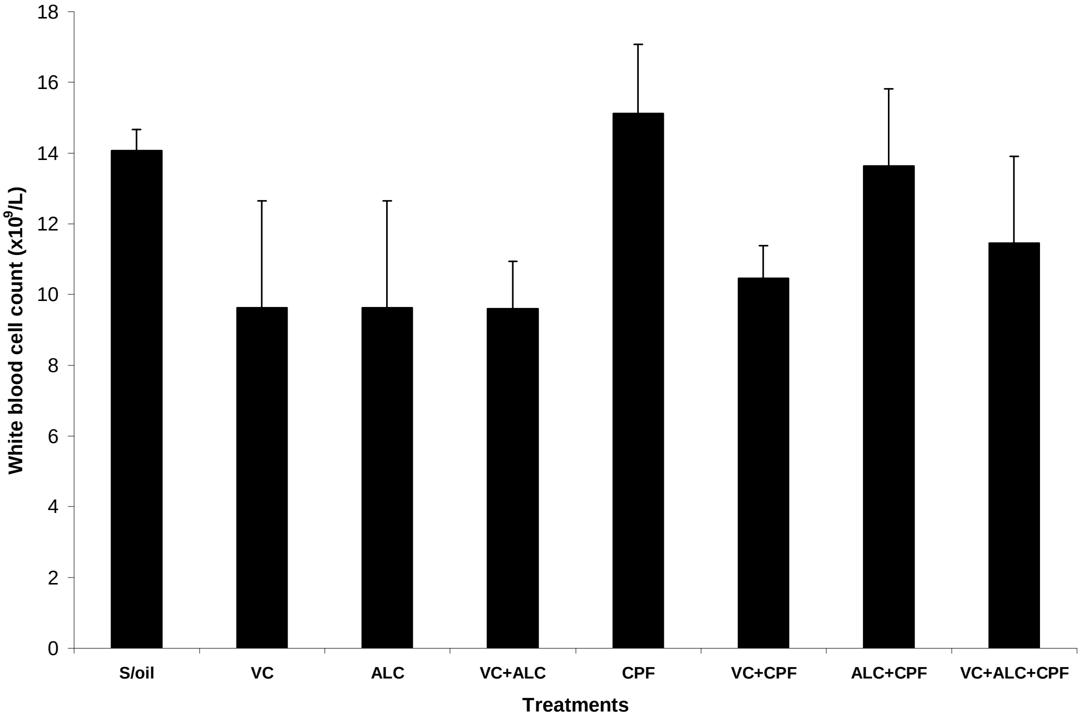


Figure 4.6: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on total white blood cell count in male Wistar rats (n = 7 per group).

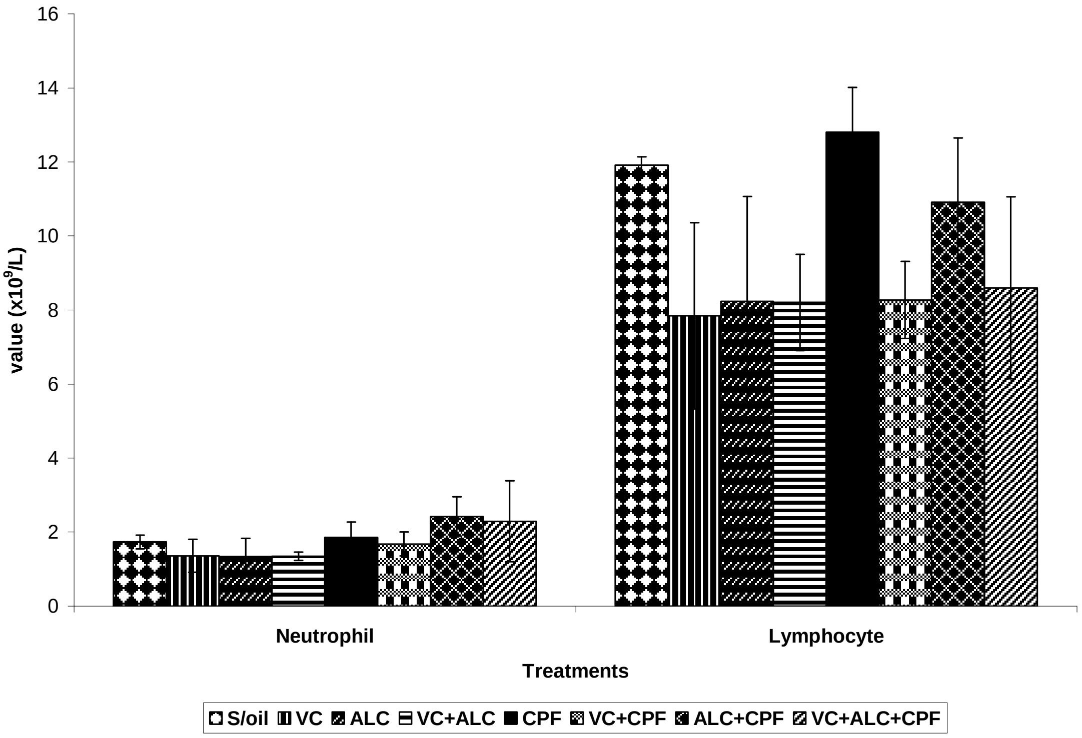


Figure 4.7: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on absolute differential white blood cell count in male Wistar rats (n = 7 rats per group).

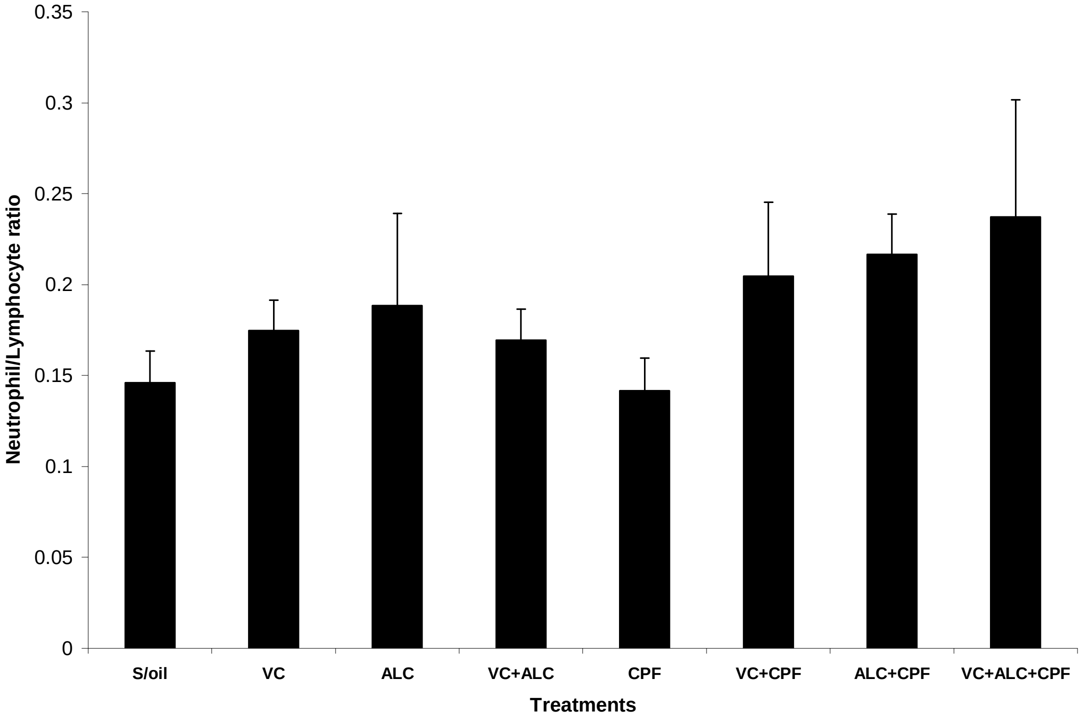


Figure 4.8: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on Neutrophil/Lymphocyte ratio in male Wistar rats (n = 7 per group).

4.3.10 Effect of Treatments on Erythrocyte Osmotic Fragility

Generally, irrespective of the treatment groups, percent haemolysis decreased with increasing NaCl concentration. Haemolysis was complete (100 %) in the standard solvent (distilled water). There was no significant difference ($P > 0.05$) in the degree of erythrocyte osmotic fragility among the rats in the various groups at 0.1, 0.3, 0.5 and 0.7% of NaCl concentration. However, at 0.5% of the NaCl solution, VC+CPF group showed the lowest mean (5.58 ± 6 %) percentage of erythrocyte fragility; while the CPF group showed the highest percentage mean (24.96 ± 11%). At 0.9% of the NaCl concentration, VC+CPF group showed no haemolysis, while there was 5.23 ± 0.9 % erythrocyte osmotic fragility in the ALC+CPF group with the CPF group showing the highest mean (6.18 ± 1.5 %) percentage of erythrocyte osmotic fragility. At 0.7% of NaCl, the CPF group showed the highest mean erythrocyte fragility (6.99 ± 2.0 %), while the VC+CPF group showed the lowest mean erythrocyte fragility (1.75 ± 1.0 %). Conversely, there was a significant decrease ($P < 0.05$) in the degree of erythrocyte osmotic fragility in the VC+CPF group, when compared to those in CPF group at 0.9% of NaCl (Figure 4.9).

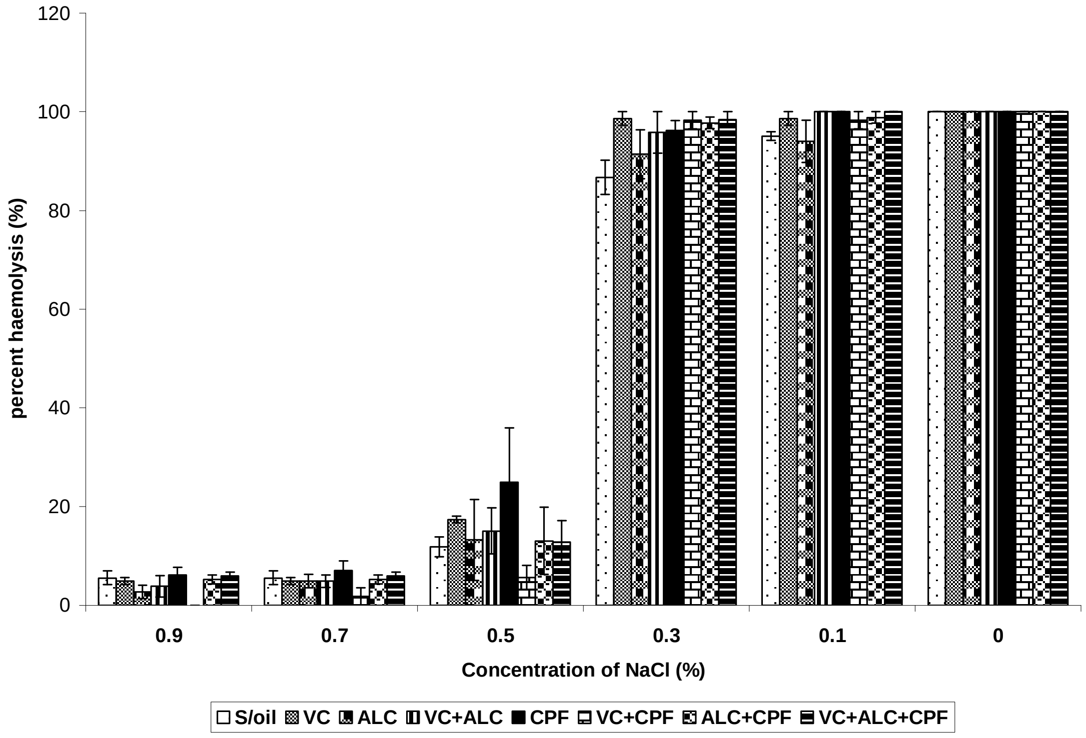


Figure 4.9: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on erythrocyte osmotic fragility in male Wistar rats (n = 7 per group).

4.4 EFFECT OF TREATMENTS ON SERUM BIOCHEMICAL PROFILE

4.4.1 Effect of Treatments on Serum Electrolyte Concentration

4.4.1.1 Effect of Treatments on Serum Sodium ion Concentration

The serum Na^+ concentration level showed no significant ($P > 0.05$) difference between the groups. The lowest mean serum Na^+ concentration was observed in the CPF group (139.3 ± 0.7 mMol/L), when compared to the other treatment groups (Figure 4.10). The Na^+ concentration slightly decreased in the CPF group by 0.5% compared to the S/oil group. However, there were 1.2%, 2.4% and 0.7% increases in Na^+ concentration in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, when compared to the CPF group.

4.4.1.2 Effect of Treatments on Serum Potassium ion Concentration

The serum K^+ concentration showed no significant ($P > 0.05$) difference between the groups. The VC group (5.667 ± 0.39 mMol/L) had the lowest mean value. Furthermore, the K^+ concentration slightly increased in the CPF group by 0.6% compared to the S/oil group. The K^+ concentration showed a slight decrease in the VC+CPF (1.1%), and a slight increase in the ALC+CPF (1.1%) and VC+ALC+CPF (2.2%) groups, respectively, when compared to the CPF group (Figure 4.10).

4.4.1.3 Effect of Treatments on Serum Chloride ion Concentration

The serum Cl^- concentration showed no significant ($P > 0.05$) difference between the groups. However, the lowest mean Cl^- concentration was observed in the CPF group (97.33 ± 1.76 mMol/L) as it marginally decreased by 2% compared to the S/oil group (Figure 4.10). There were 4%, 3% and 2% increase in Cl^- concentration in VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, when compared to the CPF group.

4.4.1.4 Effect of Treatments on Serum Bicarbonate Concentration

No significant ($P > 0.05$) difference was observed between the groups. The lowest mean value of HCO_3^- concentration was observed in the CPF group (25.00 ± 0.58 mMol/L) as it decreased by 2.6% compared to the S/oil group (Figure 4.10). However, there were 1.3%, 4% and 5% increases in HCO_3^- concentration in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, when compared to the CPF group.

4.4.2 Effect of Treatments on Serum Glucose Concentration

A significant reduction ($P < 0.001$) in serum glucose level was observed in the CPF group (2.83 ± 0.12 mg/dl) when compared to the S/oil group (5.00 ± 0.35 mg/dl). Significant changes were observed between S/oil vs. VC groups ($P < 0.001$), S/Oil vs. VC+CPF groups ($P < 0.05$), S/oil vs. ALC + CPF groups ($P < 0.05$), VC vs. ALC groups ($P < 0.001$), VC vs. VC+ALC groups ($P < 0.01$), VC vs. VC+ALC + CPF groups ($P < 0.01$), ALC vs. CPF groups ($P < 0.001$), ALC vs. VC +CPF groups ($P < 0.001$), ALC vs. ALC +CPF groups ($P < 0.05$), CPF vs. ALC + VC groups ($P < 0.001$) and CPF vs. VC + ALC+CPF groups ($P < 0.01$) (Figure 4.11). However, there were 35%, 32% and 54% increases in the serum glucose concentration in the VC+CPF, ALC+CPF and VC+ALC+ CPF groups, respectively, compared to the CPF group.

4.4.3 Effect of Treatments on Serum Total Protein, Albumin and Globulin Concentration

4.4.3.1 Effect of Treatments on Serum Total Protein Concentration

No significant (P > 0.05) change was observed in the TP concentration between the CPF and the S/oil groups. TP level significantly (P < 0.01) increased in the ALC+CPF group when compared to the CPF group. Significant changes were observed in S/oil vs. VC groups (P < 0.05), S/oil vs. ALC+CPF groups (P < 0.01), VC vs. CPF groups (P < 0.05), VC+CPF vs. ALC+CPF groups (P < 0.01), ALC+CPF vs. ALC+CPF groups (P < 0.01) and ALC+CPF vs. VC+ALC+CPF groups (Figure 4.12). The TP level showed a marginal decrease of 0.6% in the VC+CPF group compared to the S/oil group. However, there were 3%, 33% and 3% increase in TP concentration in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, relative to the CPF group.

4.4.3.2 Effect of Treatments on Serum Albumin Concentration

There was no significant (P > 0.05) difference in the serum albumin concentration between the treatment groups. The lowest serum albumin level was observed in the CPF group (34.0 ± 0.6 mg/dL) as it decreased by 14% compared to the S/oil group. The serum albumin concentration in the VC+CPF, ALC+CPF and VC+ALC+CPF groups increased by 14%, 12% and 13%, respectively, when compared to the CPF group (Figure 4.12).

4.4.3.3 Effect of Treatments on Serum Globulin Concentration

As shown in Figure 4.12, no significant ($P > 0.05$) difference in the serum globulin level was observed between the CPF and S/oil groups. Significant ($P < 0.05$) increases were observed between VC+CPF vs. ALC+CPF groups and S/oil vs. ALC+CPF groups. However, there were 22% and 15% decrease in globulin concentration in the VC+CPF and VC+ALC+CPF groups, and a 49% increase in ALC + CPF group, respectively, relative to the CPF group.

4.4.4 Effect of Treatments on Serum Urea Level

The serum urea concentration in the CPF group (8.40 ± 0.20 mMol/L) was significantly higher compared to either the VC group ($P < 0.001$) or ALC group ($P < 0.01$). However, between the groups there were significant differences between S/Oil vs. VC ($p < 0.001$), VC vs. ALC ($P < 0.05$), VC vs. VC+CPF ($P < 0.001$), VC vs. ALC+CPF ($P < 0.001$), VC vs. ALC+VC ($P < 0.001$), VC vs. VC+ALC+CPF ($P < 0.001$), ALC vs. ALC+CPF ($P < 0.05$), ALC vs. ALC+VC ($P < 0.05$) and ALC vs. VC+ALC+CPF ($P < 0.05$) (Figure 4.13). The urea concentration in the CPF group was not significantly different ($P > 0.05$) from the S/oil group but comparatively higher by 19.5%. The urea concentration in the pretreated groups, VC+CPF, ALC+CPF and VC+ALC+CPF decreased by 9%, 7% and 6%, respectively, relative to the CPF group.

4.4.5 Effect of Treatments on Serum Creatinine Concentration

The serum creatinine level showed no significant ($P > 0.05$) difference between the groups. The highest mean value was observed in the CPF group (77.0 ± 2.0 mMol/L) as it increased by 22% compared to the S/oil group (Figure 4.14). However, there were 10%, 8% and 16% decrease in creatinine concentration in the VC+CPF, ALC+CPF and VC+ALC+CPF, respectively, relative to the CPF group.

4.4.6 Effect of Treatments on Serum Enzymes

4.4.6.1 Effect of Treatments on Serum Aspartate Aminotransferase Activity

The serum AST value showed no significant ($P > 0.05$) difference between the groups. The CPF group had the lowest value (179 ± 6.9 IU/L) decreasing by 20.4% compared to the S/oil group (Figure 4.15). However, the AST activity showed an increase in the pretreatment groups, VC+CPF (13%), ALC+CPF (24%) and VC+ALC+CPF (3%) respectively, relative to the CPF group.

4.4.6.2 Effect of Treatments on Serum Alanine Aminotransferase Activity

There was no significant difference in the serum ALT activity in between groups except in the CPF group where there was a significant ($P < 0.05$) elevation when compared to the VC group. The serum ALT activity was highest (115.3 ± 2.0 IU/L) in the CPF group as it slightly increased by 7% compared to the S/oil group (Figure 4.15). However, the ALT activity showed a decrease in the VC+CPF (17%), ALC+CPF (11%) and VC + ALC+ CPF (10%) groups, respectively, relative to the CPF group.

4.4.6.3 Effect of Treatments on Serum Alkaline Phosphatase Activity

The serum ALP activity in the CPF group showed a significant ($P < 0.05$) increase when compared to the S/oil group. Significant ($P < 0.05$) increase in the ALP activity was also observed in the S/oil compared to the ALC groups. The CPF group had the highest ALP activity (372 ± 23 IU/L) as it increased by 76% compared to the S/oil group (Figure 4.15). There were 33%, 26% and 38% decrease in the ALP activities in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively, relative to the CPF group.

4.4.7 Effect of Treatments on Serum Creatine Kinase Activity

No significant ($P > 0.05$) changes were observed in CK activities between the treatment groups. The highest mean value of CK activity was observed in the CPF group (3890 ± 11 IU/L) as it slightly increased by 13% compared to the S/oil group (Figure 4.16). However, the CK activities decreased in the VC + CPF (8%), ALC+CPF (6%) and VC + ALC + CPF (40%) groups, respectively, relative to the CPF group.

Figure 4.10: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum Na^+, K^+, Cl^- and HCO_3^- concentrations in male Wistar rats (n = 7 per group).

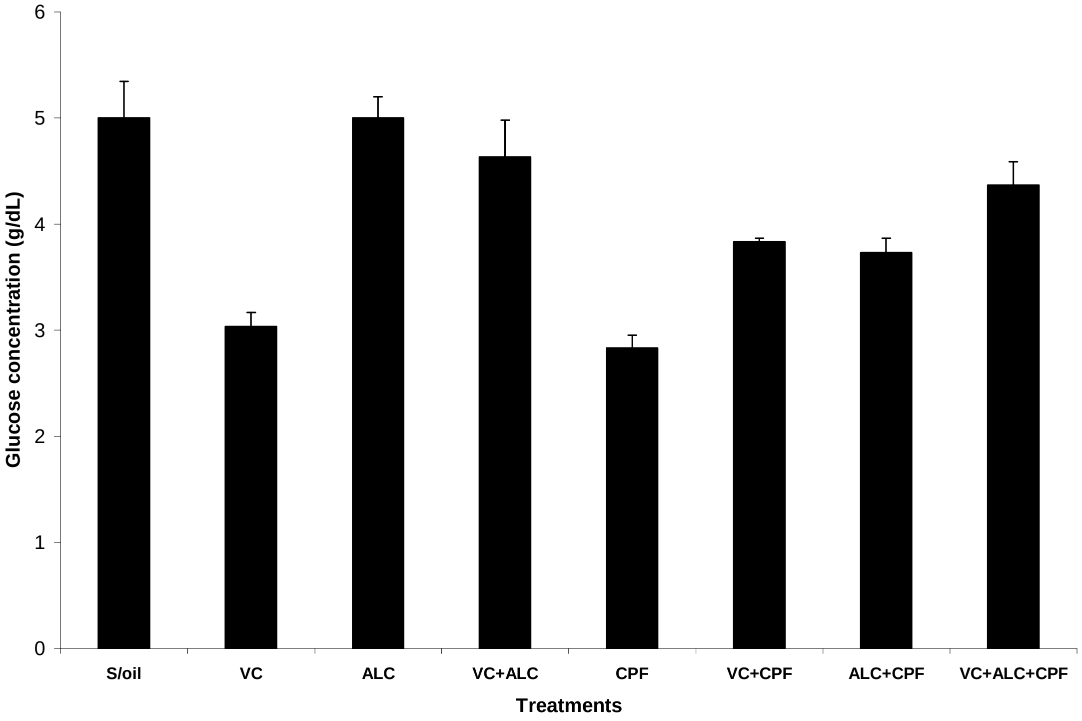

6
5
4
3
2
1
0
Glucose concentration (g/dL)
S/oil
VC
ALC
VC+ALC
CPF
VC+CPF
ALC+CPF
VC+ALC+CPF
Treatments

Figure 4.11: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum glucose concentration in male Wistar rats (n = 7 per group).

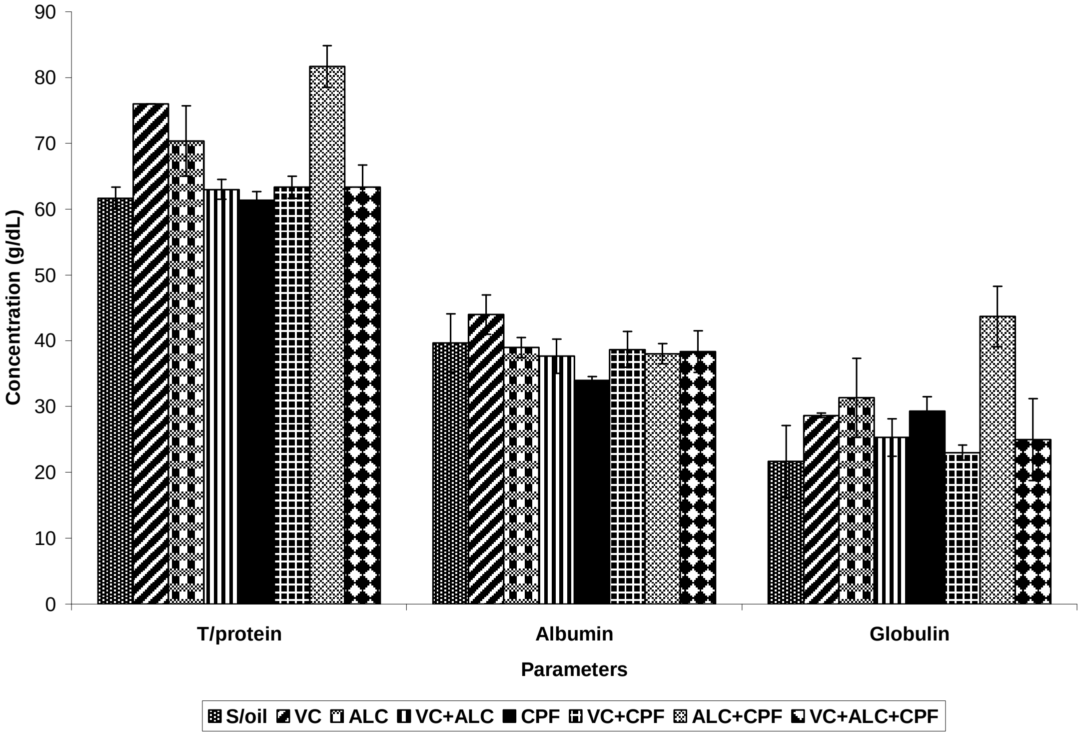


Figure 4.12: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum total protein, albumin and globulin levels in male Wistar rats (n = 7 per group).

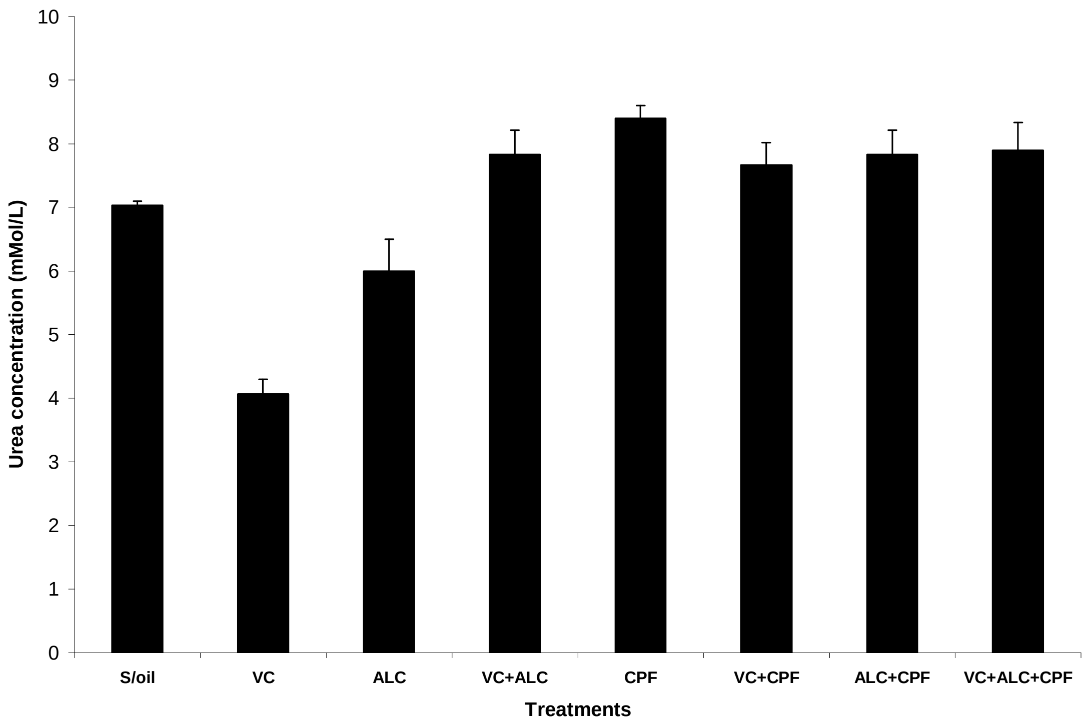


Figure 4.13: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum urea concentration in male Wistar rats (n = 7 per group).

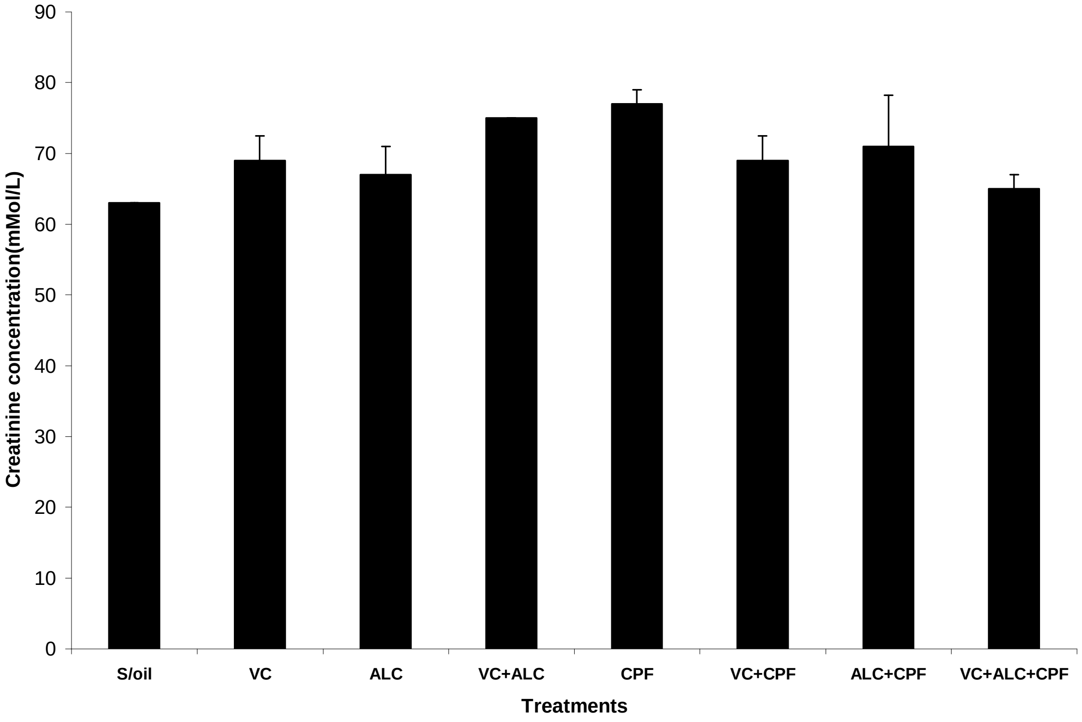


Figure 4.14 Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum creatinine concentration in male Wistar rats (n = 7 per group).

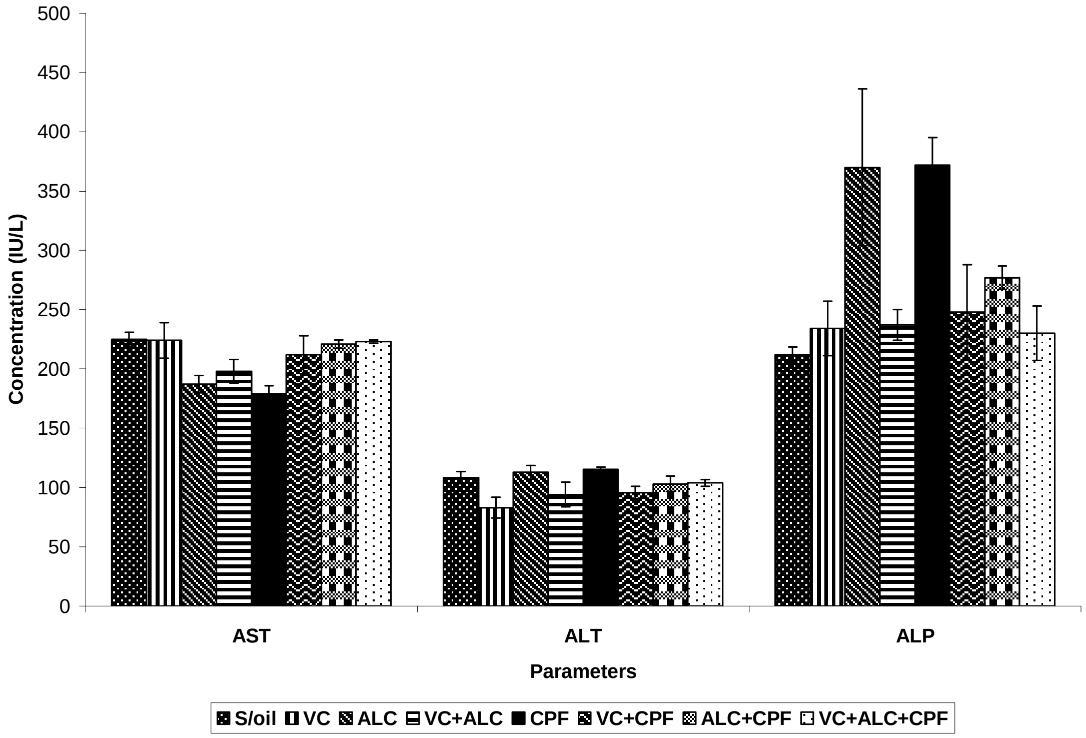
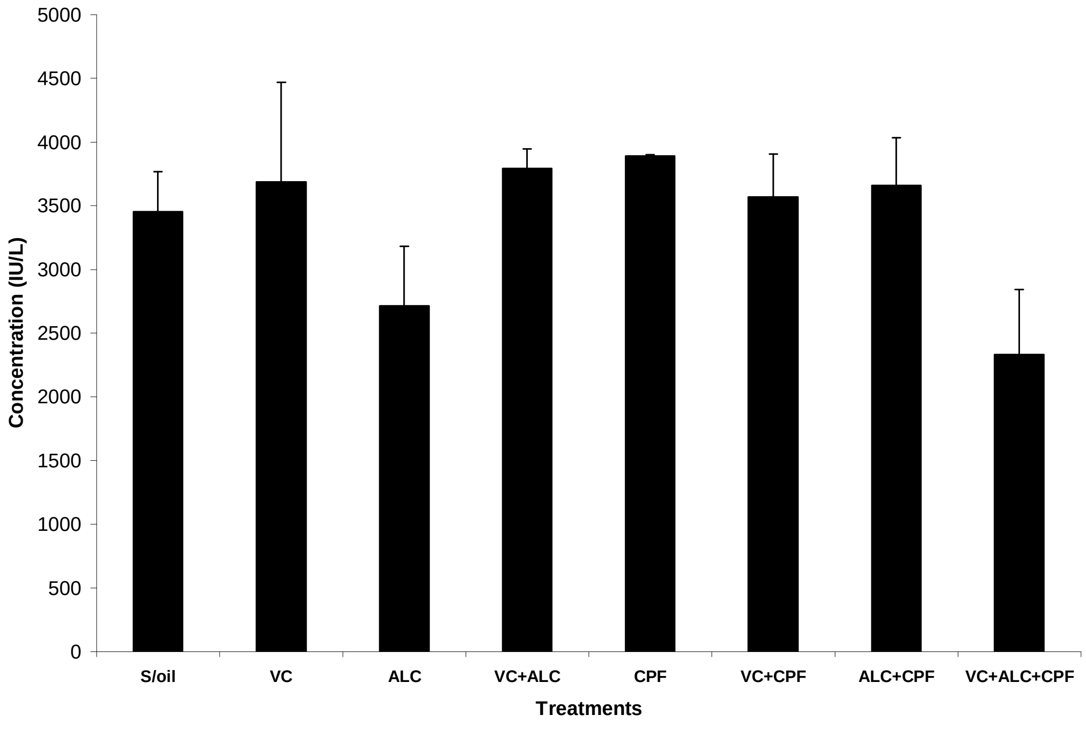


Figure 4.15: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on activities of liver enzymes in male Wistar rats (n = 7 per group).



5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Concentration (IU/L)
S/oil
VC
ALC
VC+ALC
CPF
VC+CPF
ALC+CPF
VC+ALC+CPF
Treatments

Figure 4.16: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on serum creatine kinase activity in male Wistar rats (n = 7 per group).

4.5 EFEECT OF TREATMENTS ON HEPATIC ANTIOXIDANT LEVELS

4.5.1 Effect of Treatments on Hepatic Superoxide Dismutase Activity

No significant ($P > 0.05$) change in the specific activity of SOD was observed in the CPF group (72.44 ± 14 IU/ mg protein), when compared to the S/oil group (98 ± 24 IU/ mg protein). However, the hepatic SOD activity in the CPF group decreased by 26% compared to the S/oil group. The SOD activity increased in the VC + CPF (22.5%), ALC + CPF (17%) and VC + ALC + CPF (33%) groups, respectively, relative to the CPF group (Figure 4.17).

4.5.2 Effect of Treatments on Hepatic Catalase Activity

The specific activity of CAT did not differ ($P > 0.05$) between the treatment groups. The hepatic CAT activity in the CPF group decreased by 30% compared to the S/oil group. The activity in the VC + CPF, ALC + CPF and VC + ALC + CPF groups increased by 20%, 2.4% and 34%, respectively, relative to the CPF group (Figure 4.18).

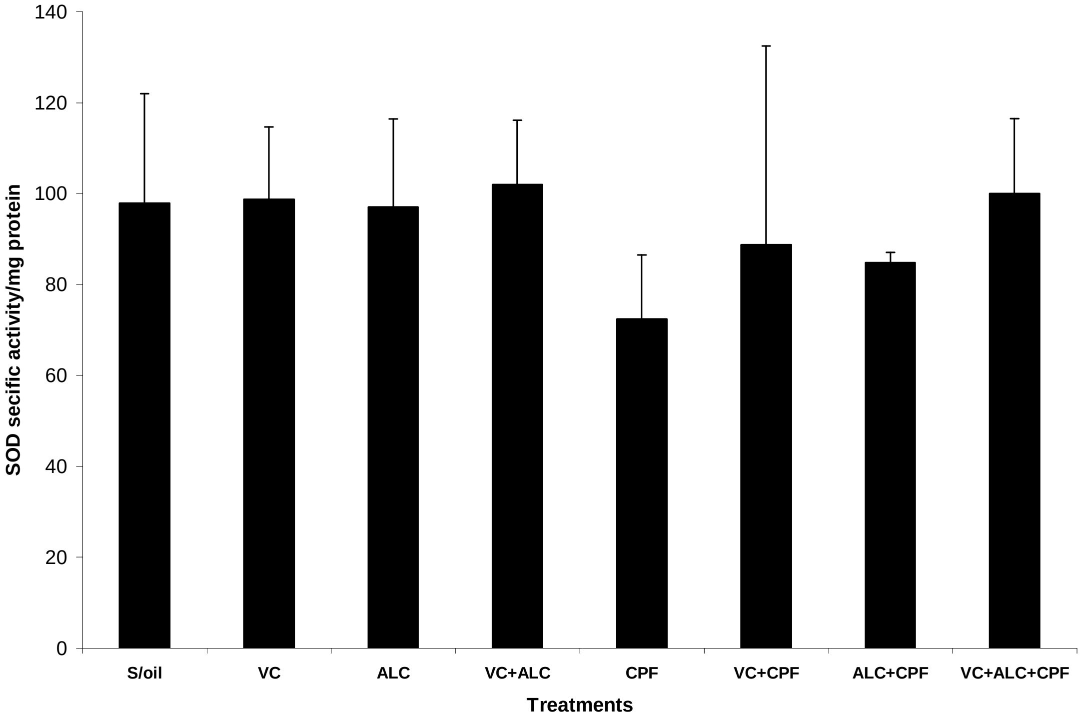


Figure 4.17: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on hepatic superoxide dismuyase specific activity in male Wistar rats (n = 7 per group).

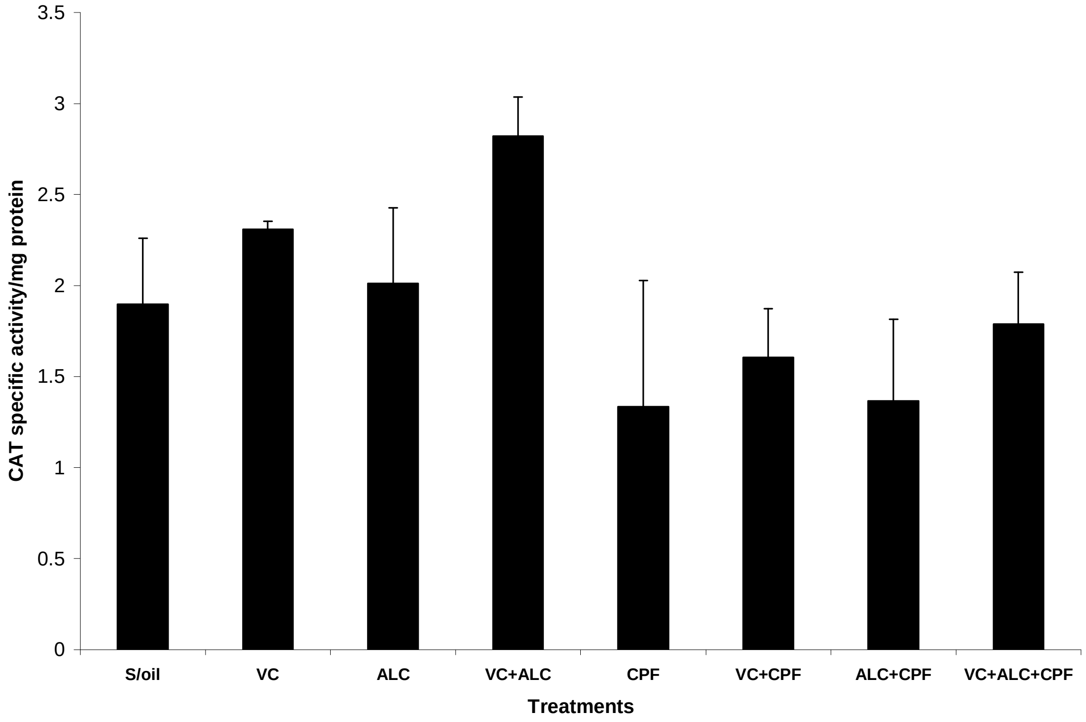


Figure 4.18: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on hepatic catalase specific activity in male Wistar rats (n = 7 per group).

4.6 EFFECTS OF TREATMENTS ON HEPATIC MALONALDEHYDE CONCENTRATION

A significant ($P < 0.001$) increase in MDA concentration was recorded in the CPF group compared to the S/oil group. Significant differences were observed also between VC vs. CPF ($P < 0.001$) groups, ALC vs. CPF ($P < 0.001$) groups, CPF vs. VC+CPF ($P < 0.01$) groups, CPF vs. ALC+CPF ($P < 0.05$) groups, CPF vs. VC+ALC ($P < 0.001$) groups and CPF vs. VC+CPF ($P < 0.001$) groups (Figure 4.19).

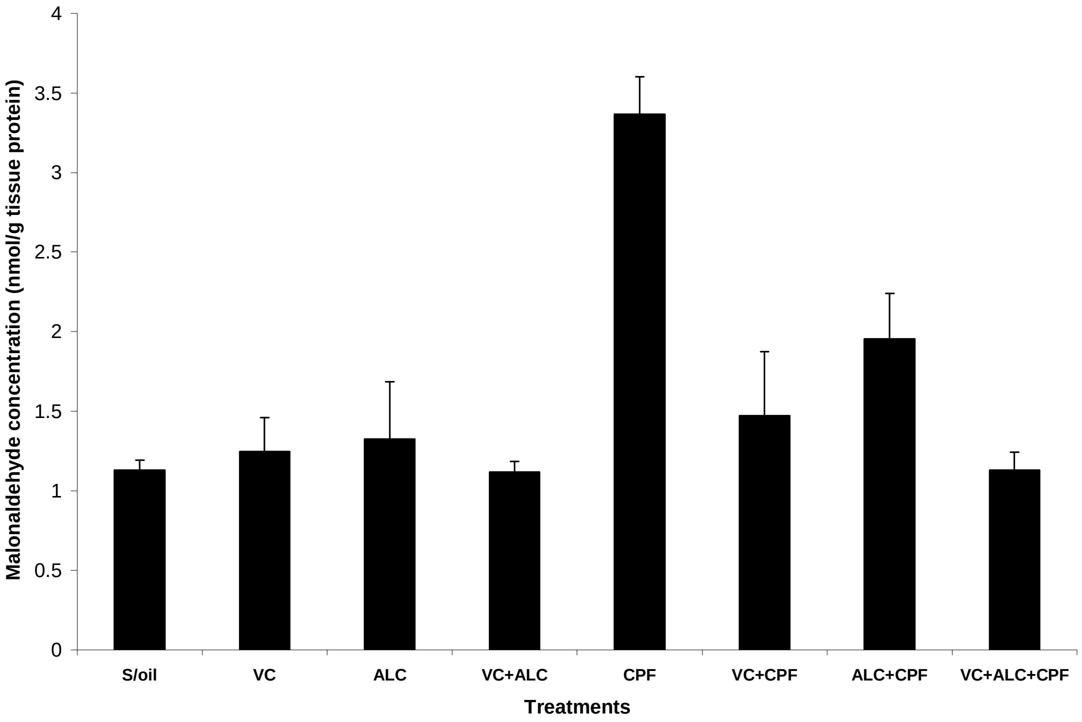


Figure 4.19: Effect of subacute administration of chlorpyrifos (CPF), soya oil (S/oil), vitamin C (VC) and acetyl-L-carnitine (ALC) on hepatic MDA concentration in male Wistar rats (n = 7 rats per group).

4.7 EFFECT OF SUBACUTE ADMINISTRATION OF SOYA OIL, CHLORPYRIFOS ONLY AND CHLORPYRIFOS AND VITAMIN C AND/OR ACETYL-L-CARNITINE ON TISSUE PATHOLOGY

4.7.1 Effect of Treatments on Gross Lesions

There were no observable gross lesions in rats in the control group. Rats exposed to CPF only showed mild congestion of the heart and brain vessels; mild congestion and enlargement of the spleen; mild congestion and petechial haemorrhages of the liver, lungs and kidney, and enteritis. Rats in VC+CPF group showed petechial haemorrhages of the lung, liver and kidneys; enteritis and splenic enlargement. Gross lesions observed in ALC+CPF group included haemorrages on the liver and spleen and mild congestion of kidney. The gross pathological lesion shown by rats in VC+ALC+CPF group were mild congestion of the lungs, spleen and heart; petechial haemorrhages on the liver. In the VC and/or ALC groups, there were no observable gross pathologic lesions in the rats.

4.7.2 Effect of Treatments on Histopathological Changes

Liver: No histopathological lesion was observed in rats in the control (Plate I) and ALC (Plate III) groups. There were diffuse fatty changes in the hepatic cells of rats in the VC group (Plate II). Fatty degeneration and focal areas of necrosis were observed in the hepatocytes of rats in the CPF group (Plate IV). Mild fatty changes were observed in the hepatic cells of rats in the ALC+CPF

(PlateV) and VC+CPF (Plate VI) groups. While, mild areas of congestion were observed in rats in the VC+ALC+CPF group (Plate VIII). There were also mild areas of fatty changes in the hepatocytes of rats in the VC+ALC group (Plate VII).

Kidneys: No histopathological lesion was observed in the kidneys of the control (Plate IX), VC (Plate X), ALC (Plate XI), ALC+CPF (Plate XIII), VC+CPF group (Plate XIV) and VC+ALC group (Plate XV). The kidneys of rats in the CPF group had focal areas of necrosis of the tubular epithelium (Plate XII). Focal areas of necrosis of the renal tubular epithelium were also observed in the rats in the VC+ALC+CPF group (Plate XVI).

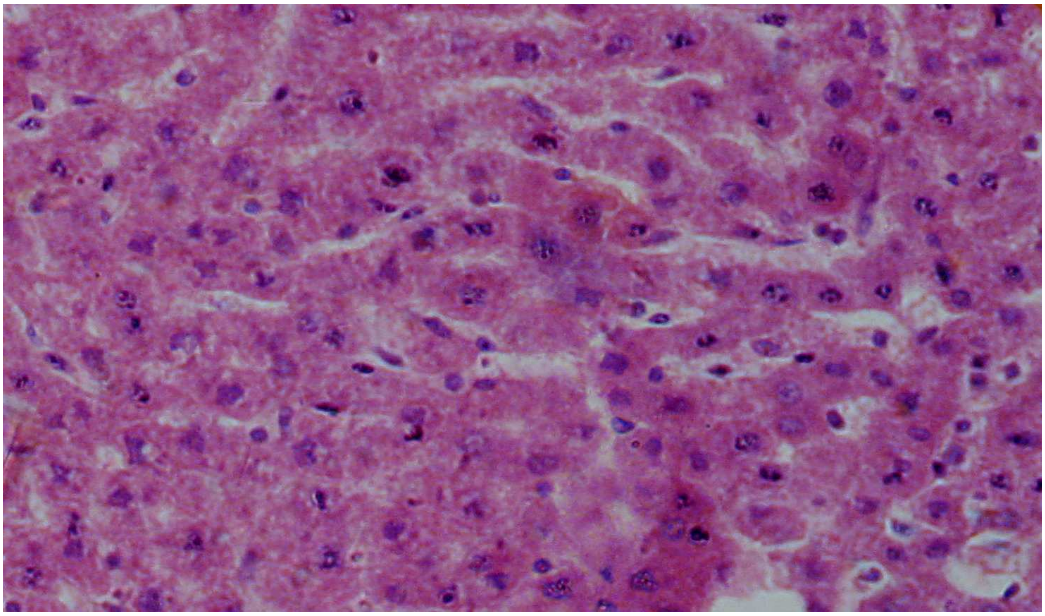


Plate I: Photomigrograph of a section of the liver of a rat exposed to soya oil showing no apparent histopathological lesions (H and E ×400).

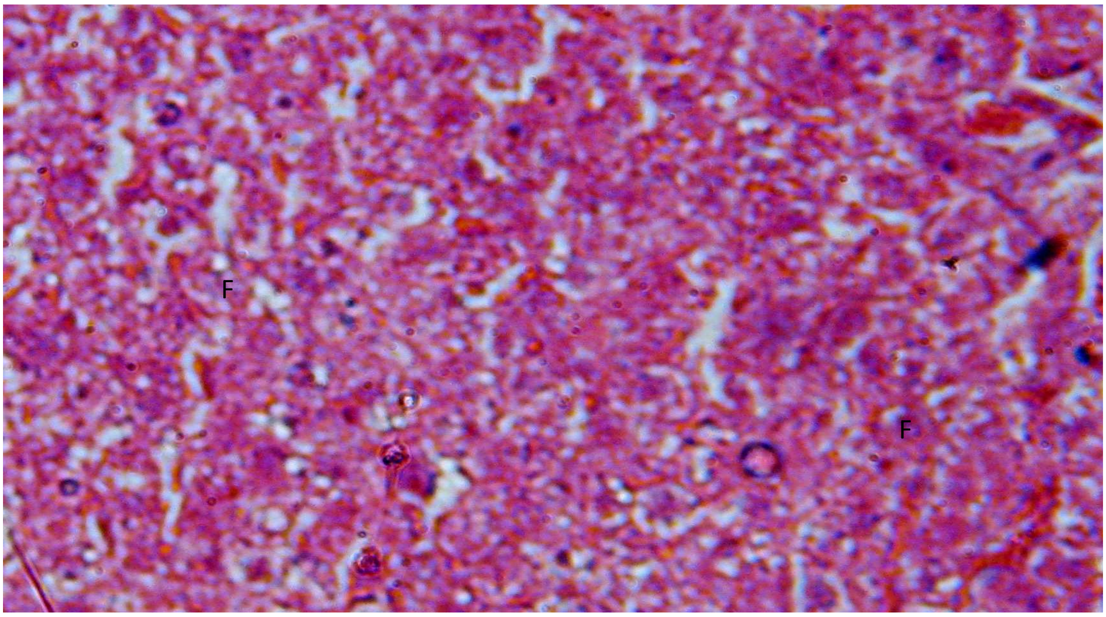


Plate II: Photomicrograph of a section of the liver of a rat exposed to vitamin C only showing diffuse fatty changes (F) in the hepatocytes (H and E ×400).

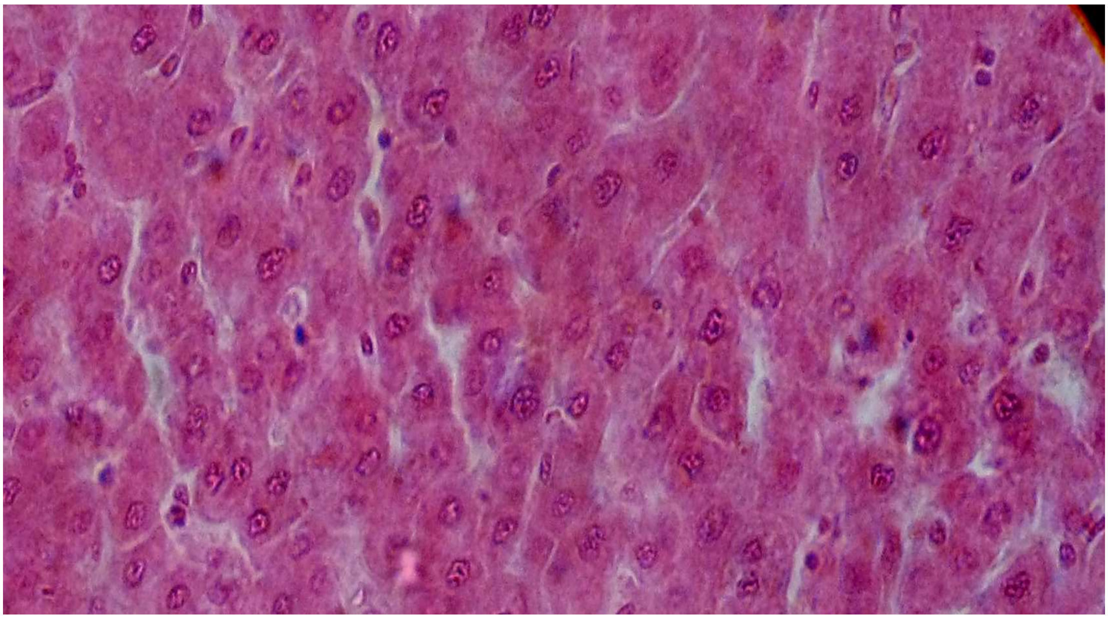


Plate III: Photomicrograph of a section of liver of a rat exposed to acetyl-L-carnitine only showing no apparent histopathological lesions (H and E ×400).

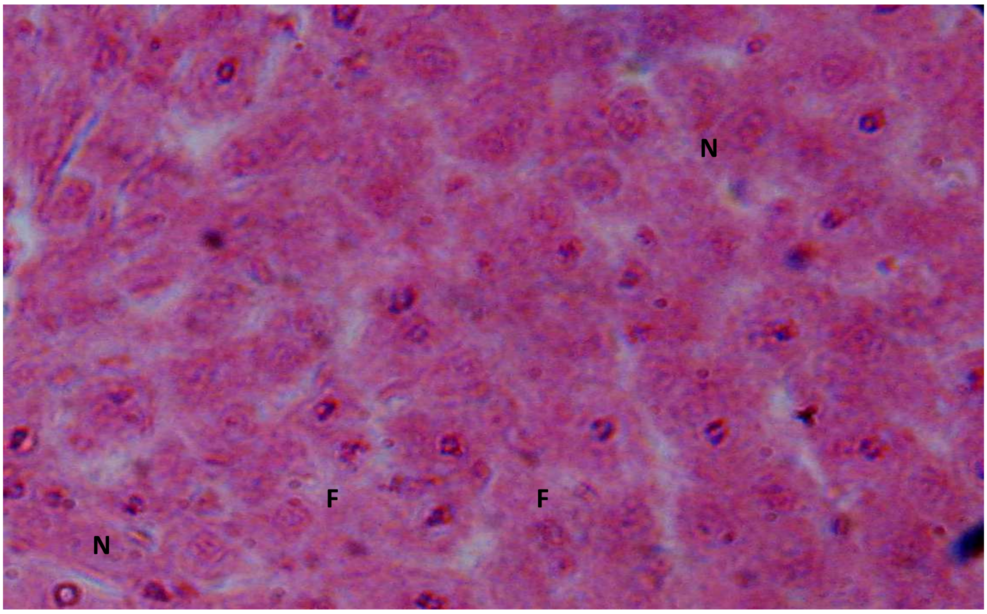


Plate IV: Photomicrograph of a section of the liver of a rat exposed to chlorpyrifos only showing fatty degeneration (F) and focal areas of necrosis (N) of the hepatocytes (H and E ×400).

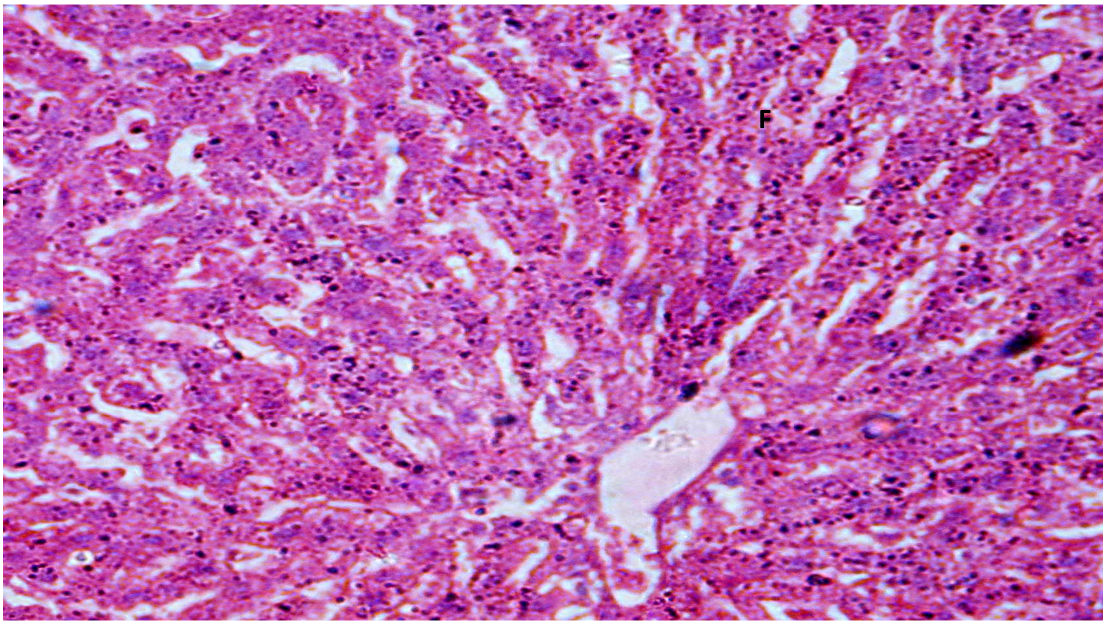

F

Plate V: Photomicrograph of a section of the liver of a rat exposed to acetyl-L-carnitine and chlorpyrifos showing mild fatty changes (F) of the hepatocyte (H and E ×400).

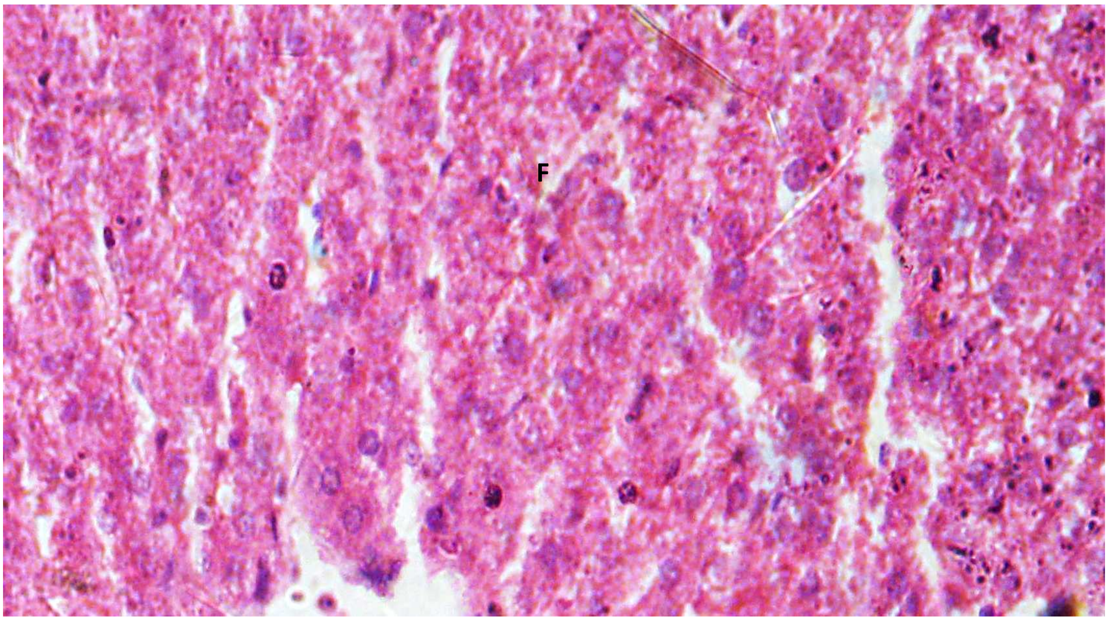


Plate VI: Photomicrograph of a section of the liver of a rat exposed to vitamin C and chlorpyrifos showing mild fatty changes (F) in the hepatocytes (H and E ×400).

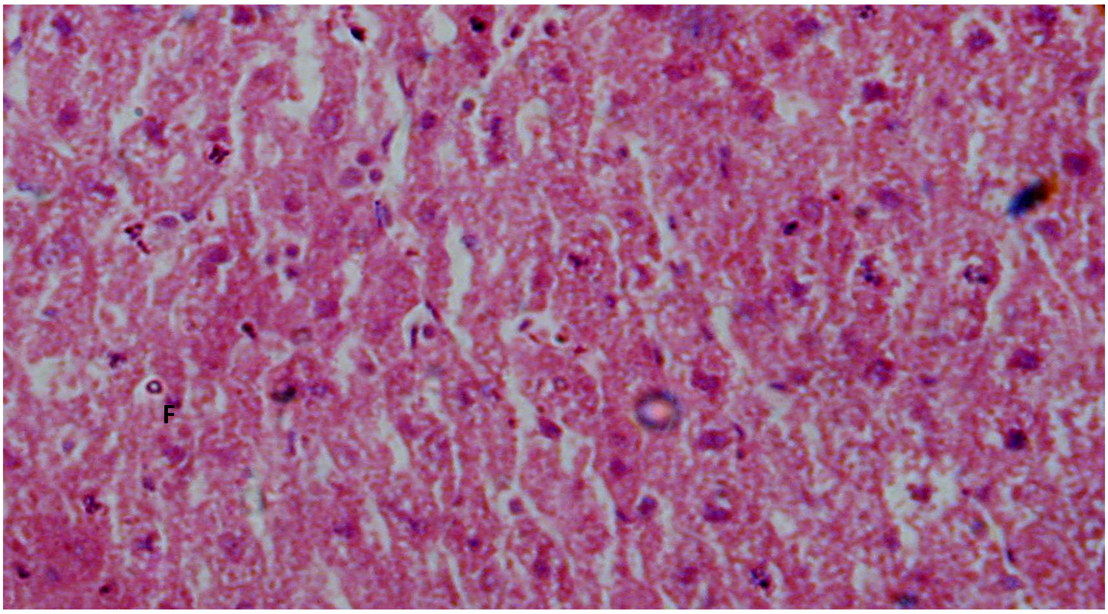


Plate VII: Photomicrograph of a section of the liver of a rat exposed to vitamin C and acetyl-L-carnitine showing mild fatty changes (F) in the hepatocytes (H and E ×400).

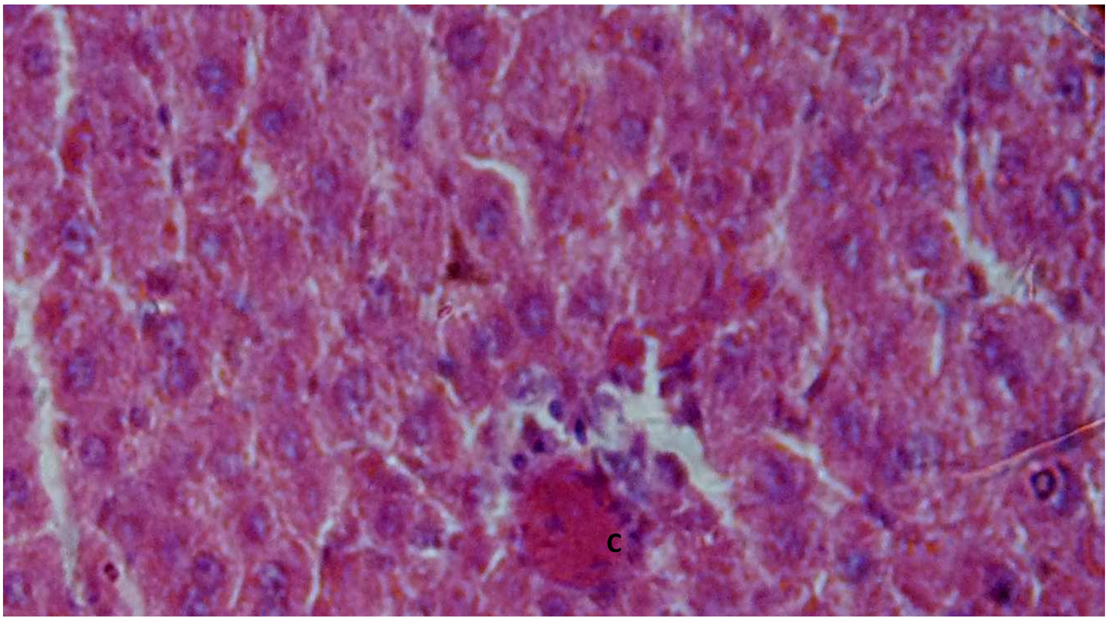

c

Plate VIII: Photomicrograph of a section of the liver of a rat exposed to vitamin C, acetyl-L-carnitine and chlorpyrifos showing mild congestion (C) (H and E ×400).

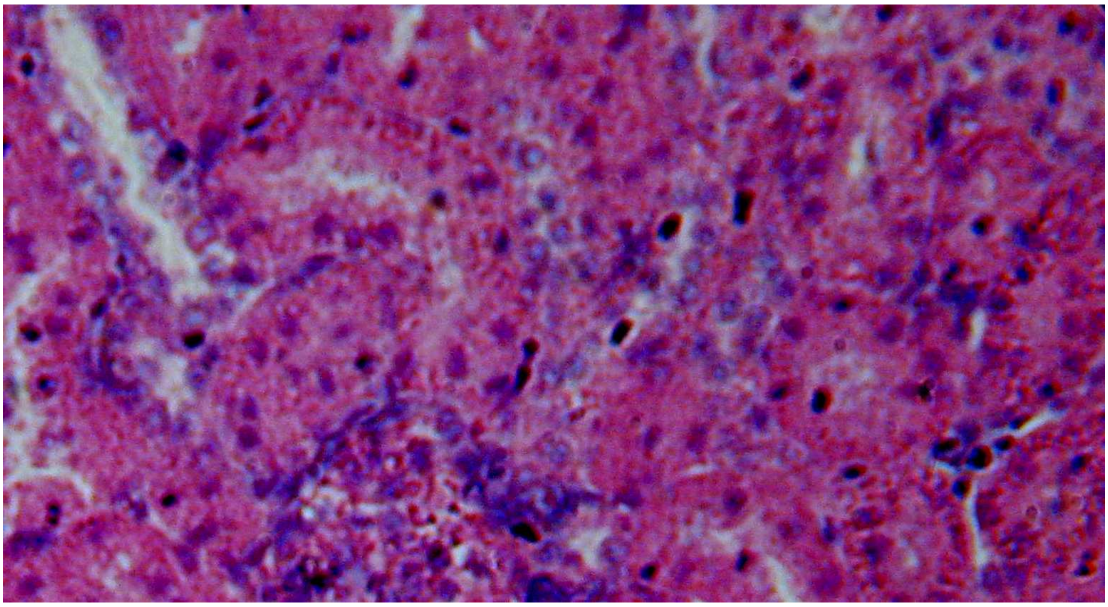


Plate IX: Photomicrograph of a section of the kidney of a rat exposed to soya oil showing no apparent histopathological lesions (H and E ×400).

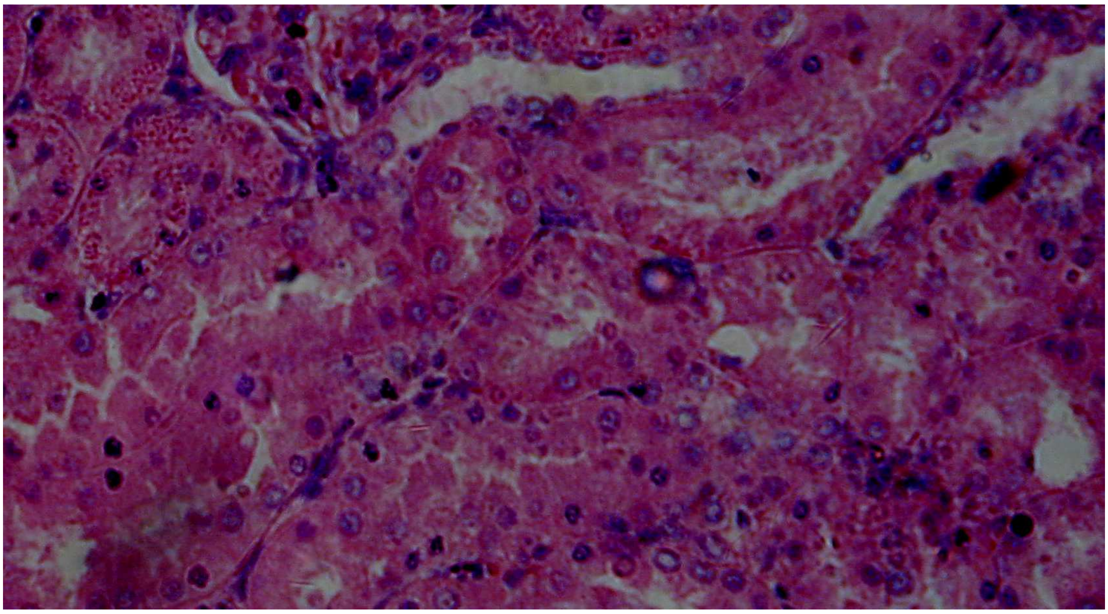


Plate X: Photomicrograph of a section of the kidney of a rat exposed to vitamin C only showing no significant histopathological lesion (H and E ×400).

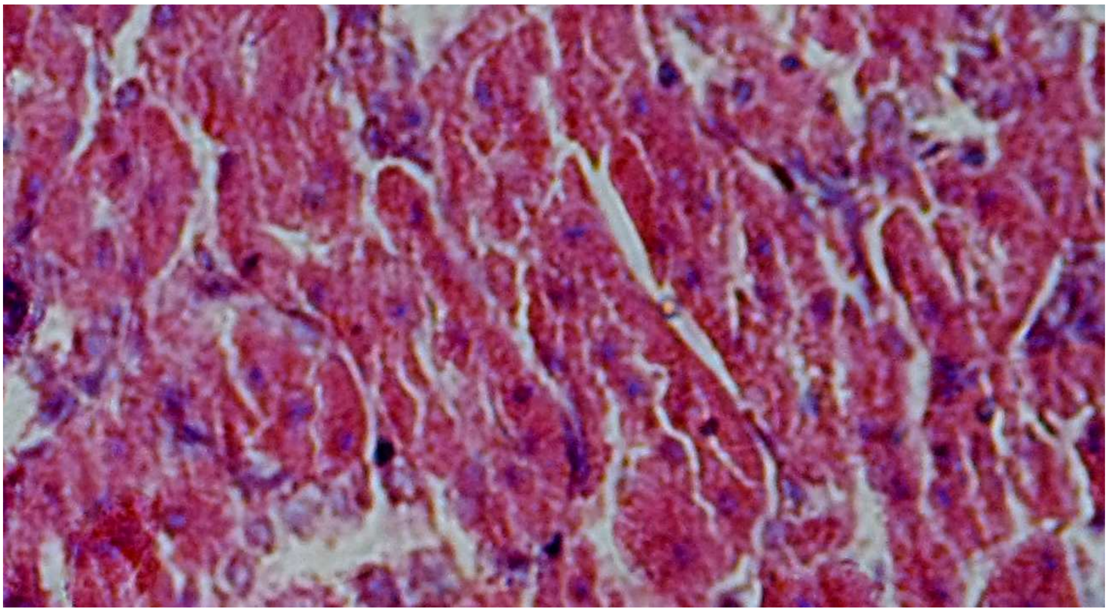


Plate XI: Photomicrograph of a section of the kidney of a rat exposed to acetyl-L-carnitine only showing no apparent histopathological lesions (H and E ×400).

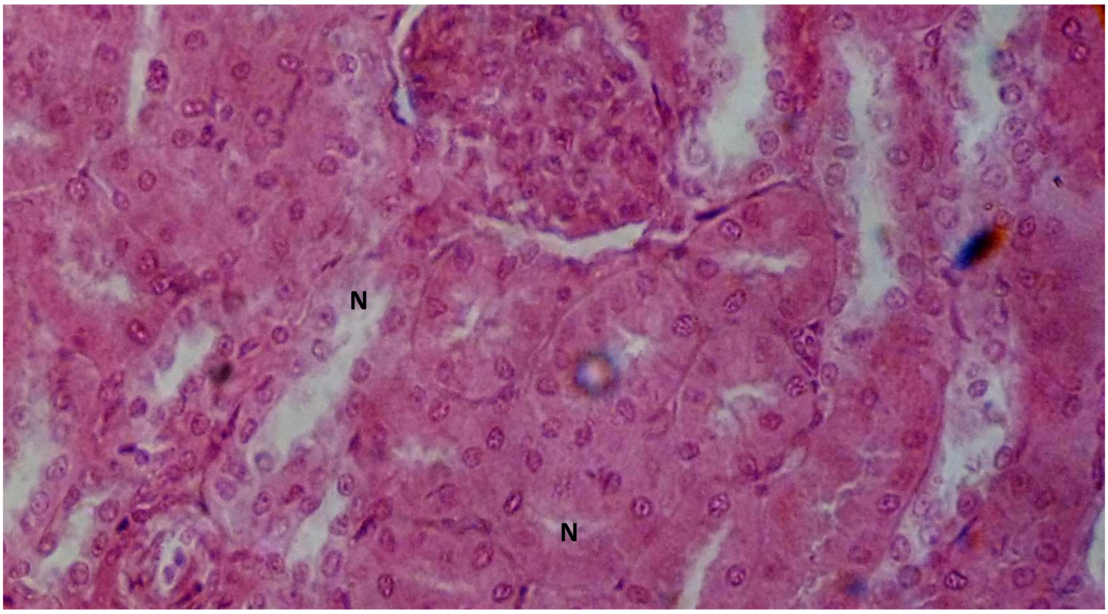

N
N

Plate XII: Photomicrograph of a section of the kidney of a rat exposed to chlorpyrifos only showing areas of necrosis (N) of the renal tubular epithelium (H and E ×400).

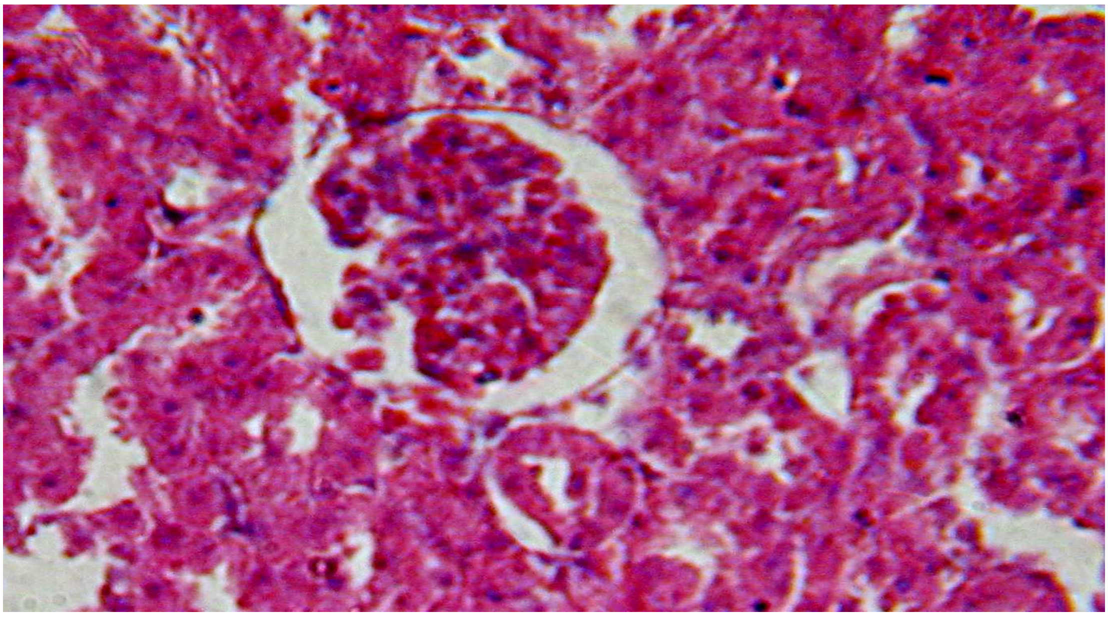


Plate XIII: Photomicrograph of a section of the kidney of a rat exposed to acetyl-L-carnitine and chlorpyrifos showing no apparent histopathological lesions (H and E ×400).

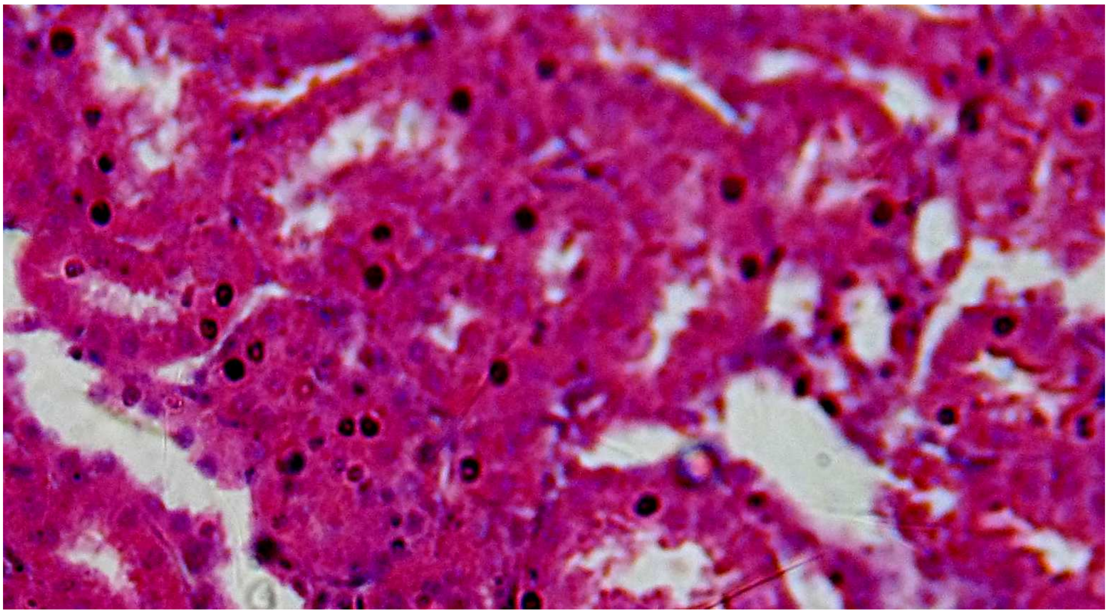


Plate XIV: Photomicrograph of a section of the kidney of a rat exposed to vitamin C and chlorpyrifos showing no apparent histopathological lesions (H and E ×400).

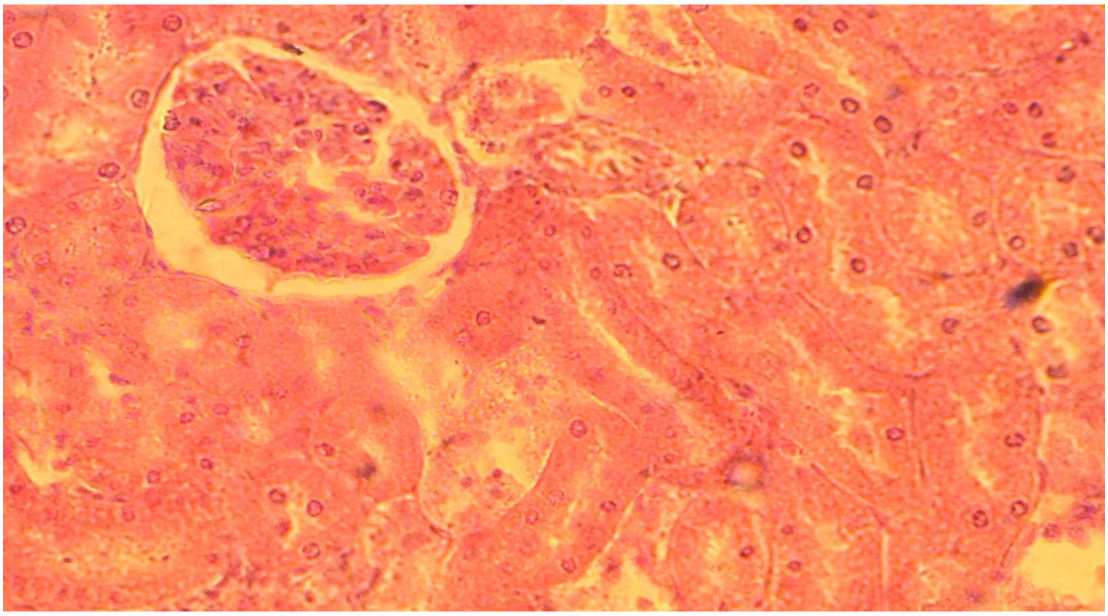


Plate XV: Photomicrograph of a section of the kidney of a rat exposed to vitamin C and acetyl-L-carnitine showing no apparent histopathological lesions (H and E ×400).

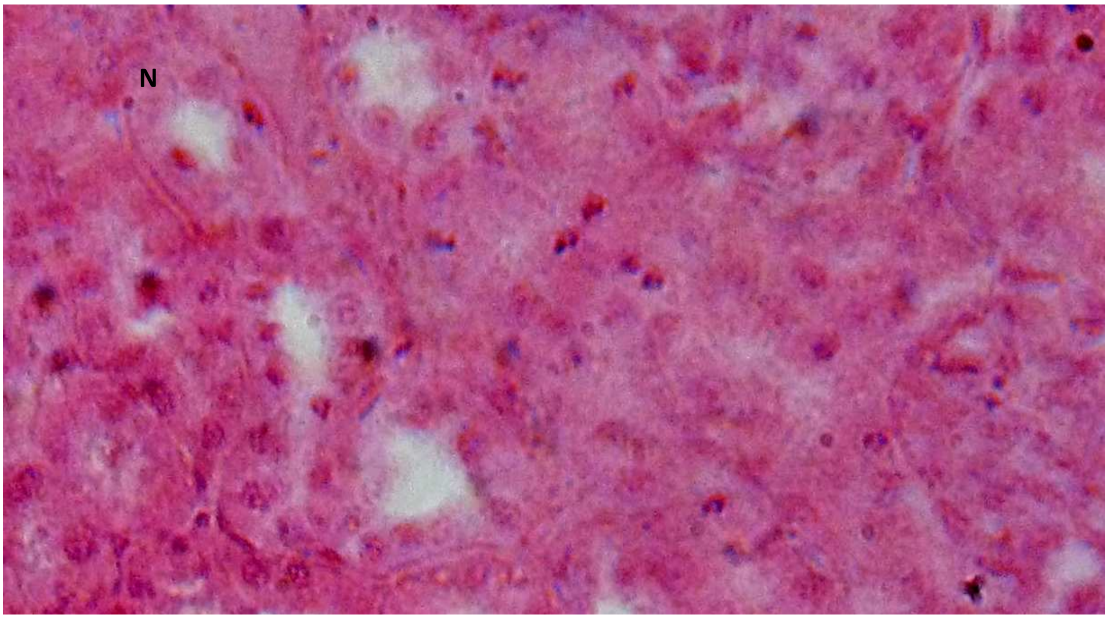


Plate XVI: Photomicrograph of a section of the kidney of a rat exposed to vitamin C, acetyl-L-carnitine and chlorpyrifos showing focal areas of necrosis (N) of renal tubular epithelium (H and E ×400).

CHAPTER 5

DISCUSSION

The LD_{50} value of 85 mg/ kg obtained in the present study was within the range of 82- 270 mg/ kg reported by other workers (Berg, 1986; Cox, 1994), indicating the moderate toxicity of CPF. The clinical signs obtained during the LD_{50} evaluation showed that CPF caused muscarinic, nicotinic and central cholinergic effects, which are characteristic signs observed in OP poisoning (Paudyal, 2008). The huddling observed may be due to induction of hypothermia (Gordon, 1994; Grue *et al*., 1997). Although the mechanism responsible for hypothermia has not been ellucidated, it has been suggested that it may be mediated through the excitation of the cholinergic synapse in the anterior hypothalamus that lowers the set point for heat release (Grue *et al*., 1997).

The clinical manifestations of poisoning in the rats exposed to CPF only were lacrimation and conjunctivitis. The lacrimations have been documented as part of the muscarinic signs observed following exposure to OP insecticides. The ocular signs were due to the inhibition of AChE by CPF, resulting in the accumulation of ACh in the muscarinic cholinergic receptors (Kozawa *et al*., 2009). However, rats in the VC, ALC, VC + CPF, ALC + CPF, VC + ALC and VC + ALC + CPF groups did not manifest any toxic sign. The amelioraion of CPF-induced cholinergic signs following VC pretreatment has been reported in previous studies (Ambali *et al*., 2007; Ambali, 2009). The result showed that VC and ALC ameliorated CPF- induced toxicosis. This may be due to the ability of the antioxidants VC and ALC to reduce oxidative damage. Pettegrew *et al*. (2000) have also reported the protective role of ALC against neurotoxic agents, while Yavuz *et al*. (2004) and Ambali *et al.* (2010b) showed that VC enhances the restoration of cholinesterase activity depressed by methidathion and CPF, respectively. Therefore, the restoration of AChE activity by VC and ALC may contribute to the reduction in cholinergic signs in the pretreated groups observed in the present study.

The results of the parameters obtained in the present study are in agreement with the findings of Rastogi *et al*. (2009), who demonstrated that the toxicity of OPs, such as CPF causes adverse effects on the body's homeiostatic parameters including those associated with haematology and clinical biochemistry changes. Haematological parameters would readily respond to incidental factors, physical stress and environmental stress due to contaminants (Ramesh and Saravanan, 2008).

The lower values of RBC, PCV and Hb observed in the CPF group in the present study agreed with those obtained in earlier studies (Goel *et al*., 2006b; Okechukwu *et al.,* 2007; Akhtar *et al*., 2009; Ambali *et al*., 2010c). This indicated that repeated exposure to CPF causes anaemia. The reason for the anaemia in the CPF group is not known. It may, however, be related to distruption of erythropoiesis or an increase in RBC destruction (Vural *et al*., 1986; Patil *et al*., 2003). The destruction of red blood cells is postulated to occur by either membrane oxidation or haemoglobin denaturation (Carrell *et al*., 1975). The anaemia may have been due to the ability of the OP compound to decrease tissue iron concentration by decreasing absorption of iron (Goel *et* al., 2006b), interference with Hb biosynthesis and shorten RBC life span (Ray, 1992) or even increase erythrocyte fragility (Ambali *et al*., 2010a, c, d). The high lipoperoxidative changes in erythrocyte of rats exposed to CPF as indicated by high MDA concentration reported by other workers (Gultekin *et al*., 2001; Mansour and Mossa, 2009; Ambali *et al*., 2010a-d) demonstrates the role of oxidative stress in the pathophysiology of CPF-induced anaemia. The RBC is vulnerable to lipoperoxidative changes because of its direct association with molecular oxygen, high content of metal ions, catalyzing oxidative reaction and availability of high amount of polyunsaturated fatty acids (PUFA) (Etlik and Tomur, 2006), which are susceptible to lipid peroxidation. The inability to repair membrane damage and regenerate, and poor antioxidant enzyme composition of the plasma medium in which they are bathed are some of the other factors that enhance the vulnerability of the RBC to lipid peroxidation (Etlik and Tomur, 2006). The overall effect of the factors on the RBC is the reduction in its membrane integrity and lifespan. The degenerative changes in kidneys of rats exposed to CPF recorded in the present study may have contributed to the anaemia in this group. This is due to interference with the production of erythropoietin by the kidney, a hormone essential to functional erythropoiesis (Ambali, 2009). Therefore, the anaemia observed in this study may be as a result of a combination of factors.

The MCH and MCHC did not alter significantly within the groups, thus indicating that CPF did not significantly alter erythrocyte indices. The slight decrease in MCV seen and the apparently normal MCH and MCHC in the CPF group may indicate an apparent normocytic normochromic anaemia.

The improvement in the PCV, concentrations of RBC and Hb in the rats pretreated with VC and/or ALC suggest their ameliorative effect on the CPF-induced decrease in these haematological parameters. This shows that oxidative stress is partly involved in CPF-induced anaemia. The improvement seen in rats pretreated with VC may be due to the ability of the antioxidant vitamin to improve the absorption of iron from the gut (Wardlaw, 1999; Iqbal *et al*., 2004) by facilitating the reduction of oxidized iron to its reduced form thus increasing the serum concentration of iron essential for haeme synthesis (Sayers *et al*., 1973). However, the improvement in the PCV, and concentrations of RBC and Hb concentration seen in the ALC+CPF group was better compared to those recorded in the VC+ CPF and VC+ALC+ CPF groups. The reason for this better improvement in the ALC pretreated group may be due to the ability of ALC to improve RBC survival and also by increasing the action of erythropoietin on bone marrow (Ardwini *et al*., 1992; Nand *et al*., 2008). Carnitine is known to have beneficial effect on sickle-cell (Ronca *et al*., 1994) and chronic renal failure anaemia (Golper *et al*., 2003). Similarly, carnitine has been shown to reduce deformability of red blood cell, increase haematocrit, haemoglobin levels, erythrocyte count and survival time (Dokmeci *et al*., 2006). The salutary effect of ALC on anaemia centers on improvement in erythrocyte survival, specifically through enhanced erythrocyte membrane stability and rise in red blood cell osmotic resistance (Nikolaos *et al*., 2000; Matsumoto *et al*., 2001). The decreased in the level of amelioration seen in the VC + ALC + CPF group compared to VC+CPF or ALC+CPF group may be due to the overwhelming presence of the antioxidants beyond the existing RBC demand, as studies have shown that antioxidants in large concentration manifest some prooxidant effect (Naidu, 2003). The result showed that administration of vitamin C and/or ALC in CPF-treated rats consolidated the erythrocyte membrane integrity, and thus reduces the degree of haemolysis. Antioxidant has been shown to counteract oxidative stress, intravascular haemolysis and prevent erythrocyte deterioration (Senturk *et al*., 2001).

The study revealed an apparent increase in the WBC concentration in the group exposed to CPF only. The reason for the apparent leukocytosis is not known. This finding agreed with those of Akhtar *et al*. (2009) in rats and Okechukwu *et al*. (2007) in cat fish. However, the finding contradicted the result of previous studies which showed that repeated CPF exposure causes leukopaenia (Goel *et al*., 2006; Ambali *et al*., 2007, 2010b). The low neutrophil/lymphocyte ratio in the CPF group goes further to confirm the lymphocytosis observed in the study and did not agree with that observed by Ambali et al. (2010c). The implication of the apparent leucocytosis is not known, especially as it relates to the immune system and deserves further studies. This is in the light of studies that have shown that pesticides are toxic to cells of the immune system through the induction of necrosis and apoptosis (Rabideau, 2001). Banerjee *et al*. (1999) stated that "inhibition of AChE could result in the accumulation of ACh and increase lymphocyte mobility and cytotoxicity". Joshi *et al*. (2002) attributed the increase in WBC to an increase in antibody production which aids in survival and recovery. Okechukwu *et al*. (2007) corroborated this, by suggesting that the increased WBC may be due to hypersensitivity of leucocytes to CPF, which may be due to immunological reaction leading to antibody production.

Pretreatment with VC did ameliorate the CPF-induced leukocytosis. The reason for the restoration of leucocytosis by VC is not known for certain but may be due to its modulatory role on the immune cells, as it is capable of producing large quantities of cytokines and by helping B cells to synthesize immunoglobulins to control inflammatory reaction. It is known to block pathways that lead to apoptosis of T-cells (Naidu, 2003). The amelioration of WBC concentration observed in the VC pretreated group was comparatively better compared to ALC+CPF and VC+ALC+CPF groups. ALC's modulatory role on the immune cells (Jirillo *et al*., 1993) may be the reason for the ameliorative effect on CPF-evoked leukocytosis observed in the present study.

The study recorded no significant difference in the platelet count of rats exposed to CPF only and those pretreated with VC and/or ALC. The comparatively lower platelet count recorded in the CPF group compared to the control, however, disagrees with that reported by Szabo *et al*. (1988) and Ambali *et al*. (2010c). In the present study, there was a decrease in platelet count in the CPF group compared to the control. The reason for this slight thrombocytopaenia in rats exposed to low-dose CPF is not known and would require further study. However, it may be due to decreased production of thrombopoietin by the liver as a result of liver damage (Wikipedia, 2010a) since the present study and others (Goel *et al*., 2005; Ambali, 2009) have shown CPF-induced hepatotoxicity. In addition, the thrombocytopaenia may be related to CPF-induced oxidative damage to platelet membranes. Apart from being less resistant to oxidative stress, the membranes of platelets are thinner than those of erythrocytes (Araujo *et al*., 2008) hence are more susceptible to lysis. Araujo *et al*. (2008) established a direct relationship between oxidative stress and thrombocytopaenia in patients infected with malaria parasites. It is expected that increased oxidative stress may lead to increase in platelet lysis. Ohyashiki *et al*. (1991) showed the platelet lipid peroxidation increases when rat platelets were exposed to ROS. Therefore, ROS may play an important role in structural and functional damage of platelets hence may be partly involved as a mechanism of thrombocytopaenia recorded in the CPF group.

Pretreatment with ALC showed a slight improvement in platelet count indicating the role of oxidative stress in the CPF-induced thrombocytopaenia. However, VC and the combination of VC and ALC did not alleviate the thrombocytopaenia. The reason for this is also not clear and will require further study.

The study showed an increase in erythrocyte fragility in the CPF group compared to the control group. This shows the ability of subacute exposure to CPF to compromise the integrity of the RBC membrane apparently from increased oxidative damage to the erythrocyte membrane (Wagner *et al*., 1988). This result agreed with those reported in earlier studies that demonstrated increase erythrocyte osmotic fragility in RBC of rats exposed to CPF as a result of increased lipoperoxidation of erythrocyte membranes (Ambali *et al*., 2010a,b,d). The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of RBC (Langsdorf and Zydney, 1993). The participation of oxygen radical species in haemolytic states has been suggested (Jacob and Lux, 1958). Erythrocyte lysis may be the end result of minor defects in the red blood cell membrane. The structural integrity of the erythrocyte membrane is an important feature for its resistance to peroxidative attack. However, erythrocytes are susceptible to oxidative stress due to large amount of unsaturated membrane phospholipids (Gain and Shohet, 1981). It is plausible to speculate from our result that CPF treatment or exposure results in peroxidation of PUFA in the membrane leading to the degeneration of phospholipids and ultimately cellular death as reported by Tappel (1973). Process of lipid peroxidation decreases hydrophobic characteristic of bilayer membrane of erythrocyte altering affinity and interaction of protein and lipids, thereby impairing the functioning and homoestasis of erythrocyte membranes (Dargel, 1991). Since CPF is lipophilic, it may enhance lipoperoxidation by directly interacting with the cellular plasma membrane (Hazarika *et al*., 2003). The increase in the *in vitro* erythrocyte osmotic fragility in rats exposed to CPF only confirmed the observation that this can be used as indirect method of evaluating lipid peroxidation in animals (Chihuailaf *et al*., 2002).

The amelioration of the erythrocyte osmotic fragility by antioxidant VC and/or ALC further confirmed the role of oxidative stress in the toxic mechanism of erythrocyte damage observed following the subacute CPF exposure. From our result, it seems paradoxical the VC showed stronger protective effect on erythrocyte fragility than the combination of VC and ALC, this may however be due to the prooxidant effect of VC and ALC combination due to their overwhelming concentration in the erythrocyte. Ambali *et al*. (2010e) showed the ability of VC to ameliorate CPF-induced increased erythrocyte fragility apparently due to decreased lipoperoxidative damage to the erythrocytes. Infact, VC completely prevented haemolysis of erythrocyte of rats exposed to CPF at 0.9% NaCl solution in this present study. Therefore, the protective effect of VC on CPF-induced erythrocyte fragility may be due to its antioxidant properties. Devi *et al*. (2007) also reported that supplementation with VC decreased the induction of oxidative stress in the erythrocyte. VC is known to protect completely lipids in plasma and low density lipoprotein against peroxidative damage (Sauberlich, 1994). VC is located in the extracellular and hydrophilic regions of the cell (Verma *et al*., 2009). Thus, VC in the extracellular matrix defends the cell first. Free radicals must pass across the membrane to interact with extracellular components (Verma *et al*., 2007).

The increase in erythrocyte fragility observed in VC+ALC, and VC+ALC+CPF groups at 0.5, 0.7 and 0.9% NaCl concentration over those in the VC+CPF and ALC+CPF may be due to overwhelming presence of the antioxidants beyond the existing demand of the RBC. Studies have shown that antioxidants in large concentration, antioxidants manifest some prooxidant effect. This results in their toxic build-up, causing damages to the erythrocyte membrane. Therefore, caution should be exercised when combining both VC and ALC as antioxidants. Furthermore, pretreatment with ALC and VC+ALC was able to reduce the percentage erythrocyte osmotic fragility in CPF exposed rats, even though it was not significant. This may be due to their antioxidant properties. ALC is known to aid in the maintenance of RBC membrane stability (Ardwini *et al.*, 1992) and also decrease osmotic fragility (Matsumura *et al.*, 1996). ALC may act as an antioxidant either having a primary antioxidant activity (inhibition of free radical propagation reaction), or, more likely functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acid esterified in membrane phospholipids) (Liu *et al.*, 2004).

The study also demonstrated that subacute CPF exposure caused no significant alteration in the serum levels of Na^+, K^+, Cl^- and HCO_3^-. This finding partly agrees with that Ambali *et al.* (2007), who reported no significant alteration in Cl^- and K^+, but a significant alteration in Na^+ concentration. However, there was a marginal decrease in the serum Cl^- level in the CPF-treated group when compared to the control and other groups. This marginal variation, apparently, associated with functional alteration in the proximal tubules of the nephron (Krishna and Ramachandra, 2009), was reversed following pretreatment with VC and/or ALC. This effect may be attributed to their protective effect on CPF-induced renal damage, suggesting their antioxidant effect.

The present study also demonstrated that subacute CPF exposure caused a significant decrease in serum glucose level. This finding was in agreement with those recorded in previous studies (Szabo *et al*., 1988; Zama *et al*., 2005; Krishnamoorthy *et al*., 2006; Akhtar *et al*., 2009). This, however, contradicted the hyperglycaemia earlier reported by Ambali (2009). The reason for the hypoglycaemia may be due to the impairment in hepatic gluconeogenesis due to liver damage, resulting in decrease in glucose synthesis (Goel *et al*., 2006a). The fatty degenerative changes and focal areas of necrosis in the hepatocytes may be the cause of the impairment in hepatic gluconeogenesis. In addition, CPF has been shown to cause damage to *zona fasciculata* of the adrenal cortex (Yano *et al*., 2000), the region responsible for the production of glucocorticoid, a hormone that stimulates gluconeogenesis during stress. This effect may also lead to decreased gluconeogenesis and hence low glucose concentration.

Pretreatment with VC ameliorated the CPF-induced changes in glucose level, indicating the role of oxidative stress in the hypoglycaemic response. VC apparently, prevented oxidative damage to the liver and preserved the integrity of the adrenal cortex probably due to its antioxidant effect. Pretreatment with ALC also ameliorated the CPF-induced changes in glucose level, similarly indicating the role of oxidative stress in the hypoglycaemic response. Pretreatment with a combination of VC and ALC brought about a higher increase in glucose level, implicating oxidative stress in the CPF-induced hypoglycemic response. The improvement in the CPF-induced hepatic lesions, hence improvement in gluconeogenesis may have been partly responsible for the apparent increase in glucose concentration in the pretreatment groups.

The present study also showed an insignificant reduction in the levels of serum total protein in rats subacutely exposed to low-dose CPF. This finding agrees with those recorded in previous studies (Khan and Kour, 2007; Akhtar *et al*., 2009; Ojezele and Abatan, 2009; Ambali, 2009). The relatively lowered TP in the CPF group was apparently due to hypoalbuminaemia. This finding of other authors that the low serum protein recorded in prolonged exposure to CPF is associated with low serum albumin (Kagan, 1971; Patil *et al*., 2003) agreed with those observed in the present study.

Albumin, apart from being a useful indicator of the integrity of glomerular membrane, is also an important indicator in determining the severity of a disease (Mukut *et al*., 2001; Iwasaki *et al*., 2008). Decrease albumin level may be due primarily to reduction in the synthesis by the liver (Luskova *et al*., 2002). Changes in serum total proteins and albumin reflect hepatocellular injury and disturbed amino acid metabolism induced by OP (Gomes *et al*., 1999; Yousef *et al*., 2006). Albumin scavenges OP molecules and, therefore, reduces the amount available for reaction with AChE (Peeples *et al*., 2005). Indeed, Peeples *et al*. (2005) have proposed albumin as a biomarker of OP exposure. Albumin represent the major and predominant antioxidant in plasma, a body compartment known to be continuously exposed to oxidative stress (Roche *et al*., 2008). Therefore, the marginal decrease in albumin observed in the present study may be partly due to increased albumin binding to CPF and increase in the antioxidant demand, which reduced its free form in the serum. Besides, lipid hydroperoxides, the lipid peroxidation products of polyunsaturated fatty acids, have been shown to cause protein degradation and denaturation (Chiba and Iwata, 2002), thereby contributing to low serum protein observed in the study. Reactive oxygen species can also induce oxidation of critical sulfhydryl (SH) groups in protein and DNA, which will alter cellular integrity and function (Fatemeh-Teimouri, 2006). Exposure to OP insecticides also causes inhibition of the cytoplasmic proteases and some lysosomal proteases in the liver tissue which are the major site for insecticide metabolism (Mantle, 1997). Kagan (1971) and Bomphard *et al*. (1981) also reported a significant reduction in serum total protein and albumin following phosphorganic and methyl-parathion exposure. To this end, decreased albumin observed in this study may be partly attributed to the hepatic fatty degeneration and focal necrosis observed in the CPF group. A decrease in biosynthetic function of the liver as shown by decreased level of serum protein and albumin is the end result of this phenomenon (Khan and Kour, 2007). This agreed with the findings of other workers (Khan and Kour, 2007; Akhtar *et al*., 2009; Ambali, 2009; Ojezele and Abatan, 2009).

Pretreatment of the rats with VC and/or ALC ameliorated the deficit in total serum protein and albumin caused by CPF, apparently due to reduction of oxidative stress-induced hepatic degenerative changes. Similarly, increased paraoxonase activity is induced by VC (Jarvik *et al*., 2002), thereby aiding OP compound detoxification (Shih *et al*., 1988) and may partly contribute to the improved serum proteins and albumin levels in the VC pretreated group. VC supplementation as reported by other workers increases serum albumin (Seyrek *et al*., 2004). Supplementation with ALC has been reported by Calabrese *et al*. (2006) to have protein sparing effect (the activation of β-oxidation pathway, mediated through carnitine, reduces the break down of branched amino acids via internal feed-back mechanism) and also ability to promote protein synthesis. Furthermore, the amelioration may have resulted from the protection offered by VC and/or ALC on the CPF-induced liver damage, which ultimately spared the VC and/or ALC pretreated rats from hypoalbuminaemia. The ability of VC and ALC to spare the proteins from degradative effect of free radicals evoked by CPF may have contributed to the improvement in the concentration of total proteins and albumins in the pretreatment groups.

The present study revealed an apparent increase in globulin concentration in the serum of rats exposed to CPF only when compared to the Soya oil treated rats. This finding agreed with that observed by Subbotina and Bellonozhko (1968) following exposure to an OP compound, sevin. The result, however, contradicted those obtained by Szabo *et al*. (1988) following repeated CPF exposure in rats. The increased level of globulin observed in this study may be due to its α-globulin fraction which may be attributed to tissue destruction and inflammatory reaction (Peace and Kaplan, 1987). OP exposures have been reported to induce the formation of antibodies to the nervous tissues (McConnell *et al*., 1999). These antibodies may be responsible for the increased globulin observed in the CPF group in the present study. The fact that pretreatment with VC reduced the serum globulin level in the present study contradicted the findings of Ambali (2009), who reported an increase in serum globulin. In contrast, pretreatment with ALC further increased the serum globulin. The reason for this was not clear. However, pretreatment with the combination of VC and ALC resulted in lowering the serum globulin, thus, implicating oxidative stress in the CPF-induced apparent increase in globulin level.

The present study demonstrated that subacute CPF exposure caused a significant increase in the serum urea concentration. This finding is consistent with those of Krishnamorthy *et al.* (2006) who reported an increase in serum urea level in chick fed with CPF, and Kerem *et al.* (2007) who obtained an increase in serum urea level in rats exposed to fenthion, an OP compound. The apparent increase in urea concentration demonstrated the ability of the subacute CPF exposure to induce low-grade pathological changes in the kidneys. This increase in urea level may also be attributed to the transient renal injury associated with the direct action of the OP, causing tubular necrosis (Kerem *et al*., 2007), or to the secondary mechanism that followed the cholinergic crisis or even oxidative damage caused by ROS generated by CPF exposure. In addition, the increase in serum urea concentration may be due to the necrosis of the renal tubular epithelial cells observed in the CPF group. Indeed, the apparent reduction of the urea concentration observed due to the pretreatment with VC and/or ALC revealed the role of oxidative stress in the renal pathological changes. This may be due to their protective role against CPF-induced renal damage as demonstrated by improved renal histoarchitecture.

The study also showed a marginal increase in creatinine level in the CPF group. This observation is in agreement with the result obtained in chicks (Krishnamoorthy *et al*., 2006), mice (Ambali *et al.,* 2007) and rats (Ambali, 2009). The elevation of creatinine may be as a result of pathological lesions in the muscle, as it is a by product of muscle metabolism (Quintanilla, 1982) and in the kidneys. Elevated creatinine has been shown to be associated with renal insufficiency and renal obstruction (Ambali *et al.,* 2007). The increased creatinine concentration agreed with the renal lesions recorded in the CPF group. However, pretreatment with VC and/or ALC restored creatinine to almost a normal level as observed in the control group. Since, no apparent renal histopathological lesion was observed in the VC and ALC pretreated group. This finding is also consistent with that reported by Ambali *et al*. (2007). The group pretreated with the combination of VC and ALC gave the best ameliorative effect, followed by VC and then ALC. The ameliorative effect may be due to their protective role against OP-induced renal and muscle damages, probably due to their antioxidant effect.

Subacute exposure of rats to CPF was shown in this study to cause a decrease in AST activity. This finding agreed with the result obtained by Ambali *et al*. (2007) in mice and that of Barna-Llyod *et* *al*. (1990) in rats. However, the finding of the present study contradicts the results of Goel *et al*. (2005), Zama *et al*. (2005), Khan and Kour (2007) and Ambali (2009) in rats. The reason for the low activity of AST is not known and besides the toxicological relevance of this remains obscure (Ambali *et al*., 2007). It is possible that CPF inhibited directly AST activity as was observed in ALT by Altuntas and Delibas (2002).

The relative increase in serum ALT activity recorded in the CPF group was in consonant with the findings from earlier studies (Goel *et al.,* 2005; Zama *et al*., 2005; Khan and Kour, 2007; Ambali, 2009). However, this finding contradicts those of Barna-Llyod *et al*. (1990) and Ambali *et al.* (2007). ALT, located mainly in cytoplasm of the hepatocytes is released to the blood stream when damage to cytoplasmic membrane occurs (Siest and Galteau, 1974; Loegering *et al*., 1975). It is highly liver specific (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2005) and one of the most reliable indicator of hepatotoxic damage (Ozer *et al*., 2007). ALT is primarily localized to liver, with lower enzyme activities found in skeletal and cardiac muscles (Ozer *et al*., 2007). High ALT activity observed in rats exposed to CPF only in the present study may be due to hepatic lesions as exemplified by increased hepatic lipoperoxidation, fatty degeneration and focal necrosis. Although, the ALC group showed an elevated ALT activity, there was however no histopathologic lesion recorded in this group. The reason for this contradiction is not known.

The present study further showed that pretreatment with VC and/or ALC resulted in normalization of the AST activity relative to the S/oil group. This demonstrated the ability of VC and/or ALC to reverse the inhibition of AST activity. This was corroborated by the presence of mild fatty degenerative changes in the hepatocytes of VC+CPF and ALC+CPF groups and mild congestion in those of VC+ALC+CPF group.

Similarly, pretreatment with VC and/or ALC resulted in a marginal decrease in the ALT activity. This demonstrated the protective tendency of the antioxidants against CPF-induced liver damage. Thus, the results obtained in the present study strongly suggest that oxidative stress plays a significant role in CPF-induced liver damage as milder fatty degenerative changes were observed in the pretreatment groups.

The significant increase in serum ALP activity in rats exposed to CPF only agreed with previous works (Goel *et al*., 2005; Ambali *et al*., 2007; Khan and Kour, 2007; Ambali, 2009). This finding shows that subacute CPF exposure may cause pathological changes all the organs involved in the synthesis and/or release of ALP such as the liver, kidneys, bones, skeletal muscles and intestinal mucosa, probably due to oxidative damage. The functional importance of ALP in animals has been demonstrated by Ozer *et al*. (2007). The enzyme is associated with glycogen and transportation of intermediate compounds in glycogenesis or glycogenolysis. The increase in the ALP activity induced by different insecticides has also been reported by Singh and Singh (2002) and Jaroli and Sharma (2005). Khan and Pandya (1985) reported that elevated ALP may be due to the destruction of the hepatic smooth endoplasmic reticulum membrane in insecticide intoxicated mice. This increase in ALP activity recorded in the present study may be due to the cellular damage caused by the toxicity of CPF or increased ROS.

Conversely, there was an increase in ALP activity in rats treated with ALC alone. The reason for this is not known, however, this could be due to the effect of ALC on growing bones and muscle as ALC is known to boost muscle and bone mass, since ALP is also synthesized in the bones and muscles (Wikipedia, 2010b). However, pretreatment with VC and/or ALC brought about apparent decrease in ALP activity, indicating their ameliorating effect. This hepatoprotective effect resulting from pretreatment with VC agreed with the results of previous studies (Ambali *et al.,* 2007; Ambali, 2009). This suggests that VC and/or ALC have protective effect on the damage induced by CPF on the organs producing these enzymes, especially the liver. Again, this indicates that oxidative stress plays an important role in CPF-induced hepatotoxicity.

Furthermore, the present study showed that subacute exposure of rats to CPF caused a slight increase or elevation in creatine kinase (CK) activity, indicating liver or muscle damage. This finding agrees with that of Zama *et al.* (2005), but contradicts that of Ambali (2009). Apart from its presence in the blood, mostly as isozyme CK-M, another isozyme of CK (CK-B) is also present in the brain and spinal cord. CK is present in neurons, astrocytes and oligodendrocytes (Manos *et al*., 1991), and is believed to play a role in the continuous replenishment of ATP from phosphocreatine in these cells (Wyss *et al.,* 1992). Elevated CK activity has also been reported in cats exposed to repeated inhalation of CPF (Jaggy and Oliver, 1990). Pretreatment with VC and ALC was shown to marginally decrease the CK activity, but a far marginal decrease was observed in the group pretreated with the combination of VC and ALC. This further demonstrated that pretreatment with VC and/or ALC ameliorated the damages induced by CPF on organs producing CK, and thus restored CK activity.

It has been shown in the present study that CPF significantly increased MDA levels in the liver of rats. This finding was in agreement with that reported by other workers (Goel *et al*., 2005; Zama *et al*., 2007; Ambali, 2009). The levels of MDA, a major product of peroxidation of PUFA in cytomembranes, have been considered as an important indicator of lipid peroxidation occurring as a result of ROS in the body (Kalender *et al*., 2004; Aly *et al*., 2010). Thus, increase formation of MDA could be due to both increases in pesticide-induced ROS generation and inhibition of antioxidant function (Gultekin *et al*., 2001). Lipid peroxidation is reported to increase on exposure to various xenobiotics (Verma and Srivastava, 2003). The increase in lipid peroxidation in the liver following repeated exposure to CPF may cause membrane damage resulting in considerable ultrastructural damage of liver cells and eventually loss of its membrane integrity (Khan and Kour, 2007). Impairment of enzymatic antioxidant system apparently favours accumulation of free radicals that is responsible for increased lipid peroxidation on CPF exposure (Gultekin *et al*., 2000). It has been reported that during liver damage there is decrease in antioxidant defences of the liver (Seven *et al*., 2004). CPF was reported in earlier studies to increase lipid peroxidation in various tissues of the body (Gultekin *et al*., 2000; Zama *et al*., 2007; Aly *et al*., 2010; Ambali *et al*., 2010a-d). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity (Kehrer, 1993). The damage caused may alter cellular function through changes in intracellular calcium or intracellular pH, and eventually result in cell death (Kehrer *et al*., 1990).

However, the present study showed that pretreatment with VC significantly decreased MDA level in the liver indicating low lipoperoxidation. This finding is consistent with other observations regarding VC and other antioxidants which were also found to be potent inhibitors of lipid peroxidation (Aly *et al*., 2010; Ambali *et al*., 2010a-c). Similarly, pretreatment with ALC has also been demonstrated by the present study to significantly decrease LPO levels in the liver of rats. ALC was shown in earlier studies to inhibit lipid peroxidation (Gulcin, 2006; Kart *et al*., 2006; Mansour, 2006). Furthermore, pretreatment with the combination of VC and ALC also significantly decreased LPO level in the liver of rats exposed to CPF. This finding is in agreement with those of Gultekin *et al*. (2001) and Ambali *et al*. (2010a), who respectively reported that melatonin and combination of vitamin E and C restored LPO levels, elevated by CPF.

The function of antioxidant systems is to modify the highly ROS to form less reactive intermediates which no longer pose a threat to the cell (Al- Omar *et al*., 2004). Despite the presence of these delicate cellular antioxidant systems, an overproduction of ROS in both intra- and extracellular spaces often occurs upon exposure of cells or individuals to certain chemicals (Verma and Srivastava, 2003). The present study demonstrates that inhibition of CAT and SOD activities in rats repeatedly exposed to CPF modified the endogenous antioxidants, SOD and CAT, which may leads to the development of oxidative stress in some tissues (Zama *et al*., 2007). This observation explains the inhibition of SOD and CAT activities in the CPF group seen in this study even though it was not significant. The result is consistent with the lower CAT and SOD activities observed in previous studies on CPF exposure (Gultekin *et al*., 2006; Zama *et al*., 2007; Aly *et al*., 2010). This inhibition in SOD activity could be due to the increased production of ROS as evident from the increased LPO level due to CPF exposure. The superoxide radical has been shown to directly inhibit the activities of enzyme catalase (Kono and Fridovich, 1982), likewise, singlet oxygen and peroxyl radical have been shown to inhibit SOD and CAT activities (Escobar *et al*., 1996). A reduction in SOD activity favours the accumulation of oxygen free radicals in the liver, and other cells leading to tissue damage as a result of oxidative binding of key intracellular molecules containing thiol groups. Lipid peroxidation of biological membranes may be of greatest importance in the cytotoxicity of pesticides responsible for cellular death (Lopez *et al*., 2007). Lower CAT activity may have resulted from increased superoxide radical production arising from decreased SOD activity (Zama *et al*., 2007). Similarly, Luskaszewicz-Hussain and Moniuszko-Jakoniuk (2005) reported that decrease in liver SOD activity results from overproduction of H_2O_2, as SOD is inactivated by the product of its own reaction. Therefore, the altered liver enzyme activity observed in the group exposed to CPF only in the present study may be due to increased lipid peroxidation, resulting in hepatic lesions. The resulting hepatic lesions observed as fatty degeneration and focal necrosis in the CPF group further reinforced the role of lipoperoxidation in the CPF-evoked hepatic damage.

In contrast, groups pretreated with VC and/or ALC in the present study showed increased hepatic SOD and CAT activities depressed by CPF. This may have been due to decreased lipoperoxidation and milder histopathological changes in the hepatocytes of these groups. The increase in SOD activity accelerates the removal of ROS (Zama *et al*., 2007) thus reducing the adverse effect of oxidative stress on hepatic tissue. This finding was in agreement with that of Gultekin *et al*. (2001) which reported a restoration to normal the activities of antioxidant enzymes by administration of melatonin and a combination of vitamins E and C, which were decreased following exposure of rat erythrocyte to CPF treatment. This result also agreed with the findings of Zama *et al*. (2007), who demonstrated that the extracts of *Paronychia argentea L* increased the activities of SOD and CAT in CPF-induced oxidative stress and tissue damage in the liver of pregnant rats.

The elevation of CAT and SOD activities in the antioxidant pretreated groups may be due to the ability to scavenge the deleterious toxic free radicals, capable of causing tissue injury (Verma *et al*., 2007). Although pretreatment with VC showed a better elevation of enzyme activity than that of ALC, pretreatment with the combination of VC and ALC showed a far better restorative SOD activity. Conversely, pretreatment with VC also demonstrated a far better restoration of specific activity of CAT than that of ALC. Pretreatment with the combination of VC and ALC resulted in the best restoration of CAT activity. The primary role of VC is to neutralize free radicals that will seek out an electron to regain their stability. VC, as an excellent source of electrons donates electrons to free radicals and quenches their reactivity (Aly *et al*., 2010). The elevated SOD and CAT activities observed in the pretreatment groups may be as a result of their antioxidant ability, which accelerated the removal of the ROS, thus preventing CPF-induced reduction in SOD and CAT activities. Therefore, the redox status of the liver was improved in the pretreatment groups.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The present study has shown that subacute exposure of adult Wistar rats to CPF caused:

1. Relative reduction in haematological parameters of PCV, Hb and total erythrocyte count.
2. Leucocytosis due to lymphocytosis.
3. Increased erythrocyte osmotic fragility, which predisposes the RBC to lysis, apparently due to free radical-mediated and compromised erythrocyte membrane.
4. Hypoglycaemia.
5. Marginal decrease in total protein, apparently due to low albumin, and hyperglobinaemia.
6. Marginal increase in serum creatinine concentration, probably arising from muscle and renal damage.
7. Increase in activity of some serum enzymes such as ALT and ALP, which may reflect some level of hepatic damage.
8. Increase in the activity of serum CK, suggestive of some level of damage to the liver and/or the muscle.
9. Significant hyperuraemia, resulting from possible protein loss or break-down and renal damage.
10. Increased lipid peroxidation in the liver as evidenced by increased TBARS, MDA in the liver.

11. Decrease in liver antioxidant enzymes of SOD and CAT, due to increased production of ROS as evident from the increased LPO levels.

The study has also shown that pretreatment with acetyl-L-carnitine and/or vitamin C to a varying degree improved levels of PCV, Hb and RBC decreased by CPF. Pretreatments with ALC and/or VC also reduced the CPF-induced leucocytosis. In addition, the study demonstrates the ability of vitamin C to completely protect the integrity of RBC membrane by ameliorating CPF-induced erythrocyte osmotic fragility. Furthermore, the study also reveals the ability of ALC to also protect the integrity of RBC membrane as revealed in the amelioration of CPF-induced erythrocyte osmotic fragility. Similarly, pretreatment with ALC/or VC protected against CPF-induced hypoproteinaemia, hypoalbuminaemia and hyperglobulinaemia. The study showed that ALC and/or VC ameliorated the hypoglycaemia induced by subacute exposure to CPF. The antioxidant effect of ALC and/or VC ameliorated CPF-induced increase in activity of ALT and ALP. The study further demonstrates the ability of ALC/or VC to protect the body against CPF-induced lipid peroxidation as evidenced by reduced concentration of MDA in the various pretreated groups. Pretreatment with ALC and/or VC reversed the decrease in antioxidant enzyme activities of the liver, depleted as a result of CPF-induced increase in ROS production.

6.2 CONCLUSION

In conclusion, the study has shown that:

1 Subacute low-dose CPF exposure in Wistar rats caused alteration in haematological and biochemical profiles.

2 Oxidative stress as exemplified by increased MDA and low antioxidant enzyme activities is an important molecular mechanism involved in haematological and biochemical changes observed in subacute CPF exposure in Wistar rats.

3 Pretreatment with ALC and/or VC differentially ameliorated the CPF-induced haematological, biochemical and histopathological changes in Wistar rats.

6.3 RECOMMENDATIONS

6.3.1 Specific Recommendations

1 Further work should be done, especially on the electrophoresis of globulin in CPF-induced toxicity, to evaluate the effect and responses of the various types of globulin to ALC and/or VC.

2. Additional work should be carried out to ascertain the reason why CPF exposure resulted in thrombocytopaenia and also why pretreatment with VC and the combination of ALC and VC resulted in lower platelet count. The implication of this on both human and animal health should be studied.

3. Attempt should be made to explain the reason for increased ALP activity in rats exposed to ALC alone, and its implication on both human and animal health.

6.3.2 General Recommendations

1. Pesticide applicators, farmers, veterinary attendants, veterinarians and other users of CPF and perhaps other OPs should limit direct contact with them by the use of effective protective clothing and masks.

2. Periodic evaluation of haematological and biochemical parameters of individuals or animals who are frequently exposed to low-dose CPF should be carried out, to enable professional counseling of individuals on the risk of exposure.

3. Sensitization of the general public via awareness campaigns on the dangers of indiscriminate use of pesticides should be embarked upon.

4. Government should put in place policy that will check the indiscriminate importation into the country and the use of dangerous and banned pesticides through effective border police, control and public enlightment programmes.

5. Individuals who are at risk of having contact with CPF and, probably, other OPs should be pretreated with ALC and VC to reduce damages caused by frequent low-dose exposure.

REFERENCES

Aardema, H., Meertens, J.H.J.M., Ligtenberg, J.J.M., Peters-Polman, O.M., Tulleken, J.E. and Zijlstra, J.G. (2008). Organophosphorus pesticide poisoning: cases and developments. *The Netherlands Journal of Medicine*, 66(4): 149-153.

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S. and Rezale, A. (2004). Pesticide and oxidative stress: a review. *Medical Science Monit*, 10(6): 141-147.

Abou-Donia, M.B. (2003). Organophosphorus ester-induced chronic neurotoxicity. *Archives of Environmental Health*, 58(8): 484-497.

Adams, J.D. and Odunze, I.N. (1991). Review. Oxygen free radicals and Parkinson's disease. *Free Radical Biology and Medicine*, 10: 161-169.

Adenkola, A.Y., Ayo, J.O. and Sackey, A.K.B. (2009). Ascorbic acid-induced modulation of rectal temperature fluctuation in pigs during the harmattan season. *Journal of Thermal Biology*, 34: 152-154.

Aguirre, R.A. and May, J.M. (2008). Inflammation in the vascular bed: Importance of vitamin C. *Pharmacology and Therapeutics*, 119: 96-103.

Ahmad, R., Tripathi, A.K., Tripathi, P., Singh, R., Singh, S. and Singh, R.K. (2010). Studies on lipid peroxidation and non- enzymatic antioxidant status as indices of oxidative stress in patients with chronic myeloid leukaemia. *Singapore Medical Journal*, 51(2): 110-115.

Aiub, C.A.F., Coelho, E.C.A., Sodre, E., Pinto, L.F.R. and Felzenswalb, I. (2002). Genetoxic evaluation of the organophosphorus pesticide temephos. *Genetics and Molecular Research*, 1(2): 159-166.

Akhtar, N., Srivastava, M.K. and Raizada, R.B. (2006). Transplacental disposition and teratogenic effects of chlorpyrifos in rats. *The Journal of Toxicological Science*, 31(5): 521-527.

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

Al-Badrany, Y.M.A. and Mohammad, F.K. (2007). Effect of acute and repeated oral exposure to the organophosphate insecticide chlorpyrifos on open field activity in chicks. *Toxicology Letters*, 174: 110-116.

Albers, J.W., Garabrant, D.H., Schweitzer, S.J., Garrison, R.P., Richardson, R.J. and Berent, S. (2005). The effects of occupational exposure to chlorpyrifos on the peripheral nervous system: a prospective cohort study. *Occupational and Environmental Medicine*, 61: 201-211.

Ali, S.M. and Chia, S.E (2008). Interethnic variability of plasma paraoxonase (PON1) Activity towards organophosphates and PON1 polymorphisms among Asian population. A short review. *Industrial Health*, 46: 309-317.

Al-Majed, A.A., Sayed-Ahmed, M.M., Al-Omar, F., Al-Yahya, A., Aleisa, A.M. and Al-Shabanah, O.A. (2006). Carnitine esters prevent oxidative stress damage and energy depletion following transcient forebrain ischaemia in the rat hippocampus. *Clinical and Experimental Pharmacology and Physiology*, 33: 725-733.

Al-Omar, M.A., Beedham, C. and Alsarra, I.A. (2004). Pathological roles of reactive oxygen species and their defence mechanisms. *Saudi Pharmaceutical Journal*, 12: 1-17.

Almeida, M.G., Fanini, F., Davino, S.C, Aznar, A.E., Koch, O.R.and Barros S.B.M. (1997).Pro- and anti-oxidant parameters in rat liver after short-term exposure to hexachlorobenzene. *Human and Experimental Toxicology*, 16: 257-261.

Altuntas, I. and Delibas, N. (2002). The effect of fenithion on lipid peroxidation and some liver enzymes: the possible protective role of vitamin E and C. *Turkish Journal of Medical Science*, 32: 293-297.

Aly, N., El-Gendy, K., Mahmoud, F. and El-Sebae, A.K. (2010). Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. *Pesticide Biochemistry and Physiology*, 97: 7-12.

Ambali, S.F. (2009). Ameliorative effects of vitamin C and E on neurotoxicological, haematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats. *Doctor of Philosophy Dissertation*, Ahmadu Bello University, Zaria, Nigeria, 355 pp.

Ambali, S., Akanbi, D., Igbokwe, N., Shittu, M., Kawu, M. and Ayo, J. (2007). Evaluation of subchronic chlorpyrifos poisoning on haematology and serum biochemical changes in mice and the protective effect of vitamin C. *Journal of Toxicological Sciences,* 32(2): 111-120.

Ambali, S.F. Abbas, S.O., Shittu, M., Dzenda, T., Kawu, M.U., Salami, O. and Ayo, J.O. (2009). Effect of gestational exposure to chlorpyrifos on implantation and neonatal mice. *Journal of Cell and Animal Biology*, 3(4): 50-57.

Ambali, S.F., Shittu, M., Yaqub, L.S., Aliyu, H., Kobo, P., Kawu, M.U., Aluwong, T. and Ayo, J.O. (2009). Adherence to pesticide withdrawal periods on stored products among farmers in Nigeria: a case study of Zaria, Kaduna State. *A Paper presented at the International Field Toxicology Symposium in Africa*, University of Zambia, Lusaka, October 15, 2009.

Ambali, S.F., Adeniyi, S.A., Makinde, A.O., Shittu, M. and Yaqub, L.S. (2010a). Methanol extract of *Phyllanthus niruri* attenuates chlorpyrifos-evoked erythrocyte fragility and lipoperoxidative changes in Wistar rats. *Archives of Applied Science Research,* 2(4): 191-198.

Ambali, S.F., Abubakar, A.T., Shittu, M., Yaqub, L.S., Anafi, S.B. and Abdullahi, A. (2010b). Chlorpyrifos-induced alteration of haematological parameters in Wistar rats: Ameliorative effect of zinc. *Research Journal of Environmental Toxicology*, 4(2): 55-66.

Ambali, S.F., Abubakar, A.T., Shittu, M., Yaqub, L.S., Kobo, P.I. and Giwa, A. (2010c). Ameliorative effect of zinc on chlorpyrifos-induced erythrocyte fragility in Wistar rats. *New York Science Journal,* 3(5): 117-122.

Ambali, S.F., Ayo, J.O., Ojo, S.A. and Esievo, K.A.N. (2010d). Ameliorative effect of vitamin C on chronic chlorpyrifos-induced erythrocyte osmotic fragility in Wistar rats. *Human and Experimental Toxicology*, Epub ahead of print.

Ambali, S.F., Ayo, J.O., Ojo, S.A. and Esievo, K.A.N. (2010e). Co-administration of vitamins C and E ameliorates chronic chlorpyrifos-induced erythrocyte osmotic fraility in Wistar rats. *Australian Journal of Basic and Applied Sciences*, 4(6): 1051-1021.

Ambali, S.F., Idris, S.B., Onukak, C., Shittu, M. and Ayo, J.O. (2010f). Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats. *Toxicology and Industrial Health,* 26(9): 547-558.

Araujo, C.F., Lacerda, M.V.G., Abdalla, D.S.P. and Lima, E.S. (2008). The role of platelet and plasma markers of antioxidant status and oxidative stress in thrombocytopaenia among patients with vivax malaria. *Institute of Oswoldo Cruz*, 103: 517-521.

Ardwini,A., Mancinelli,G., Raclatti,G.L., Dottori, S., Molajoni, F. and Ramsay, R.R. (1992). Role of L-carnitine and carnitine palmitoyltransferase as integral component of pathway for membrane phospholipids fatty acid turnover in intact human erythrocytes. *Journal of Biological Chemistry*, 267 (18):12673-12681.

Ather, N.A., Ara, J., Khan, E.A., Sattar, R.A. and Durrani, R. (2008). Acute organophosphate insecticide poisoning. *Journal of Surgery Pakistan (International)*, 13(2): 71-74.

Aquil, F.R., Ahmad, I. and Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology*, 30: 177-183.

Balali-Mood, M. and Balali-Mood, K. (2008). Neurotoxic disorders of organophosphorus compounds and their managements. *Archives of Iranian Medicine*, 11(1): 65-89.

Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K. (1999). Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. *Toxicology Letters*, 107: 33-47.

Barna-Lloyd, T., Szabo, J.R. and Davis, N.L. (1991). Chlorpyrifos-methyl (Reldan R) rat chronic dietary toxicity and recovery study. Unpublished report TXT: K-046193-026 from Dow chemical, Texas, USA. Submitted to WHO by Dow Elanco, Indianapolis, USA.

Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry,* 195: 133-140.

Bender, D.A. and Mayes, P.A. (2003). Vitamins and Minerals. *In*: Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, C.W. (Eds.). *Harper's Illustrated Biochemistry*. 26th Edition, Lange Medical Books/McGraw-Hill. Medical Publishing Division. New York, Pp. 494-497.

Berg, G.L. (Ed). (1986). *Farm Chemical Handbook*. Willoughby, OH. Meister Publishing Company.

Binienda, Z.K. (2003). Neuroprotective effect of L-carnitine in induced mitochondrial dysfunction. *Annals of New York Academy of Science,* 993: 289-295.

Bjørling- Poulsen, M., Andersen, H.R. and Grandjean, P. (2008). Potential developmental neurotoxicity of pesticides used in Europe. *Environmental Health*, 7(50): 1-23.

Bloomer, R.J., Fisher-wellman, K. H. and Tucker, P.S. (2009). Effect of oral acetyl- L- carnitine arginate on resting and postprandial blood biomarkers in pre-diabetics. *Nutrition and Metabolism*, 6: 25.

Bomphard, E., Loser,E. and Schilde, B. (1981). [E605-methyl] [Methyl-parathion] Chronic toxicological studies in rats (2 years feeding trial). *Wupparatal-Elber Field Bayer AG, Institute of Toxicoloy IPCS*, 145-155.

Breslin, W.J., Liberacki, A.B., Dittenber, A. and Quast, J.F. (1996). Evaluation of the developmental and reproductive toxicity of chlorpyrifos in rats. *Fundamental and Applied Toxicology*, 29: 119-130.

Calabrese, V., Stella, A.M.G., Calvani, M. and Butterfield, D.A. (2006). Acetycarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *Journal of Nutritional Biochemistry*, 17: 73-88.

Calo, L.A., Pagnin, E., Davis, P.A., Semplicini, A., Nicolai, R., calvani, M. and Pessina, A.C. (2005). Antioxidant effect of L-carnitine and its short-chain esters relevance for the protection from oxidative stress related cardiovascular damage. *International Journal of Cardiology*, 107: 54-60.

Calvert, G.M., Karnik, J., Mehler, L., Beckman, J., Morrissey, B., Sievert, J., Barrett, R., Lackovic, M., Mabee, L., Schwartz, A., Mitchell, Y. and Moraga-Mc Haley, S. (2008). Acute pesticide poisoning among agricultural workers in the United States, 1998- 2005. *American Journal of Industrial Medicine*, 51: 883-898.

Canadas, F., Cardona, D., Davila, E. and Sanchez-santed, F. (2005). Long-term neurotoxicity of chlorpyrifos: spatial learning impaired on repeated acquisition in a water maze. *Toxicological Science*, 85: 944-951.

Carr, A.C. and Frei, B. (1999). Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB Journal*, 3: 1007-1024.

Carrell, R.W., Winterbourn, C.C. and Rachmilewitz, E.A. (1975). Activated oxygen and haemolysis. *British Journal of Haematology*, 30: 259-264.

Casida, J.E. and Quistad, G.B. (2004). Organophosphate toxicity: Safety aspects of non-acetylcholinesterase secondary targets. *Chemical Research in Toxicology*, 17: 983-998.

Cetinkaya, A., Bulbuloglu, E., Kantarceken, B., Ciralik, H., Kurutas, E.B., Buyukbese, M.A. and Gumusalan, Y. (2006). Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. *Digestive Diseases and Sciences*, 51(3): 488-494.

Chakraborti, T. K., Farrar, J.D. and Pope, C.N. (1993). Comparative neurochemical and neurobehavioural effects of repeated chlorpyrifos exposures in young rats. *Pharmacology, Biochemistry and Behaviour*, 46: 219-224.

Cheeseman, K.H. and Slater, T.F. (1993). An introduction to free radical biochemistry. *British Medical Bulletin*, 49: 481-493.

Chheteri, H.P., Khan, G.M., Acharya, A., Maharjan, S., Manandhar, M., Manandhar, R. and Gautam, S. (2008). Acute organophosphate poisoning: Pattern, management and outcomes. *Pharmacologyonline*, 1*:* 48-54.

Chiba, H. and Iwata, E. (2002). Studies on protein denaturation and enzyme inactivation caused by lipid hydroperoxides containing radicals. *Proceedings of The Japanese Academy*, 78 Serial B: 256-260.

Chiuailaf, R.H., Contreras, P.A. and Wittwer, F.G. (2002). Pathogenesis of oxidative stress: consequences and evaluation in animal health. *Vet Méx*, 33(3): 265-283.

Clarke, E.G.C. and Clarke, M.L. (1975). Organic compounds (II) pesticides. In: Veterinary Toxicology, (First Edition).Bailliere Tindall. A Division of Cassell and Collier Macmilliam Publishers Limited, New York, Pp 180-191.

Cohn, J. and Macphail, R.C. (1997). Chlorpyrifos produces selective learning deficit in rats working under a schedule of repeated acquisition and performance. *Journal of Pharmacology and Experimental Therapeutics*, 283 (1): 312-320.

Cope, W.G., Leidy, R.B. and Hodgson, E. (2004). Classes of toxicants: use classes. In: *Textbook of Modern Toxicology*, 3rd Edition, John Wiley and Sons, Inc., New Jersey, Pp. 58-70.

Costantini, D. and Verhulst, S. (2009). Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology*, 23: 506-509.

Cox, C. (1994). Chlorpyrifos, Part 1 (Toxicology). *Journal of Pesticide Reform*, 14(4): 15-20.

Cox, C. (1995). Chlorpyrifos, Part 2: human exposure. *Journal of Pesticide Reform,* 15(1): 14-20.

Cremlyn, R. (1978). Pesticides: Preparation and mode of action. John Wiley and Sons, New York, Pp 50-130.

Dargel, R. (1991). Lipid peroxidation - a common pathogenic mechanism? *Experimental Toxicology and Pathology*, 44: 169-181.

Davis, S.L., Tanaka, D., Aulerich, R.J. and Bursian, S.J. (1999). Organophosphorus- induced neurotoxicity in the absence of neuropathy target esterase inhibition. The effects of triphenylphosphine in the European ferret. *Toxicological Science*, 49: 78-85.

Davis, K.J.A. (2000). Oxidative stress, antioxidant defences and damage removal, repair and replacement systems, *International Union of Biochemistry and Molecular Biology Life*, 50: 279-289.

Devi, S.A., Vani, R., Subramanyam, M.V., Reddy, S.S. and Jeevratnam, K. (2007). Intermittent hypobaric hypoxia-induced oxidative stress in rat erythrocytes: protective effects of vitamin E, vitamin C and creatine. *Cell Biochemistry and Function*, 25: 221-231.

Dewey, C.W. (2005). Disorders of the peripheral nervous system. In: 50˚ Congresso Nazionale Multisala , *Societa Culturale Italiana Veterinari Per Animali Da Compaloma (SCIVAC),* Rimini, Italia.

Dille, J.R. and Smith, P.W. (1964). Central nervous system effects of chronic exposure to organophosphate insecticides. *Aerospace Medicine*, 35: 475-478.

Dokmeci, D., Apolat, M., Aydogdu, N., Uzal, C., Doganay, L. and Turan, F.N. (2006). The protective effect of L-carnitine on ionizing radiation-induced free oxygen radicals. *Scandinavian Journal of Animal Science*, 33(2): 75-83.

Draper, H.H. and Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186: 421-31.

Duarte, T.L. and Lunec, J. (2005). When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. *Free Radical Research*, 38(7): 671-686.

Duffy, F.H., Burchfield, J.L., Bartels, P.H., Gaon, M. and Sim, V.M. (1979). Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicology of Applied Pharmacology*, 47: 161-167.

Dundar, Y. and Aslan, R. (2000). Antioxidative stress. *Eastern Journal of Medicine*, 5(2): 45-47.

Durak, D.K., Uzun, F.G., Uzunhisarcikli, M., Kalender, S., Kalender, Y. and Ogutcu, A. (2008). Malathion-induced oxidative stress in human erythrocyte and the protective effect of vitamins C and E *in vitro*. *Environmental Toxicology*, 24: 234-242.

Eaton, D.L., Daroff, R.B., Autrop,H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., Reubert, D., Schulte-Hermann, R. and Spencer, P.S. (2008). Review of toxicology of chlorpyrifos with emphasis on human exposure and neurodevelopment. *Critical Review in Toxicology*, 52: 1-125.

Ecobichon, D.J. (1995). Toxic effects of pesticides. *In*: Klaassen, C.D., Amdur, M.O., Doull, J. (Eds.), *Casarett and Doull's Toxicology*. *The Basic Science of Poisons*, 5th Edition, McGraw-Hill, Health Professions Division, New York, Pp. 643-689.

Eipper, B.A. and Mains, R.E. (1991). The role of ascorbate in the biosynthesis of neuroendocrine peptides. *American Journal of Clinical Nutrition*, 54: 1153S-1156S.

El-Hossary, G.G., Mansour, S.M. and Mohamed, A.S. (2009). Neurotoxic effects of chlorpyrifos and the possible protective role of antioxidant supplements: an experimental study. *Journal of Applied Science Research*, 5(9): 1218-1222.

Environmental Justice Foundation (EJF), (2003). What's your poison? Health threats posed by pesticides in developing countries. *Environmental Justice Foundation*, London, U.K.

Escobar, J.A., Rubio, M.A. and Lissi, E.A. (1996). Superoxide dismutase and catalase inactivation by singlet oxygen and peroxyl radicals. *Free Radical Biology and Medicine*, 20: 285-290.

Eskenazi, B., Bradman, A. and Castorina (1999). Exposure of children to organophosphate pesticide and their potential adverse effects. *Environmental Health Perspective*, 107: 409-419.

Etlik, O. and Tomur, A. (2006). The oxidant effect of hyperbaric oxygenation and air pollution in erythrocyte membranes (hyperbaric oxygenation in air pollution). *European Journal of General Medicine*, 3: 21-28.

Eyer, P. (1995). Neuropsychopathological changes by organophosphorus compounds: a review, *Human Experimental Toxicology*, 14: 857-864.

Fatemeh, T., Amirkabirian, N., Esmaily, H., Mohammadirad, A., Aliahmadi, A. and Abdollahi, M. (2006). Alteration of hepatic cell glucose metabolism as a non-cholinergic detoxification mechanism in counteracting diazinon-induced oxidative stress. *Human and Experimental Toxicology,* 25: 697-703.

Faulkner, W.R. and King, J.W. (1970). *Manual of Clinical Laboratory Procedures*, Cleveland, Ohio, 354 pp.

Fenske, R.A. (1997). Pesticide exposure assessment of workers and their families. *Occupational medicine*, 12:221-237.

Fenske, R.A. and Day, E.W. (2005). Assessment of exposure for pesticide handlers in agricultural, residential and institutional environment. *In*: Franklin, C.A. and Worgan, J.P.(Eds.). *Occupational and Residential Exposure Assessment for Pesticides*. John Wiley and Sons, Limited, pp 14-18.

Ferreira, A., Maroco, E., Yonamine, M. and de Oliveira, M.L.F. (2008). Organophosphate and carbamate poisonings in the north west of Parana State, Brazil from 1994 to 2005: Clinical and epidemiological aspects. Revista Brasileira de Ciências Formacêutias. (*Brazilian Journal of Pharmaceutical Sciences)*, 44(3): 407-415.

Fishel, F.M. (2005). *Pesticide Toxicity Profile: Organophosphate Pesticides*. University of Florida, IFAS extension, P1-50. Available at: http://edis.ifas.ufl.edu.

Flatters, S.J.L., Xiao, W. and Bennett, G.J. (2006). Acetyl-carnitine prevents and reduces paclitaxel-induced painful peripheral neuropathy. *Neuroscience Letter*, 397(3): 219-223.

Frei, B. (1991). Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *American Journal of Clinical Nutrition*, 54: 11135-11185.

Furlong, J.H. (1996). Acetyl-L-carnitine: Metabolism and applications in clinical practice. *Alternative Medicine Review*, 1(2): 85-93.

Gain, S.K. and Shodet, S.B. (1981). Cacium potentiates the peroxidation of erythrocyte membrane lipids. *International Journal of Biochemistry, Biophysics and Molecular Biology*, 642: 46-54.

Galloway, T. and Handy, R. (2003). Immunotoxicity of organophosphorous pesticides. *Ecotoxicology*, 12(1-4): 345-363.

Gbaruko, B.C., Ogwo, E.I., Igwe, J.C. and Yu, H. (2009). Organophosphate induced chronic neurotoxicity: Health, environmental and risk exposure issues in developing nations of the world. *African Journal of Biotechnology,* 8(20): 5137-5141.

Gershon, S. and Shaw, F.B. (1961). Psychiatric sequelae of chronic exposure to organophosphate insecticides. *Lancet*, 1: 1371-1374.

Goel, A. and Aggarwal, P. (2007). Pesticide poisoning. *The National Journal of India*, 20(4): 182-191.

Goel, A., Dani, V. and Dhawan, D.K. (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in CPF-induced toxicity. *Chemico-Biological Interaction*, 156:131-140.

Goel, A., Dani, V. and Dhawan, D.K. (2006a). Chlorpyrifos-induced alterations in the activities of carbohydrate metabolizing enzymes in rat liver. The role of zinc. *Toxicology Letters*, 163: 235-241.

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

Golper, T.A., Goral, S., Becker, B.N. and Langman, C.B. (2003). L-carnitine treatment of anaemia. *American Journal of Kidney Diseases*, 41: 27-34.

Gomes, J., Dawodu, A.H., Lioyd, O., Revitt, D.M. and Anilal, S.V. (1999). Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphate insecticides. *Human and Experimental Toxicology*, 18(1): 33-37.

Gordon, C.J. (1994). Thermoregulation in laboratory animals and humans exposed to anticholinesterase agents. *Neurotoxicology Teratology,* 16: 427-453.

Gotoh, M., Saito, I., Huang, J., Fukaya, Y., Matsumoto, T., Hisanaga, N., Shibata, E., Ichihara, G., Kamijima, M. and Takeuchi, Y. (2001). Changes in cholinesterase activity, nerve conduction, velocity and clinical signs and symptoms in termite control operators exposed to chlorpyrifos. *Journal of Occupational Health*, 43: 157-164.

Greter, R.D., Kan, H.L., Lowe, E.R., Rick, D.L., Charles, G.D., Gallapudi, B.B. and Mattsson, J.L. (2008). Investigations of oxidative stress, antioxidant response and protein binding in chlorpyrifos exposed rat neuronal PC 12 cells. *Toxicology Mechanism and Methods*, 18: 17-23.

Grue, C.E., Gilbert, P.L. and Seeley, M.E. (1997). Neurophysiological and behavioural changes in non- target wildlife exposed to organophosphate and carbamate pesticides. Thermoregulation, food consumption and reproduction. *American Zoology*, 37: 369-388.

Guetens, G., DeBoeck, G., highly, M., Van Costerom, A.T. and de Bruyn, E.A. (2002). Oxidative DNA damage: Biological significance and methods of analysis. *Critical Review in Clinical Laboratory Sciences*, 39(4 &5): 331-457.

G�lcin, I. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Science*, 78: 803-811.

Gultekin, F., Ozturk, M. and Akdogan, M. (2000). The effect of organophosphate insecticide, chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in-vivo*). *Archives of Toxicology*, 74: 533-538.

Gultekin, F., Delibas, N., Yasar, S. and Kilinc, I. (2001). *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Archives of Toxicology*, 75(2): 88-96.

Gultekin, F., Patat, S., Akca, M.and Akdogan, M. (2006). Melatonin can suppress the cytotoxic effect of chlorpyrifos on human Hep G2 cell lines. *Human and Experimental Toxicology*, 35: 47-55.

Hagen, T.M., Liu, J., Lykkesfeldt, J., Wehr, C.M., Ingersoll, R.T., Vinarsky, V., Bartholomew, J.C. and Ames, B.N. (2002). Feeding acetyl-L- carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proceedings of the National Academy of Sciences*, 99(4): 1870-1875.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research. Comms*, 9(1): 1-32.

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35(5): 1147-1150.

Halliwell, B. (2009). The wonderings of a free radical. *Free Radical Biology and Medicine*, 46: 531-542.

Halliwell, B. and Gutteridge, J.M.C. (1990). The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics*, 280(1): 1-8.

Hammadeh, M.E., Filippos, A. and Hamad, M.F. (2009). Reactive Oxygen species and antioxidant in seminal plasma and their impact on male fertility. *International Journal of Fertility and Sterility,* 3(3): 87-110.

Hantson, P., Hainaut, P., Stappen, M.V. and Mahieu, P. (1996). Regulation of body temperature after acute organophosphate poisoning. *Canadian Journal of Anaesthesia*, 43(7): 755-758.

Hao, Y., Basile, A.S., Chen, G. and Zhang, L. (2004). Glutamate-induced over expression of GAD is down regulated by acetyl-L-carnitine in rats *islet cells*. *Endocrine Research*, 30(1): 107-116.

Harrison, F.E. and May, J.M. (2009). Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radical Biology and Medicine*, 46: 719-730.

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

Ilias, I., Manoli, I., Blackman, M.R., Gold, P.W. and Alesci, S. (2004). L-carnitine and acetyl-L-carnitine in the treatment of complications associated with HIV infection and antiretroviral therapy. *Mitochondrion*, 4: 163-168.

Iqbal, K., Khan, A. and Khattak, M.A.K. (2004). Biological significance of ascorbic acid (vitamin C) in human health - A review. *Pakistan Journal of Nutrition*, 3(1): 5-13.

Iyer, P., Kaufman, F. and Wu, K.L. (2008). Pharmacokinetics and metabolism. *In*: Evidence on the Developmental and Reproductive Toxicity of Chlorpyrifos. Reproductive and Cancer Hazard Assessment Branch Office of Environmental Health Hazard Assessment California Environmental Protection Agency, Pp. 7-12.

Iwasaki, M., Yoshihara, A., Hirotomi, T., Ogawa, H., Hanada, N. and Miyazaki, H. (2008). Longitudinal study on the relationship between serum albumin and periodontal diseases. *Journal of Clinical Periodontology*, 35: 291-296.

Jacob, H.S. and Lux, S.E. (1958). Degradation of membrane phospholipids and thiols in peroxide haemolysis: Studies in vitamin E deficiency. *Blood*, 32: 549-568.

Jaga, K. and Dharmani, C. (2003). Sources of exposure to and public health implications of organophosphate pesticides. *American Journal of Public Health*, 14(3): 171-185.

Jaggy, A. and Oliver, J.E. (1990). Chlorpyrifos toxicosis in two cats. *Journal of Veterinary internal Medicine*, 4(30): 135-139.

Jamal, G. (1997). Neurological syndromes of organophosphorus compounds. *Adverse Drug Reaction Toxicology Review*, 16: 133-170.

Jamal, G.A., Hansen, S., Pilkington, A., Buchanan, D., Gillham, R.A., Abdel-Azis, M., Julu, P.O.O., Al-Rawas, S.F., Hurley, F. and Ballantyne, J.P. (2002). A clinical neurological, neurophysiological, and neuropsychological study of sheep farmers and dippers exposed to organophosphate pesticides. *Occupational and Environmental Medicine*, 59: 434-441.

Jamil, K., Das, G.P., Shaik, A.P., Dharmi, S.S. and Murthy, S. (2007). Epidemiological studies of pesticide exposed individuals and their clinical implications, *Current Science*, 93(3): 340-345.

Jaroli, D.P. and Sharma, B.L. (2005). Effect of organophosphate insecticides on the organic constituents in liver of *Channa punctatus*. *Asian journal of Experimental Science*, 19 (1): 121-129.

Jarvik, G.P., Tsai, T.N., Mc Kinsty, L.A., Wani, R., Brophy, V., Richter, R.J., Schellenberg, G.D., Heagerty, P.J. Hatsukami, T. and Furlong, C.E. (2002). Vitamin C and E intake is associated with increase paraoxonase activity. *Arteriosclerosis*, *Thrombosis and Vascular Biology*, 22: 1329-1333.

Jirillo, E., Altamura, M., Marcuccio, C., De Simone, C. and Antonaci, S. (1993). Immunological responses in patients with tuberculosis and in vivo effects of acetyl-L-carnitine oral administration. *Mediators of inflammation*, 2:S17-S20.

Joshi, P., Deep, H. and Smith, J. (2002). Effect of lindane and malathion exposure to certain blood parameters in a fresh water teleost fish *Claria batrachus*. *Pollution Resources*, 21: 55-57.

Julvez, J. and Grandjean, P. (2009). Neurodevelopmental toxicity risk due to occupational exposure to industrial chemicals during pregnancy. *Industrial Health*, 47: 459- 468.

Jusman, S.W.A. and Halim, A.S. (2009). Oxidative stress in liver tissue of rat induced by chronic systemic hypoxia. *Makara, Kesehatan*, 13(1): 34-38.

Kagan, J. (1971). Topical questions about the toxicology of phosphorganic insecticides. *Ernahrungs-Forschung,* 4: 503-514. (in German).

Kalender, S., Kalender, Y., Ogutcu, A., Uzunhisarcikli, M., Durak, D. and Acikgoz, F. (2004). Endosulfan-induced cardiotoxicity and free radical metabolism in rats: The protective effect of vitamin E. *Toxicology,* 3: 227-235.

Kamanyire, R. and Karalliede, L. (2004). Organophosphate toxicity and occupational exposure. *Occupational Medicine*, 54: 69-75.

Karalliede, L. and Senanayake, N. (1989). Organophosphate insecticide poisoning. *British Journal of Anaesthesia*, 63: 736-750.

Kart, A. (2005). Neuropathic aspect of organophosphate toxicity: A review. *Journal of Animal and Veterinary Advances*, 4(8):723-725.

Kart, A., Yapar, K., Karaphehlivan, M., Tunca, R., Ogun, M. and Citil, M. (2006). Effects of L-carnitine on kidney histopathology, plasma and tissue total sialic acid, malonaldehyde and glutathione concentrations in response to gentamicin administration in Balb/C mice. *Revue de Medecine Veterinaire*, 157(4): 179-184.

Kehrer, J.P., Jones, D.P., Lemaster, J.J., Farber, L. and Jaeschke, H. (1990). Summary of the symposium presented at the 1990 Annual Meeting of the Society of Toxicolgy. *Toxicology and Applied Pharmacology,* 106: 165-178.

Kehrer, J.P. (1993). Free radicals as mediators of tissue injury and disease. *Critical Review in Toxicology*, 23: 21-48.

Kerem, M., Bedirli, N., Gürbüz, N., Ekinci, Ö., Bedirli, A., Akkaya, T., Sakrak, Ö. and Pasaoğlu, H. (2007). Effects of acute fenthion toxicity on liver and kidney function and histology in rats. *Turkish Journal of Medical Science*, 37(5): 281-288.

Khan, S.M. and Kour, G. (2007). Subacute oral toxicity of chlorpyrifos and the protective effect of green tea extract. *Pesticide Biochemistry and Physiology*, 89: 118-123.

Khan, S. and Pandya, K.P. (1985). Hepatotoxicity in albino rats exposed to n- octane and n-nonane. *Journal of Applied Toxicology*, 5: 63.

Kidd, P.M. (1999). A review of nutrients and botanicals in the integrative management of cognitive dysfunction. *Alternative Medicine Review*, 4: 144-161.

Kono, Y. and Fridovich, I. (1982). Superoxide radical inhibits catalase. *Journal of Biological Chemistry,* 257: 5751-5754.

Konradsen, F., Van der Hoek, W., Cole, D.C., Hutchison, G., Daisley, H., Singh, S. and Eddleston, M. (2003). Reducing acute poisoning in developing countries - options for restricting the availability of pesticides. *Toxicology,* 192: 249-261.

Kousba, A.A., Sultatos, L.G., Poet, T.S. and Timchalk, C. (2004). Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. *Toxicological Science*, 80: 239-248.

Kozawa, K., Aoyama, Y., Mashimo, S. and Kimura, H. (2009). Toxicity and actual regulation of organophosphate pesticides. *Journal of Toxicology: Toxin Review*, 28(4): 245-254.

Krishna, H. and Ramachandran, A.V. (2009). Biochemical alterations induced by the acute exposure to combination of chlorpyrifos and lead in Wistar rats. *Biology and Medicine*, 1(2): 1-6.

Krisnamoorthy, P., Vairamuthu, S., Balachandran, C. and Muralimanohar, B. (2006). Chlorpyrifos and T-2 toxin induced haemato-biochemical alterations in broiler chicken. *International Journal of Poultry Science*, 5(2): 173-177.

Kukucka, M.A. and Misra, H.P. (1993). The antioxidant defence system of isolated guinea pig Leydig cells. *Molecular and Cellular Biochemistry*, 126: 1-7.

Langsdorf, L.J. and Zydney, A.L. (1993). Effect of uraemia on the membrane transport characteristic of red blood cells. *Blood*, 81: 820-827.

Leeuwenburgh, C. and Heinecke, J.W. (2001). Oxidative stress and antioxidants in exercise. *Current Medicinal Chemistry*, 8: 829-838.

Leibson, T. and Lifshutz, M. (2008). Organophosphate and carbamate poisoning: review of the current literature and summary of clinical and laboratory experience in Southern Isreal. *Isreal Medical Association Journal*, 10: 767-770.

Li, Q. (2007). New mechanism of organophosphorus pesticide- induced immunotoxicity. Review. *Journal of Nippon Medical School*, 74(2): 92-105.

Liu, J., Head, E., Ghrib, A.M., Yuan, W., Ingersoll, R.T., Hagen, T.M., Cotman, C.W. and Ames, B.N. (2002). Memmory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L- carnitine and/or – lipoic acid. *Proceedings of the National Academy of Sciences*, 99(4): 2356-2361.

Liu, J., Head, E., Kuratsune, H., Cotman, C.W. and Ames, B. N. (2004). Compaism of the effects of L-carnitine and acetyl-L-carnitine on carnitine levels, ambulatory activity, and oxidative stress biomarkers in the brain of old rats. *Annals of New York Academy of Sciences*, 1033: 117-131.

Loegering, D.J., Bonin, M.C., Smith, J.J. (1975). Effect of exercise, hypoxia, and epinephrine on lysosomes and plasma enzyme. *Experimental Molecular Pathology*, 22: 242

López, O., Hermandez, A.F., Rodrigo, L., Gil, F., Pena, G., Serrano, J.L., Parron, T., Villanenva, E. and Pla, A. (2007). Changes in antioxidant enzymes in humans with long-term exposure to pesticides. *Toxicology Letters*, 171: 146-153.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 54: 250-287.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal Biology Chemistry*, 193: 265-275.

Lukaszewicz-Hussain, A. and Moniuszko-Jakoniuk, J. (2005). A low dose of chlorfenvinphos affects hepatic enzymes in serum and antioxidant enzymes in the erythrocytes and liver of rats. *Polish Journal of Environmental Studies*, 14(2): 199-202.

Luna, G.H. (1960). *Manual of Histologic Staining Method of Armed Forces Institute of Pathology*. 35th Edition, McGraw-Hill Book Company, New York, 46 pp.

Luskova, V., Svoboda, M. and Kolarova, J. (2002). The effect of diazinon on blood plasma biochemistry in Carp (*Cyprinus carpio L*). *ACTA Veterinaria. BRNO,* 71: 117-123.

Mahan, D.C., Ching, S. and Dabrowski, K. (2004). Developmental aspects and factors influencing the synthesis and status of ascorbic acid in pig. *Annual Review of Nutrition*, 24: 79-103.

Manos, P., Bryan, G.K. and Edmond, J. (1991). Creatine Kinase activity in postnatal rat brain development and in cultured neurons, astrocytes, and oligodendrocytes. *Journal of Neurochemistry*, 56: 2101-2107.

Mansour, H.H. (2006). Protective role of carnitine ester against radiation-induced oxidative stress in rats. *Pharmacological Research*, 54 (3): 165-171.

Mansour, S.A. and Mossa, A.H. (2009). Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pesticide Biochemistry and Physiology*, 93: 34-39.

Mansour, M.K., El-Kashaury, A.A.I., Rasheed, M.A. and Koretem, K.M. (2009). Oxidative and biochemical alterations induced by profenofos insecticide in rats. *Nature and Science*, 7 (2): 1-14.

Mantle, D. (1997). Effect of pirimphos methyl on proteolytic enzyme activities in rat heart, kidney, brain and liver tissues *in-vivo. International Journal of Clinical Chemistry,* 262: 89-97.

Matsumoto, Y.I., Amano, S., Hirose, Y., Tsuruta, Y., Hara, S., Murata, M. and Imai, T. (2001). Effects of L-carnitine supplementation on renal anaemia in poor responders to erythropoietin. *Blood purification*, 19: 24-32.

Matsumura, M., Hatakeyana, S., Koni, L., Mabuchi, H. and Muramoto, H. (1996). Correlation between serum carnitine levels and erythrocyte osmotic fragility in haemodialysis patients. *Nephron,* 72: 574-578.

McCall, M.R. and Frei, B. (1990). Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radical Biology and Medicine*, 26(7/8): 1034-1053.

McConnell, R., Delgado-Tellez, E., Cuadra, R., Torres, E., Keifer, M., Simpson, D. and Lundberg, I. (1999). Organophosphate neuropathy due to methamidophos; biochemical and neurophysiological markers. *Archives of Toxicology*, 73: 296-300.

Mehta, A., Verma, R.S. and Srivastava, N. (2009). Chlorpyrifos-induced alterations in the levels of hydrogen peroxide nitrate and nitrite in rat brain and liver. *Pesticide Biochemistry and Physiology*, 94:55-59.

Menvielle- Bourg, F.J (2005). Superoxide dismutase (SOD), a powerful antioxidant is now available orally. *Phytotherapie*, 3:1-4.

Metcalf, D.R. and Holmes, J.H. (1969). EEG, psychological and neurological alterations in human organophosphorus exposure. *Annals New York Academy of sciences*, 160: 357-365.

Miguel, F., Augusto, A.C. and Gurgueira, S.A. (2009). Effect of acute vs. chronic H_2O_2- induced oxidative stress on antioxidant enzyme activities. *Free Radical Research*, 43(4): 340-347.

Miranda, J, (2003). Neurotoxicity after poisonings with organophosphate pesticides in Nicaragua. *National Institute for Working Life,* S-11279 Stockholm Sweden. Pp 1-29.

Miranda, J., Mc Connell, R., Wesseling, C., Cuadra, R., Delgado, E., Torres, E., Keifer, M. and Lundberg, I. (2004). Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticides. *Occupational and Environmental Medicine*, 61: e4.

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

Mitra, N.K., Siong, H.H. and Nadarajah, V. D. (2008). Evaluation of neurotoxicity of repeated dermal application of chlorpyrifos on hippocampus of adult mice. *Annals of Agricultural and Environmental Medicine,* 15: 211-216.

Moretto, A. and Lotti, M. (1998). Poisoning by organophosphorus insecticides and sensory neuropathy. *Journal of Neurology, Neurosurgery and Psychiatry*, 64: 463-468.

Mukut, S., Ran, S., Xiu, L.G., Brain, L.F., Ellen, T.M., Virginia, J.S., Eric, P.C. and John, E.M. (2001). Early detection of radiation-induced glomerular injury by albumin permeability assay. *Bio one*, 155(3): 414-480.

Naidu, K.A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutitional Journal,* 2: 7-16.

Nand, N., Aggarwal, H.K., Bharti, K. and Chakrabarti, D. (2007). Organophosphate induced delayed neuropathy. *Journal of Association of Physicians of India*, 55: 72-73.

Nand, N., Yadav, R.K., Aggarwal, H.K., Yadav, A. and Sharma, M. (2008). To study the effect of L-carnitine administration on anaemia and oxidative stress in haemodialysed patients. *Journal India Academy of Clinical Medicine*, 9 (4): 268-273.

Nikolaos, S., George, A., Telemachos, T., Maria, S., Yannis, M., Konstantinos, M. (2000). Effect of L-carnitine supplementation on red blood cell deformability in haemodialysis patients. *Renal Failure*, 22: 73-80.

Noguchi, N., Watanabe, A. and Shi, H. (2000). Diverse functions of antioxidants. *Free Radical Research*, 33: 809-817.

Odenkirchen, E.W. and Eisler, R. (1988). Chlorpyrifos hazards to fish, wildlife, and invertebrates: A synoptic review. *Contaminant Hazard Reviews*. Report No. 13: 1-24.

Ohyashiki, T., Kobayashi, M. and Matsui, K. (1991). Oxygen radical mediated lipid peroxidation and inhibition of ADP-induced platelet aggregation. *Archives Biochemistry and Biophysics*, 288: 282-286.

Ojezele, O.M. and Abatan, M.O. (2009). Toxicological effects of chlorpyrifos and methidathion in young chickens. *African Journal of Biochemistry Research*, 3: 48-51.

Okechukwu, E.O., Auta, J. and Balogun, J.K. (2007). Effect of acute norminal doses of chlorpyrifos-ethyl on some haematological indices of African catfish *Clarias gariepinus- teugels. Journal of Fisheries International*, 2(2):190-194.

Onem, G., Aral, E., Enli, Y., Oguz, E.O., Coskun, E., Aybek, H., Ozcan, A.V., Sacar, M., Bir, L.S., Baltalarli, A. and Baycu, C. (2006). Neuroprotective effects of L-carnitine and vitamin E alone or in combination against ischaemia-reperfusion injury in rats*. Journal of Surgical Research*, 131: 124-130.

Otitoju, O., Onwurah, N.E.I., Otitoju, G.T.O. and Ugwu.C.E. (2008). Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin. *Biokemistri,* 20(2): 93-98.

Oyagbemi, A.A., Azeez, O.I. and Saba, A.B. (2009). Interactions between reactive oxygen species and cancer: the roles of natural dietary antioxidant and their molecular mechanism of action. *Asian Pacific Journal of Cancer Prevention*, 10: 535-544.

Oyewale, J.O. (1993). Effects of storage of blood on the osmotic fragility of mammalian erythrocyte. *Journal of Veterinary Medicine*, 40: 258-264.

Ozer, J., Ratnerb, M., Shawc, M., Baileya, W. and Schomakerd, S. (2007). The current state of serum biomarkers of hepatotoxicity. *Toxicology*, 245(3): 194-205.

Packer, L. and Cadenas, E. (2007). Oxidant and antioxidant revisited. New concept of oxidative stress. *Free Radical Research*, 41(9): 951-952.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Kwan, O., Lee, J., Chen, S., Corpe, C., Dutta, A., Dutta, S.K. and Levine, M. (2003). Vitamin C as an antioxidant: Evaluation of its role in diseases prevention. *Journal of the American College of Nutrition*, 22(1): 18-35.

Parsons, K.C., Schmidt, S.R., Tarbill, G. and Tucker, K.R. (2006). Sublthal effects of exposure to cholinesterase-inhibiting pesticides. Pesticide and you. *Beyond Pesticides/ National Coalition against the Misuse of Pesticides*, 26(2): 19-24.

Patil, J.A., Patil, A.J. and Govindwar, S.P. (2003). Biochemical effects of various pesticides on sprayers of Grape gardens. *Indian Journal of Clinical Biochemistry*, 18(2): 16-22.

Paudyal, B.P. (2008). Organophosphorus poisoning. Review article. *Journal of Nepal Medical Association*, 47(172): 251-258.

Pazdnerniki, T.L., Emerson, M.R., Cross, R. Nelson, S.R. and Samson, F.E. (2001). Soman-induced seizure: Limbic activity, oxidative stress and neuroprotective proteins. *Journal of Applied Toxicology*, 21: 587-594.

Peace, A.J. and Kaplan, L.A. (1987). *Clinical Chemistry*. Mosby, St.Loius, U.S.A.

Peeples, E.S., Schopter,L.M., Duysen, E.G., Spaulding, R., Voelkei, T., Thompson, C.M. and Lockridge, O. (2005). Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry. *Toxicological Science*, 83(2): 303-312.

Peirrs-John, R.J. and Wickremasinghe, R. (2008). Impact of low-level exposure to organophosphates on human reproduction and survival. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102:239-245.

Perry, G., Sayre, L.M., Atwood, C.S., Castellani, R.J., Cash, A.D., Rottkamp, C.A. and Smith, M.A. (2002). The role of iron and copper in the aetiology of neurodegenerative disorders: therapeutic implications. *CNS Drugs*, 16(5): 339-352.

Pettegrew, J.W., Levine, J. and Mcclure, R.J. (2000). Acetyl-L- carnitine physico-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimers disease and genetic depression. *Molecular Psychiatry*, 5(6): 616-632.

Picconi, B., Barone, I., Pisani, A., Nicolai, R., Benatti, P., Bernardi, G., Caivani, M. and Calabresi, P. (2006). Acetyl-L-carnitine protects striatal neurones against *in-vitro* ischaemia: The role of endogenous acetylcholine. *Neuropharmacology*, 50: 917-923.

Pilkington, A., Buchanan, D., Jamal, G.A., Hansen, S., Gilham, R., Hansen, S., Kidd, M., Hurley, J.F. and Soutar, C.A. (2001). An epidemiological study of the relationship between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occupational and Environmental Medicine*, 58(11): 702-710.

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. *Environment Development and Sustainability*, 7: 229-252.

Poet, T.S., Wu, H., Kousba, A. and Timchalk, C. (2003). *In-vitro* rats hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. *Toxicological Science*, 72: 192-200.

Pohanka, M., Jun, D. and Kuca, K. (2008). Amperometric biosensors for real time assays of organophosphates. *Sensors*, 8:5303-5312.

Pope, C.N., Chakraborti, T.K., Chapman, M.L. and Ferrar, J.D. (1992). Long-term neurological and behavioural effects induced by acute chlorpyrifos treatment. *Pharmacology and Biochemistry of Behaviour*, 42: 251-256.

Pruett, S.B., Han, Y., Munson, A.E. and Fuchs, B.A. (1992). Assessment of cholinergic influence on a primary humoral immune response. *Immunology*, 77(3): 428-435.

Quintanilla, A.P. (1982). Pigment nephropathy. *In*: Earle, D.P. (Ed.). *Manual of Clinical Nephrology*, Saunders, Philadlphia, Pp. 269-276.

Rabideau, C.L. (2001). Pesticide mixtures induce immunotoxicity: potentiation of apoptosis and oxidative stress. *M.Sc. Thesis,* Virginia Polytechnic and State University, Blackburg, Virginia, 170 pp.

Ramesh, M. and Saravanan, M. (2008). Haematological and biochemical responses in a fresh water fish Cyprinus carpio exposed to chlorpyrifos. *International Journal of Integrative Biology*, 3(1): 80-83.

Rangan, U. and Bulkley, G.B. (1993). Prospects for treatment of free radical medicated tissue injury. *In*: Cheeseman, K.H. and Slater, T.F. (Eds.). Free Radicals in Medicine, New York: Churchill Livingstone, Pp.700-1718.

Rani, P.J.A. and Panneerselvam, C. (2001). Carnitine as a free radical scavenger in ageing. *Experimental Gerontology*, 36: 1713-1726.

Rastogi, S.K., Satyanarayan, P.V.V., Ravishankar, D. and Tripathi, S. (2009). A study of oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spraying. *Indian Journal of Occupational and Environmental Medicine*, 13(3):131-134.

Ray, D.E. (1992). *Pollution and Health*. Wiley Eatern Ltd., New Delhi.

Ray, D. E. and Richards, P.G. (2001). The potential for toxic effects of chronic low- dose exposure to organophosphates. *Toxicology Letters*, 120: 343-351.

Rees, H. (1996). Exposure to sheep dip and the incidence of acute symptoms in a group of welsh sheep farmers. *Occupational and Environmental Medicine*, 53: 258-263.

Reinecke, S.A. and Reinecke, A.J. (2005). The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. *Ecotoxicity and Environmental Safety*, 143: 261-266.

Rice-Evans, C.A. and Diplock, T.A. (1993). Current status of antioxidant therapy. *Free Radical Biology and Medicine*, 15: 77-96.

Richardson, R.J., Moore, T.B., Kayyali, U.S. and Randall, J.C. (1993). Chlorpyrifos: assessment of potential for delayed neurotoxicity by repeated dosing in adult hens with monitoring of brain acetylcholinesterase, brain and lymphocyte neurotoxic esterase and plasma butyrylcholinesterase activities. *Fundamental and Applied Toxicology*, 21: 89-96.

Roche, M., Rondeau, P., Singh, N.R., tarnus, E. and Bourdon, E. (2008). The antioxidant properties of serum albumin. *Federation of European Biochemical Societies Letters*, 582: 1783-1787.

Ronca, F., Palmieri, L., Malengo, S. and Bertelli, A. (1994). Effect of L-propionylcarnitine on *in-vitro* membrane alteration of sickle-cell anaemia erythrocytes. *International Journal of Tissue Reaction*, 16: 187-194.

Rosety-Rodriguez, M., Ordonez, F.J., Rosety, I., Rosety, J.M. and Rosety, M. (2005). Erythrocyte antioxidant enzymes of gilthead as early warning bio-indicators of oxidative stress induced by malathion. *Haematology*, 8 (2): 237-240.

Rubin, C., Esteban, E., Kieszak, S., Hill, R.H., Dunlop, B., Yacovac, R., Trottier, J., Boylan, K., Tomasewski, T. and Pearce, K. (2002). Assessment of human exposure and human health effects after indoor application of methyl parathion in Lorain county, Ohio, 1995-1996. *Environmental Health Perspective*, 110(6): 1053-1056.

Sams, C., Cocker, J. and Lennard, M.S. (2004). Biotransformation of chlorpyrifos and diazinon by human liver microsomes and recombinant human cytochrome p450s (CYP). *Xenobiotica*, 34(10): 861-873.

Satyanarayana, M.L., Advithi, R., Venkateshwari, A. and Nallari, P. (2009). Pro and antioxidants in cardiomyopathy. *International Journal of Human Genetics*, 9(3-4): 263-267.

Sauberlich, H.E. (1994). Pharmacology of vitamin C. *Annual Review of Nutrition,* 14: 371-391.

Saulbury, M.D., Heyliger, S.O., Wang, K. and Johnson, D.J. (2009). Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. *Toxicology*, 259: 1-9.

Sayers, M.H., Lynch, S.R. and Jacobs, P. (1973). The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. *British Journal of Haematology*, 4: 209-218.

Schneider, C.D. and deOliveira, A.R. (2004). Oxygen free radicals and exercise: Mechanisms of Synthesis and adaptation to the physical training. *Review Bras Medical Esparte*, 10(4): 314-318.

Scremin, O., Shih, T.M., Huynh,L., Roch, M., Booth, R. and Jenden, D.J. (2003). Delayed neurologic and behavioural effects of subtoxic doses of cholinesterase inhibitors. *Journal of Pharmacology Experimental Therapeutics*, 304: 1111-1119.

Senanyake, N. and Karallied, L. (1987). Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. *New England Journal of Medicine*, 316: 761-763.

Senturk, U.K., Gunduz, F., Kuru,O., Aktekin, M.R., Kipmen, D., Yalcin, O., Bor-Kucukatay, M., yesikaya, A. and Baskurt, O.K. (2001). Exercise-induced oxidative stress affects erythrocytes in sedentary rats but not in exercise trained rats. *Journal of Applied Physiology*, 91: 1999-2004.

Seven, A., Guzel, S., Seymen, O., Civelek, S., Bolayirh, M., Uncu, M. and BurCak, G. (2004). Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma. *Yonsei Medical Journal*, 45: 703-710.

Seyrek, K., Yenisey, C., Serter, M., Kiral, F.K., Ulutas, P.A. and Bardakcio-Glu, H.E. (2004). Effects of dietary vitamin C supplementation on some serum biochemical parameters of laying Japanese quails exposed to heat stress (34.8 C). *Revue de Médecine Véterinaire*., 155(6): 339-342.

Shah, M.A.A. (2002). Toxicology of agrochemicals: organochlorine insecticide. *In*: Garg, S.K. and Jain, S.K. (Eds.). *Veterinary Toxicology*, (First Edition), CBS Publishers and Distributors, 4596/1A, 11 Darya Ganj, New Delhi, India. Pp. 151-157.

Shah, M.A.A. and Dumka, V.K. (2002). Toxicology of agrochemicals: organophosphorus insecticides. *In*: Garg, S.K. and Jain, S.K. (Eds.). *Veterinary Toxicology*, (First Edition), CBS Publisher and Distributors, 4596/1A, 11 Darya Ganj, New Delhi, India. Pp. 158-171.

Shah, M.A.A. and Srivastava, A.K. (2002). Toxicology of agrochemicals. *In*: Garg, S.K. and Jain, S.K. (Eds.). *Veterinary Toxicology*, (First Edition), CBS Publisher and Distributors, 4596/1A, 11 Darya Ganj, New Delhi, India. Pp. 146-191.

Shih, D.M., Gu, L., Xia, Y.K., Navab, M., Li, W.F., Hama, S., Castellani, L.W., Furlang, C.E., Costa, L.G., Fogelman, A.M. and Lusis, A.J. (1998). Mice lacking serum paraoxonase are susceptible to organophosphorus toxicity and atherosclerosis. *Nature*, 374(6690): 284-287.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, 82: 291-295.

Siest, G. and Galteau, M.M. (1974). Variation of plasmatic enzymes during exercises. *Enzymes,* 19: 179.

Singh, G. and Khurana, D. (2009). Neurology of acute organophosphate poisoning. *Neurology India*, 57(2): 119-125.

Singh, R.P., Sharad, S. and Kapur, S. (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *Journal of Indian Academy of Clinical Medicine,* 5(3): 218-225.

Slotkin, T.A. (2004). Cholinergic systems in the brain development and disruption by neurotoxins: nicotine, environmental tobacco smoke, organophosphates. *Toxicology and Applied Pharmacology*, 198: 132-151.

Slotkin, T.A., Levin, E.D. and Seidler, F.J. (2006). Comparative developmental neurotoxicity of organophosphate insecticides: Effects on brain development are separable from systemic toxicity. *Environmental Health Perspectives*, 114: 746-751.

Smegal, D.C. (2000). Human health risk assessment: Chlorpyrifos. *US Environmental Protection Agency, Office of Pesticide Programmes Health Effects Division (7509C*), Pp. 1-138.

Srivastava, A.K. and Dumka, V.K. (2002). Toxicology of agrochemicals: organophosphorus insecticides. *In*: Garg, S.K. and Jain, S.K. (Eds.). *Veterinary Toxicology*, (First Edition), CBS Publisher and Distributors, 4596/1A, 11 Darya Ganj, New Delhi, India. Pp. 158-171.

Stephens, R., Spurgeon, A., Calvert, I.A., Beach, J., Levy, L.S. Berry, H. and Harrington, I.M. (1995). Neuropsychological effects of long- term exposure to organophosphates in sheep dip. *Lancet*, 345: 1135-1139.

Subbotina, S.G. and Belonozhko, G.A. (1968). (The effect of sevin on the thermoresistance and fractionary protein content of blood serum). *Gig. Primen Toxicolo Pestic. Klin. Otrav*, 442-444.

Sungur, M. and Guven, M. (2001). Intensive care management of organophosphate insecticide poisoning. *Critical Care*, 5: 211-215.

Sunil, K. and Dinesh, K. (2009). Antioxidant and free radical scavenging activities of edible weeds. *African Journal of Food Agriculture Nutrition and development,* 9(5): 1174-1190.

Szabo, J.R., Young, J.T. and Granjean, M. (1988). Chlorpyrifos: 13-week dietary toxicity study in Fisher 344 rats. *Jackson Research Centre, Health and Environmental Sciences-Texas: Laboratory Study No: TXT: K-044793-071. Report dated December 28, 1988.*

Tang, J., Cao, Y., Rose, L.R., Brimfield, A.A., Dai, D., Goldstein, J.A. and Hodgson, E. (2001). Metabolism of chlorpyrifos by human cytochrome P_{450} isoforms and human, mouse, and rat liver microsomes. *Drug Metabolism and Disposition*, 29(9): 1201-1204.

Tappel, A.L. (1973). Lipid peroxidation damage to cell components. *Federation Proceedings*, 32: 1870-874.

Thrasher, J.D., Madison, R. and Broughton, A. (1993). Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. *Archives of Environmental Health*, 48(2): 89-93.

Thrasher, J.D., Heuser, G. and Broughton, A. (2002). Immunological abnormalities in humans chronically exposed to chlorpyrifos. *Archives of Environmental Health*, 57: 181- 187.

Timchalk, C., Nolan, R.J., Mendrala, A.L., Dittenber, D.A., Brzak, K.A. and Mattson, J.L. (2002). A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicologic Sciences*, 66: 34-53.

Timchalk, C., Kousba, A.A. and Poet, T.S. (2007). An age dependent physiologically base pharmacokinetics/pharmacodynamic model for the organophosphorous insecticide chlorpyrifos in the preweaning rat. *Toxicology Science*, 98(2): 348-365.

Tkaczyk, J. and Vizek, M. (2007). Oxidative stress in lung tissue: Sources of reactive oxygen species and antioxidant defence. *Praque Medical Report*, 108(2): 105-114.

Tripathi, S. and Srivastava, A.K. (2010). Liver profile of rats after long-term ingestion of different doses of chlorpyrifos. *Pesticide Biochemistry and Physiology*, 97(1): 60-65.

Tuzmen, N., Canadan, N., Kaya, E. and Demiryas, N. (2008). Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidants defence mechanisms and lipid peroxidation in rat liver. *Cell Biochemistry and Function*, 26: 119-124.

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

Valko, M., Moriss, H. and Cronin, M.T.D. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, 12: 1161-1208.

Vandana, S., Ram, S., Ilavazhagan, M., Kumar, G.D. and Banerjee, P.K. (2006). Comparative cytoprotective activity of vitamins C, E and beta-carotene against chromium-induced oxidative stress in murine macrophages. *Biomedicine and Pharmacotherapy*, 60: 71-76.

Verma, R.S. and Srivastava, N. (2003). Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues. *Indian Journal of Biochemistry and Biophysics*, 40: 423- 428.

Verma, R.S., Mehta, A. and Srivastava, N. (2007). *In vivo* chlorpyrifos induced oxidative stress: attenuated by antioxidant vitamins. *Pesticide Biochemisty and Physiology*, 88: 191-196.

Verma, R.S., Mehta, A. and Srivastava, N. (2009). Comparative studies on chlorpyrifos and methyl parathion induced oxidative stress in different parts of rat brain: Attenuation by antioxidant vitamins. *Pesticide Biochemistry and Physiology*, 95: 152-158.

Vermani, M.A., Biselli, R., Spadoni, A., Rossi, S., Corsico, N., Calvani, M., Fattorossi, A., De Simone, C. and Arrigoni-Martelli, E. (1995). Protective action of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. *Pharmacological Research*, 32: 383-389.

Vural, S., Cetin, E.T. and Tuzlaci, U. (1986). *Klinik tehiste Laboraturvar. Nutrettin.Uycan Ciltve, Basim. Sanayii*, Istanbul.

http://www.nadirkitap.am/klinik-teshiste-laboratuvar-1986-kiptap635537.html.

Wadia, R.S., Sadagopan, C., Amin, R.B. and Sardesai, H.V. (1974). Neurological manifestation of organophosphorus insecticide poisoning. *Journal of Neurology and Neurosurgery Psychiatry*, 37: 841-847.

Wagner, G.M., Lubin, B.H. and Chiu, D.T.Y. (1988). Oxidative damage to red blood cells. *In*: Chow, C.K. (Ed.). Cellular antioxidant defence mechanisms, Vol. 1, CRC press Inc. Boca Raton, FL. Pp. 185-195.

Wall, J. (2000). Antioxidant in prevention of reperfusion damage of vascular endothelium, *Trinity Student Medical Journal*, 1: 67-71.

Wardlaw, G.M. (1999). *Perspectives in Nutrition.* 4th Edition, Mc Graw-Hill, United States of America, pp240.

Warren, M., Spencer, H.C., Churchill, F.C., Francois, V.J., Hippolyte, R. and Staiger, M.A. (1985). Assessment of exposure to organophosphate insecticide during spraying in Haiti: monitoring of urinary metabolites and blood cholinesterase levels. *Bulletin of the World Health Organization*, 63(2): 353-360.

Weiner, S.W. and Hoffman, R.S. (2004). Nerve agent: A comprehensive review. *Journal of Intensive Care Medicine*, 19(1): 22-37.

Weiss, B., Amler, S. and Amler, R.W. (2004). Pesticides. *Paediatrics*, 113: 1030-1036.

Wesseling, C., Keifer, M., Ahlbom, A., Mc Connell, R., Moon, J., Rosenstock, L. and Hogstedt, C. (2002). Long-term neurobehavioural effects of mild poisonings with organophosphate and n-methylcarbamate pesticide among Banana workers. *International Journal of Occupational Environmental Health*, 8: 27-34.

Wikipedia (2010a). Platelets. Available at http://en.wikipedia.org/wiki/platelets. Accessed on 11th November, 2010.

Wikipedia (2010b). Acetyl-L-carnitine. Available at http://en.wikipedia.org/wiki/acetylcarnitine. Accessed on 12th November, 2010.

Wilson, J.X. (2005). Regulation of vitamin C transport. *Annual Review of Nutrition*, 25: 105-125.

Winrow, C.J., Hemming, M.L., Allen, D.M., Quistad, G.B., Casida, J.E. and Barlow, C. (2003). Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. *Nature Genetics*, 33: 477-485.

World Health Organization/United Nation Educational Programmes (WHO/UNEP) Working Group (1990). *Public Health Impact of Pesticides used in Agriculture*. World Health Organization, Geneva, Switzerland, 18: 321-325.

Worthington Enzyme Manual. Catalase (Beef Liver). (1972). Freehold, NJ. Worthington Biochemical Corporation.

Wysong, R.L. (2003). *Clinical veterinary nutrition*. Technical Monograph and Product Details. www.wysong.net.

Wyss, M., Smeltink, J., Wevers, R.A. and Walliman, T. (1992). Mitochondrial creatine kinase. A key enzyme of aerobic energy metabolism. *Biochimica et Biophysica Acta*, 1102: 119-166.

Yang, C. and Deng, J. (2007). Intermediate syndrome following organophosphate insecticide poisoning. *Journal of Chinese Medical Association*, 70 (11): 467-472.

Yano, B.L., Young, J.T., Mattsson, J.L. (2000). Lack of carcinogenicity of chlorpyrifos insecticide in a high-dose, 2-years dietary toxicity study in Fischer 344 rats. *Toxicology Science*, 53: 135-144.

Yavuz, T., Delibo, N., YÂalÂrÂm, B., Altuntao, I., CandÂr, O., Cora, A., Karahan, N., Âbrioim, E. and Kutsal, A. (2004). Vascular wall damage in rats induced by methidation and ameliorating effect of vitamins E and C. *Archives of Toxicology*, 78: 655-659.

Yokoyama, K., Arak, S., Murata, K., Nishikitani, M., Okumura, T., Ishimatsu, S., Takasu, N. and White, R.F. (1998). Chronic neurobehavioural effects of Tokyo subway sarin poisoning in relation to post-traumatic stress disorder. *Archives of Environmental Health*, 53: 249-256.

Yousef, M.I., Awad, T.J. and Mohamed, E.H. (2006). Deltamethrin-induced oxidative damage and biochemical alteration in rats and its attenuation by vitamin E. *Toxicology*, 29: 227(3): 240-247.

Zama, D., Meraihi, Z., Bouberkri, N., Amrani, A., Tebibel, S. and Baali, N. (2005). Assessment of the changes in some diagnostic enzymes and other parameters in Wistar albino rats treated with pesticides during gestation*. Sciences and Technologie C- N 23.*, 51-55.

Zama, D., Meraihi, Z., Tebibel, S., Benayssa, W., Benayache, F., Benayache, S. and Vlietinck, A.J. (2007). Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidneys, brain and foetus in pregnant rats: The protective roles of the butanolic extract of *Paronychia argentea L. Indian Journal of Pharmacology*, 39(3): 145-150.

Zhao, Q., Gadagbui, B. and Dourson, M. (2004). The critical effect of chlorpyrifos: decreased birth weight or inhibition of acetylcholinesterase? *Toxicology Excellence for Risk Assesment (TERA).* Cincinnati, Ohio, USA.

Zheng, Q., Oliver, K., Won, Y.K. and Pope, C.N. (2000). Comparative cholinergic neurotoxicity of chlorpyrifos exposure in preweaning and adult rats. *Toxicological Sciences*, 55: 124-132.