INFLUENCE OF ZINC SUPPLEMENTATION ON THE PATHOLOGY OF SUBCHRONIC EXPOSURE OF WISTAR RATS TO GLYPHOSATE

BY

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(M. Sc/ Vet – Med / 10007/ 2008 – 2009)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

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

DEPARTMENT OF PATHOLOGY, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

SEPTEMBER, 2012

DECLARATION

I declare that the work in this thesis entitled, "Influence of Zinc Supplementation on the Pathology of Subchronic Exposure of Wistar Rats to Glyphosate", has been performed by me in the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. N. D. G. Ibrahim, Prof. M. Y. Fatihu and Prof. B. D. J. George. 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.

Emmanuel Vandi TIZHE ---------- ----------

Signature Date

CERTIFICATION

This thesis, entilled "Influence of Zinc Supplementation on the Pathology of Subchronic Exposure of Wistar Rats to Glyphosate" by **Emmanuel Vandi TIZHE** meets the regulations governing the award of **Master of Science degree** in Veterinary Pathology of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to my beloved late mother, Mrs. Mary Tizhe Vandi, in memory of her motherly love and care and above all, the impetus she gave me to aspire high in life. Mummy, may your soul continue to rest in peace in the bosom of the Lord until we meet to part no more.

ACKNOWLEDGEMENT

First and foremost, I want to acknowledge God for granting me the ability and willingness to carry out this research. My sincere heartfelt gratitude goes to my supervisory committee, Prof. N. D. G. Ibrahim, Prof. M. Y. Fatihu and Prof. B. D. J. George, without whom this work would have been impossible. I therefore with all sense of humility pray that God in His infinite mercy should reward you bountifully. I am short of words to acknowledge Prof. M. Y. Fatihu and Dr. S. F. Ambali who tirelessly stood by me in the design and criticism of this work in understanding and mentorship,Sirs, i owe you a lot and pledge my life – long loyalty to your mentorship.

My unreserved appreciation and love goes to my parents, Supol. And Mrs. Tizhe Vandi and my siblings for their spiritual, moral and financial support in understanding and love for the success of this work. I cannot repay you, but God will forever allow us to soar to our peaks in life.

To my colleaques, Drs. Uchendu Chidiebere, Peter Ehizibolo, Blessed Madaki, Josua Mallum Shallangwa, Kelechi Lucius Okoro, Moses Ewhe, Mohammed Ahmad Talba, Sani Nuhu Abdulazeez, David Pindar, Suleiman Abubakar, Ruth Paul Mutah, Watirahyel Samson Mshelia, Veronica Ochigbo, Esemeje Amupitan, Deborah Arimie Iroro, Sylvanus Adah, Mercy Didel Namang, Kassim Ibrahim, Mark Francis Isa, Ibe Chikera Samuel and Ayo Adeyeye, thank you for being there. God bless you and reward you abundantly.

I cannot appreciate Drs. S. J. Sambo, Ayo Adeyeye and Chikera Samuel Ibe enough for their services of love. Oh! God you that see what no man can see should reward them more than their mind can picture and grant me the grace to do likewise to others.

To my friend Ocheze Nneoma Nwosu, thanks for your support and companionship during the study period. To my friends, Dr. Ussa Delia, Dr. Joshua Mallum Shallangwa, Dr. Friday James Ogbe, Dr. Mark Francis Isa, Dr. Taye Gladys Adeyanju, Andrew Tikus Thomas, Christopher Nelson, Lazarus Markus, Sunday Soja, Isaac Daniel, Ugwoke Ebere Vivian, thank you for your prayers and encouragement. I sincerely appreciate the brotherly love shown to me by Dr. Andrew Patrick Fashie, Dr. Ishaku Mshelizah and Barka Samuel Andrew.

I shall forever remain grateful to my mothers, Mrs. Ladi Jonah Kwaghe and Mrs. Elizabeth Wale for their selfless sacrifices of love for the success of this work. Your contribution for the realization of this work shall be rewarded by God beyond imagination. To Prof. J. K. P. Kwaga, I say thank you for your encouragement, concern and love during my study period.

To my discipler, Dr. Yusuf Mudasiru, i say thank you for ensuring that my academic pursuit and spiritual growth is brought to this level. To all 2008 – 09 Masters Class, you are the best I have ever met. Thank you and remain blessed. I shall always remain grateful to Prof. I. O. Igbokwe for his contributions and concern for the success of this work.

Indeed many names there are, tremendous help they were to me, but limited space have i. To all who aided me in one way or the other and whose names are not reflected in the acknowledgement, you shall forever remain in my heart and I pray that Almighty God should reward you immeasurably.

ABSTRACT

Glyphosate used in this study is the active ingredient of the glyphosate – based herbicide, Bushfire®. The study was conducted to determine the influence of zinc supplementation on the pathology of subchronic exposure of Wistar rats to glyphosate. Sixty adult male and female Wistar rats were used. Twelve rats were used to determine the LD_{50} which was 3750 mg kg^{-1} and forty eight were randomized in to six groups of eight rats each, four males and four females. Group I (DW) served as control and were administered only distilled water (2 ml kg^{-1}), group II (Z) were administered zinc only (50 mg kg^{-1}), group III (G) were administered glyphosate only (375 mg kg^{-1}, 10 % of the LD_{50}), group IV (Z + G) were pretreated with zinc (50 mg kg^{-1}) and glyphosate was administered (375 mg kg^{-1}) while those in group V (GC) were administered glyphosate (14.4 mg kg^{-1}, 1: 50 glyphosate : distilled water respectively, as being used for field application) and those in group VI (Z + GC) were pretreated with zinc (50 mg kg^{-1}) and glyphosate was administered (14.4 mg kg^{-1} , 1 : 50 glyphosate : distilled water respectively). The treatment regimens were administered orally by gavage once daily for eight weeks. At the end of the study period, blood samples were collected and analysed for haematological and serum – biochemical parameters which included packed cell volume, hemoglobin, total leucocyte count, absolute and differential leucocyte count, total protein, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, Na^{+}, K^{+}, Cl^{-}, HCO^{-}_{3}, Ca^{2+}, $PO^{-}_{4,}$ urea and creatinine. Alterations in the values of haematological and serum – biochemical parameters induced by glyphosate exposure were differentially ameliorated by pretreatment with zinc. Histopathologic examination revealed neuronal degeneration, degenerated hepatic cells especially at the portal areas and congested central vein,

depopulated splenic cells in both red and white pulps, degenerated glandular pancreatic acinar cells, glomerular degeneration, few mononuclear cells infiltration into the interstices with renal tubular necrosis and degenerated mucosal epithelial cells of the stomach that involved the muscularis mucosa and the glands. No observable lesions in the testis and the uterus. Supplementation with zinc ameliorated the microscopic lesions induced by glyphosate exposure in these organs. In conclusion, this study showed the ameliorative effect of zinc on subchronic glyphosate – induced pathological changes in Wistar rats.

Keywords: Glyphosate, Zinc, Haematology, Serum – biochemistry, Pathology and Ameliorate.

TABLE OF CONTENTS

TITLE PAGE

Title page - - - - - - - - - - i

Declaration - - - - - - - - - - ii

Certification - - - - - - - - - - iii

Dedication - - - - - - - - - - iv

Acknowledgement - - - - - - - - - v

Abstract - - - - - - - - - - vii

Table of Contents - - - - - - - - - ix

List of Tables - - - - - - - - - - xii

List of Figures - - - - - - - - - - xiii

List of Plates - - - - - - - - - - xv

List of Abbreviations - - - - - - - - xvii

CHAPTER 1 INTRODUCTION - - - - - - - 1

1.1 Statement of Research Problem - - - - - - - 3

1.2 Justification - - - - - - - - - 4

1.3 Research Hypothesis - - - - - - - - 4

1.4 Aim - - - - - - - - - - 5

1.5 Objectives - - - - - - - - - - 5

CHAPTER 2 LITERATURE REVIEW - - - - - - 6

2.1 Classification of Pesticides - - - - - - - 6

2.2 Routes of Exposure to Organophosphates - - - - - - 14

2.3 Mode of action of Organophophates - - - - - - 16

2.4 Kinetics - - - - - - - - - - 17

2.5 Free Radicals - - - - - - - - - 20

2.5.1 Reactions involving Free Radicals - - - - - - 21

2.5.2 Free Radical Formation - - - - - - - - - 21

2.6 Oxidative Stress: What is it? - - - - - - - 23

2.7. What is an Antioxidant? - - - - - - - - 24

2.7.1 Types of Antioxidants - - - - - - - - 24

2.8 Effect of Organophosphates on Haematology in Animals and the Role of Antioxidants - - - - - - - - - - - - - 27

2.9 Effect of Organophosphates on Serum – Biochemical Parameters in Animals and the Role of Antioxidants - - - - - - - 33

2.10 Histopathological Effect of Organophosphates in Animals and the Role of Antioxidants - - - - - - - - 39

2.11 Effect of Zinc Supplementation on Oxidative Stress in Animals - - 47

CHAPTER 3 MATERIALS AND METHODS - - - - - 49

3.1 Experimental Animals - - - - - - - - 49

3.2 Chemical Source - - - - - - - - - 49

3.3 Experimental Design - - - - - - - - 50

3.3.1 Determination of Median Lethal Dose (LD_{50}) - - - - 50

3.3.2 Subchronic Toxicity Study - - - - - - - 50

3.3.3 Haematological Analysis - - - - - - - 51

3.3.4 Serum-Biochemical Analysis - - - - - - - 51

3.3.5 Histopathological examination - - - - - - - 52

3.3.6 Statistical Analysis - - - - - - - - 52

CHAPTER 4 RESULTS - - - - - - - - 53

4.1 Clinical Signs - - - - - - - - - 53

4.2. Haematology - - - - - - - - - 53

4.2.1 Effect of Treatments on Packed Cell Volume (PCV) - - - - 53

4.2.2 Effect of Treatments on Haemoglobin (Hb) Concentration - - - 54

4.2.3 Effect of Treatments on Total White Blood Cell (WBC) Counts - - 54

4.2.4 Effect of Treatments on Differential Leucocyte Counts (DLC) - - 54

4.3 Blood Chemistry - - - - - - - - - 55

4.3.1 Effect of Treatments on Serum Protein, Albumin and Globulin Concentrations 55

4.3.2 Effect of Treatments on Serum Enzymes- - - - - 56

4.3.3 Effect of Treatments on Serum Electrolyte Concentrations - - - 56

4.3.4 Effect of Treatments on Serum Urea Concentration - - - - 58

4.3.5 Effect of Treatments on Serum Creatinine Concentration - - - 58

4.4 Histopathological Findings - - - - - - - 71

CHAPTER 5 DISCUSSION - - - - - - - 92

CHAPTER 6 SUMMARY, CONCLUSION AND RECOMMENDATIONS - 107

6.1 Summary - - - - - - - - - - 107

6.2 Conclusion - - - - - - - - - - 108

6.3 Recommendations - - - - - - - - - 108

REFERENCES - - - - - - - - - 110

APPENDIX - - - - - - - - - - 124

LIST OF TABLES

Tables **Page**

Table 2.1: Pesticide Classification Based on Toxicity Category - - - 13

Table 2.2: Antioxidant Metabolites and their Solubility - - - - 26

Table 4.1: Determination of Median Lethal Dose (LD_{50}) for Glyphosate – Phase I - 59

Table 4.2: Determination of Median Lethal Dose (LD_{50}) for Glyphosate – Phase II - 60

LIST OF FIGURES

Figures		Page
Figure 4.1	Effect of sub – chronic exposure to distilled water (DW), Zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled Water (GC) on packed cell volume in Wistar rats	61
Figure 4.2	Effect of sub – chronic exposure to distilled water (DW), Zinc (Z) and Glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on haemoglobin concentration in Wistar rats	62
Figure 4.3	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on white blood cell count in Wistar rats	63
Figure 4.4	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on differential leucocyte counts in Wistar rats	64
Figure 4.5	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in total protein, albumin and globulin concentrations in Wistar rats, a = $p < 0.05$	65
Figure 4.6	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) changes in serum enzymes activities in Wistar rats, a = $p < 0.05$ versus GC	66
Figure 4.7a	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in serum electrolytes concentrations in Wistar rats	67
Figure 4.7b	Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) changes in serum electrolytes in Wistar rats. a = $P < 0.01$ versus DW and Z, $P < 0.001$ versus G and GC, $P < 0.05$ versus Z + G	68

Figure 4.8 Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in serum urea concentration in Wistar rats 69

Figure 4.9 Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on serum creatinine concentration in Wistar rats 70

LIST OF PLATES

Plates		Page
Plate I	Photomicrograph of a section of the brain of a rat exposed to distilled water only (H and E Stain)	72
Plate II	Photomicrograph of a section of the brain of a rat exposed to zinc only at 50 mg / kg (H and E Stain)	73
Plate III	Photomicrograph of a section of the brain of a rat exposed to glyphosate only at 375 mg/kg (H & E Stain)	74
Plate IV	Photomicrograph of a section of the brain of a rat exposed to zinc and glyphosate at 375 mg / kg (H and E Stain)	75
Plate V	Photomicrograph of a section of the liver of a rat exposed to distilled water only (H and E Stain)	76
Plate VI	Photomicrograph of a section of a liver of a rat exposed to glyphosate only at 375 mg/kg (H and E Stain)	77
Plate VII	Photomicrograph of a section of the liver of a rat exposed to zinc and glyphosate at 375 mg / kg (H and E Stain)	78
Plate VIII	Photomicrograph of a section of the spleen of a rat exposed to distilled water only (H and E Stain)	79
Plate IX	Photomicrograph of a section of the spleen of a rat exposed to zinc only at 50 mg / kg (H and E Stain)	80
Plate X	Photomicrograph of a section of the spleen of a rat exposed to glyphosate only at 375 mg/kg (H & E Stain)	81
Plate XI	Photomicrograph of a section of the spleen of a rat exposed to zinc and glyphosate at 375 mg/kg (H&E Stain)	82
Plate XII	Photomicrograph of a section of the pancreas of a rat exposed to distilled water only (H and E Stain)	83
Plate XIII	Photomicrograph of a section of the pancreas of a rat exposed to glyphosate at 375 mg/kg (H and E Stain)	84

Plate XIV	Photomicrograph of a section of the pancreas of a rat exposed to zinc and glyphosate at 375 mg/kg (H & E Stain)	85
Plate XV	Photomicrograph of a section of the kidney of a rat exposed to distilled water only (H and E Stain)	86
Plate XVI	Photomicrograph of a section of the kidney of a rat exposed to glyphosate at 375 mg/kg (H and E Stain)	87
Plate XVII	Photomicrograph of a section of the kidney of a rat exposed to zinc and glyphosate at 375 mg/kg (H and E Stain)	88
Plate XVIII	Photomicrograph of a section of the stomach of a rat exposed to distilled water only (H and E Stain)	89
Plate XIX	Photomicrograph of a section of the stomach of a rat exposed to glyphosate only at 375 mg/kg (H and E Stain)	90
Plate XX	Photomicrograph of a section of the stomach of a rat exposed to zinc and glyphosate at 375 mg/kg (H and E Stain)	91

ABBREVIATIONS

ALP	-	Alkaline phosphatase
ALT	-	Alanine aminotransferase
AST	-	Aspartate aminotransferase
ATP	-	Adenosine triphosphate
ATPase	-	Adenosine triphosphatase
BDH	-	British drug house
Ca^{2+}	-	Calcium ion
CAT	-	Catalase
Cd	-	Cadmium
Cl^-	-	Chloride ion
CPF	-	Chlorpyrifos
CS	-	Microencapsulate or Capsule
Cu	-	Copper
D	-	Dust
DF	-	Dry flowable
DLC	-	Differential leucocyte count
DNA	-	Deoxyribonucleic acid
DW	-	Distilled water
EC	-	Emulsifiable concentrate
EPA	-	Environmental protection agency
Fe	-	Iron

FIFRA	-	Federal insecticide, fungicide and rodenticide ACT
FPA	-	Food production association
G	-	Glyphosate
g	-	Gramme
GC	-	Glyphosate at the concentration used in the field
GHS	-	Global harmonized system of classification and labeling
GPX	-	Glutathione peroxidase
GR	-	Granule
GR	-	Glutathione reductase
GSH	-	Glutathione
H_2O_2	-	Hydrogen peroxide
Hb	-	Hemoglobin
HCO^-_3	-	Bicarbonate
H_O	-	Null hypothesis
IU	-	International unit
K^+	-	Potassium ion
Kg	-	Kilogramme
L	-	Litre
LD_{50}	-	Lethal dose fifty
LF	-	Liquid flowable
LPO	-	Lipid peroxidation
LTD	-	Limited
MC	-	Mean corpuscular volume
Mg	-	Magnesium
Mg	-	Miligramme

mMol	-	Milimole
Na^+	-	Sodium ion
NAD^+	-	Nicotinamide adenine dinucleotide
NADH	-	Hydrated nicotinamide adenine dinucleotide
NPIC	-	National pesticide information centre
NTE	-	Neurotoxic esterase
O_2	-	Oxygen ion
O_2^-	-	Superoxide
$O_2^=$	-	Peroxide
^-OH	-	Hydroxyl radical
OP	-	Organophosphate
P	-	Pellets
PANAP	-	Pesticide action network Asia and the Pacific
Pb	-	Lead
PCV	-	Packed cell volume
PO^-_4	-	Phosphate
Rbc	-	Red blood cell
ROS	-	Reactive oxygen species
SC	-	Suspension concentrate
SEM	-	Standard error of the mean
SL	-	Solutions
SO_4	-	Sulphate
SOD	-	Superoxide dismutase
SP	-	Soluble powder
TLC	-	Total leucocyte count

TP	-	Total protein
U.S.A	-	United State of America
ULVC	-	Ultra – low volume concentrate
WBC	-	White blood cell
WG	-	Water dispersable granule
WHO	-	World Health Organization
WP	-	Wettable powder
WSC	-	Water soluble concentrate
Z	-	Zinc
Z + G	-	Zinc plus glyphosate at 10 % of the LD_{50}
Z + GC	-	Zinc plus glyphosate at the concentration used in the field
Zn	-	Zinc
ZnO	-	Zinc oxide
α	-	Alpha

CHAPTER 1

1 INTRODUCTION

Pesticide is any substance or mixture of substances intended for preventing, repelling or mitigating any pest. Pest include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes and microbes that destroy property, spread disease or are a vector for disease or cause of nuisance (EPA, 2007; FAO, 2002). Organophosphates are the most widely used pesticides owing to their high efficacy, less persistence and more biodegradable nature than the organochlorines (Jirachayabhas *et al*., 2004). Although there are benefits to the use of pesticides, there are also setbacks such as potential toxicity to humans and other animals (FAO, 2002).

Glyphosate (phosphonomethyl glycine) was first reported as a herbicide in 1971, and is said to be the world's biggest selling herbicide (Monsanto, 1992). It is a broad-spectrum herbicide widely used to kill unwanted plants both on agricultural and non-agricultural landscapes (Temple *et al*., 1992). As a herbicide, glyphosate works by being absorbed into the plant mainly through its leaves, but also through soft stalk tissues. It is then transported throughout the plant where it acts on various enzyme systems, inhibiting amino acid m

metabolism in what is known as shikimic acid pathway. This pathway exists in higher plants and microorganisms but not in animals. Plants treated with glyphosate slowly die over a period of days or weeks, and because the chemical is transported throughout the plant, no part survives (Cox, 1995; Malik *et al*., 1989). In animals, mechanisms of toxic action have not been fully elucidated. A reduced respiratory control ratio, enhanced ATPase activity and stimulated oxygen uptake rate were observed in liver mitochondria obtained

from rats given glyphosate. Based on these results, the authors suggested that these toxicological effects may be primarily due to the uncoupling of oxidative phosphorylation (Olorunsogo *et al.,*1979).

Oxidative stress have been implicated in the molecular mechanisms of glyphosate toxicity (Beuret *et al*., 2004).The body responds to oxidative stress by evoking the enzymatic defence system within the body (Vivian *et al*., 2007).

In pure chemical terms, glyphosate is an organophosphate (OP) in that it contains carbon and phosphorus. However, it does not affect the nervous system in the same way as organophosphate insecticides, and is not a cholinesterase inhibitor (Rebecca *et al*., 1991). Most glyphosate containing products are either made or used with a surfactant. The surfactant used in a common glyphosate product (Roundup®) is more acutely toxic than glyphosate itself, but the combination of the two is yet more toxic (Santillo *et al*., 1989). The most widely used surfactant in glyphosate formulation is an ethylated amine. Polyoxy-ethyleneamine has been frequently mentioned as a surfactant, but in fact it refers to a group of ethylated amine products used in glyphosate formulations (WHO, 1994a).

Zinc is an essential trace mineral, which means that it must be obtained from the diet since the body cannot produce enough. It is the second most abundant mineral in the body, stored primarily in the muscle; it is also found in high concentrations in red and white blood cells, the retina of the eye, bones, skin, kidneys, liver and pancreas (Belongia *et al*., 2001). Zinc plays important role in the immune system, regulation of appetite, stress level, taste and smell (McClain *et al*., 1992). Two antioxidant mechanisms of Zinc have been identified: Zinc ions may replace redox active molecules such as iron and copper at critical sites in cell

membranes and proteins; alternatively, Zinc ions may induce the biosynthesis of mettallothione, sulfhydryl-rich proteins that protect against free radicals (Rostan *et al*., 2002). Owing to its mechanisms of action, Zinc has been used in the amelioration of organophosphate (Chlorpyrifos) induced alterations in haematological and serum – biochemical changes in Wistar rats (Ambali *et al*., 2010a). It has also been used to attenuate oxidative stress in arsenic and cadmium exposed rats (Kumar *et al*., 2010; Amara *et al*., 2008).

1.1 STATEMENT OF RESEARCH PROBLEM

Glyphosate, the active ingredient which is 48% acid equivalent of the 180 propylamine salt of glyphosate (phosphonomethyl glycine), is used as a non- selective herbicide and for control of a great variety of annual, biennial and perennial grasses, broad-leaved weeds and woody shrubs in orchards, vineyards, conifer plantations and many plantation crops. It is perhaps the most important herbicide ever developed (WHO, 1994b). Glyphosate has low persistence and, because repeated applications of this herbicide are practiced for the control of weeds in agricultural fields, large quantities find their way into water bodies. The indiscriminate use of the herbicide therefore makes it a potential source of danger to animals, not only in grazing fields, but also in the water bodies (Ayoola, 2008).

The manufacturers of glyphosate-based herbicides claim their 'low toxicity and environmental friendliness', however, evidence indicates that the herbicide may not be as safe as previously thought (Franz *et al.,* 1997). In addition the surfactant used in a common glyphosate product (Roundup®) is more acutely toxic than the glyphosate itself, the combination of the two is yet more toxic (Santillo *et al*., 1989; Howe *et al*., 2004; Santos *et*

al., 2005). Glyphosate had also been reported to induce oxidative stress in animals (Vivian *et al*., 2007). Also, there have been reports of numerous deaths of livestock and domestic animals as a result of the aerial spraying of glyphosate in Colombia (PANAP, 2009). Therefore, this work was designed to determine the pathological changes associated with subchronic glyphosate exposure in the blood and some organs of Wistar rats as well as the effect therein of supplementation with zinc.

1.2 JUSTIFICATION

In order to increase plant yields, decrease farm inputs requirements and save cost, the use of glyphosate to control weeds both on the agricultural and non-agricultural lands is on the increase. The widespread use of this agent has led to its widespread misuse because the farmers are predominantly not educated on the dangers of the agent and this result in residues being found on grasses and water bodies. This constitutes an important risk to both humans and animals. Although, the herbicide is acutely toxic, the danger posed by low-level exposure in humans and animals has not been given considerable attention. Similarly, the amelioration of the long-term low-dose glyphosate exposure has not been given due consideration, especially in the light of widespread use of this agent. In addition, the literature about the toxicity of glyphosate – based herbicide, Roundup®, is scarce, especially on how it affects animal health. (Martinez and Modesto, 2010).

1.3 RESEARCH HYPOTHESIS

$H_{O:}$ Zinc supplementation does not ameliorate subchronic glyphosate – induced pathological changes in Wistar rats.

1.4 AIM

The aim of this study was to determine the influence of zinc supplementation on the pathology of subchronic exposure of Wistar rats to glyphosate.

1.5 OBJECTIVES

The specific objectives of this study were to evaluate:

(i) The haematological changes associated with subchronic exposure to glyphosate in Wistar rats

(ii) The serum-biochemical changes caused by subchronic exposure to glyphosate in Wistar rats

(iii) The histopathological changes induced by subchronic exposure to glyphosate in Wistar rats

(iv) The effects of Zinc on haematological, serum-biochemical, and pathological changes induced by subchronic glyphosate exposure in Wistar rats.

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CHAPTER 2

LITERATURE REVIEW

2.1 CLASSIFICATION OF PESTICIDES

Pesticide is any substance or group of substances intended for preventing, destroying or controlling pests (Josephine, 2011). Generally speaking, pesticides can be classified based on toxicity class; and assignment of a pesticide in to a particular toxicity class is based typically on results of acute toxicity studies such as the determination of LD_{50} values in animal experiments (notably rodents) via oral, inhaled, or external application. The experimental design measures the acute death rate of an agent. The toxicity class generally does not address issues of other potential harm by the agent, such as bioaccumulation, carcinogenicity, teratogenicity, mutagenic effects, or the impact on reproduction (Liska and Kolesar, 1982).

The United States Environmental Protection Agency (EPA) uses four Toxicity classes. Classes I, II and III are required to carry a signal word on the label. Pesticides are regulated by the Federal Insecticide, Fungicide, and Rodenticide ACT (FIFRA) (EPA, 2010).

Toxicity Class I

- Most toxic
- Requires signal word:" Danger – Poison," with skull and cross bones symbol, possibly followed by: " Fatal if swallowed," "Poisons if inhaled," " Extremely harzardous by skin contact – rapidly absorbed through skin," or "Corrosive –

causes eye damage and severe skin burns" Class I materials are expected to be fatal to an adult human at a dose of < 5 grams (EPA, 2010).

Toxicity Class II

- Moderately toxic
- Signal word: "Warning," possibly followed by: "Harmful or fatal if swallowed," "Harmful or fatal if inhaled," or "causes skin and eye irritation" Class II materials are estimated to be fatal to an adult human at a dose ranging from 5 to 30 grams.(EPA, 2010).

Toxicity Class III

- Slightly toxic
- Signal word: caution, possibly followed by: "Harmful if swallowed," " May be harmful if absorbed through the skin," " May be harmful if inhaled," or " May irritate eyes, nose, throat, and skin" Class III materials are estimated to be fatal to an adult human at some dose in excess of 30 grams (EPA, 2010).

Toxicity Class IV

- Practically non – toxic
- No signal word required since 2002 (EPA, 2010).

Josephine (2011) reported that pesticide can be classified on the basis of the following:

A. Pesticide Classification Based on Target Pest

- Algae – Algicide
- Bacteria – Bactericide
- Birds – Avicide
- Fish – Piscicide
- Fungi – Fungicide
- Insects – Insecticide
- Mites – Miticide/Acaricide
- Mollusks – Molluscicide
- Nematodes – Nematicide
- Rodents – Rodenticide
- Spiders – Arachnidicide
- Trees – Arboricide
- Weed – Herbicides

B. Pesticide Classification Based on Usage

- General usage – products may be handled by the general public in conformity with label directions.
- Restricted usage – products in this category shall be handled only by trained and certified applicators.
- Banned – all usage of pesticides in group have been cancelled (Josephine, 2011).

C. Pesticide Classification Based on Age

- Proprietary (New) – exclusively manufactured and formulated by companies other than the inventing company.
- Commodity (Old) – patents have expired and products can be manufactured by companies other than the inventing company (Josephine, 2011).

D. Pesticide Classification Based on Chemical Nature

- Inorganic – do not contain carbon (Lead arsenate, Paris green, Sulfur, Zinc phosphate).
- Synthetic organic

  a. Chlorinated hydrocarbons

  b. Organophosphate

  c. Carbamate

  d. Synthetic pyrethroid

  e. New chemicals (Neonicotinoid, pyrrole, phenylpyrazole)
- Biorational – derived from various biological sources (Pheromone, Insect Growth Regulator, Microbial Naturalyte, Macrolactone – Avermectin, Botanical) (Josephine, 2011).

E. Insecticide Classification Based on Effect on Pest

- Antifeedant – inhibits feeding resulting to death due to starvation
- Attractant – lures pest to a treated location
- Chemosterilant – destroys the ability of the pest to reproduce
- Descicant –dries up plant parts or insect
- Feeding stimulant – causes the organism to feed more vigorously

- Growth Regulator – speeds up or retards growth processes
- Repellant – drives away pests without killing them
- Respiratory/Asphysiate – blocks air passage
- Direct poisons (Nerve, Muscle, Protoplasm/Cytolytic poisons, Metabolic inhibitors, Alkalyting agents e.t.c) (Josephine, 2011).

F. Pesticide Classification Based on Formulation Type

- Emulsifiable Concentrate (EC) – The active ingredient dissolved in an organic solvent. Designed to be mixed with water at the time of application and the resulting mixture is an emulsion.
- Liquid Flowable (LF) or suspension concentrate (SC) – finely divided solid particles in a liquid carrier forming a thick but pourable opaque product.
- Dry Flowable (DF) or Water Dispersible Granule (WG) – A wettable powder which is aggregated to small pellets or granules.
- Solutions (SL) or Water Soluble Concentrate (WSC) – water soluble active ingredient dissolved in water to be further diluted prior to application. It forms a true solution.
- Ultra – Low Volume Concentrate (ULVC) – contains very high active ingredient concentration, designed to be undiluted and applied only using ultra – low volume equipment.
- Microencapsulate or Capsule Suspension (CS) – A suspension with active ingredient in microcapsules.

- Aerosol – a liquid with one or more solvents. Ready to use in pressurized containers.
- Gel – high assay semi – liquid emulsifiable concentrate. Used with water – soluble packaging or placed in tubes or syringe to be applied as bait or as part of vapourizer system.
- Dust (D) or powder – finely ground material of low active ingredient such as talc, clay or chalk. Small size permits delivery of insecticide in to cracks and crevices but pose drift and inhalation hazard.
- Granular (GR) – active ingredients to sorbed on to larger particles like clay or sand. Granules are applied dry and intended for soil application.
- Pellets (P) – the active ingredient is combined with inert ingredient to form a slurry, which is then extruded under pressure and cut to produce particles of relatively uniform size and shape.
- Wettable Powder (WP) – a dry formulation containing finely ground active ingredient plus wetting and dispersing agents. Sometimes packaged in water soluble bags.
- Soluble Powder (SP) – similar to WP and DF except that it is highly soluble in water. Not common.
- Fumigants – solid or liquid product but delivers the active ingredients in the form of gas. Highly effective when properly done but highly toxic as well (Josephine, 2011).

G. Insecticide Classification Based on Mode of Entry

(i) Stomach poison – insecticide enters the body through the mouth of the pest and acts in the stomach.

(ii) Contact poison – toxic action is through direct contact on the cuticle of the pest. Insecticides enter the body through the integument in to the blood or in to the respiratory system through the spiracles.

(iii) Fumigant poison – these are volatile poisons that enter the body through the respiratory system (Josephine, 2011).

Table 2.1: H. Pesticide Classification Based on Toxicity Category

Pesticide Classification Based on harzard Category		
Category	Oral LD_{50} (mg/kg BW)	
WHO	FPA	GHS
Ia	Up to 50	< 5
Ib	50 – 500	5 – 50
II	500 – 5000	50 – 300
III	>5000	300 – 2000
U		2000 – 5000

(Josephine, 2011).

FPA: Food Production Association, USA; GHS: Global Harmonized System of Classification and Labelling.

I. Pesticide Classification Based on Mode of Action

- Physical Toxicant – mechanically blocks a physiological process (oils and soaps).
- Growth Regulator – Disrupts molting, metamorphosis and cuticle formation.
- Muscle poison – have a direct action on muscle tissue
- Nerve poison (Axonic/Synaptic) affects electronic transmission of nerve impulses causing repetitive discharges resulting to paralysis.
- Metabolic inhibitor - affects electron transport chain related to energy production and metabolism.
- Cytolytic/ protoplasmic toxin – cause cells to rupture and disintegrate.
- Alkylating agent – reacts directly with chromosomes and enzymes in cells (Josephine, 2011).

2.2 ROUTES OF EXPOSURE TO ORGANOPHOSPHATES

- **Oral**

Accidental ingestion by children or animals, ingesting food containing organophosphorous pesticide residues after incorrect treatment of foodstuffs or harvested prematurely before residues decline to acceptable levels. Oral ingestion may also occur through placing contaminated objects in the mouth during eating, drinking or smoking, or through violation of proper procedures e.g blowing out clogged spray nozzles by mouth (Teitelman *et al*., 1975).

- **Inhalation**

The majority of organophosphorous pesticides are liquids that have different vapour pressures at room temperature (e.g dichlorvos is much more volatile than parathion); thus hazardous due to inhalation of vapour vary from compound to compound. Respiratory exposure is greater when dusts are applied than when dilute sprays are used. However, aerosols of concentrated pesticide may be an even greater hazard (WHO, 1986).

- **Dermal**

Many accidental acute poisonings have occurred after spillage of a pesticide on skin and clothing. The extent of uptake will depend on persistence time (related to volatility, clothing, coverage, and thoroughness of washing after exposure), and also on the presence of solvents and emulsifiers that may facilitate uptake. Powder formulations also have a potential for skin absorption (Wolfe *et al*., 1978).

Skin absorption is somewhat greater at high temperatures and may be much greater in the presence of dermatitis, thus, leading to serious poisoning after an exposure that would ordinarily cause no effects (Gallo and Lawryk, 1991).

- **Eye**

Exposure to vapours, dusts, or aerosols can cause local effects on the smooth muscles of the eyes. Systemic poisoning may follow (Lyon *et al*., 1987).

- **Parenteral**

Accidental or intentional by injecting the organophosphates parenterally into the body (Lyon *et al*., 1987).

2.3 MODE OF ACTION OF ORGANOPHOSPHATES

Organophosphorous pesticide may mostly exert their acute effects by inhibiting acetylcholinesterase in the nervous system with subsequent accumulation of toxic levels of acetylcholine. They may also inhibit butylcholinesterases as well as other esterases. The function of butylcholinesterase is unknown, but its inhibition can provide an indication of exposure to an organophosphate (WHO, 1986).

In many cases, the organophosphorylated enzyme is fairly stable, so that recovery from intoxication may be slow. Reactivation of inhibited enzyme may occur spontaneously, the rates of reactivation depending on the tissue as well as on the chemical group attached to the enzyme (WHO, 1986).

Delayed neuropathy is initiated by an attack on a nervous tissue esterase distinct from acetylcholinesterase. The target has esterase activity and is called neuropathy target esterase (NTE) (formerly called neurotoxic esterase). The disorder develops not because of loss of esterase activity but because of change brought about in the protein molecule that results from the process of ageing of inhibited catalytic activity of NTE which appears in the nervous tissue even during the period of development of neuropathy (WHO, 1986).

Organophosphate such as glyphosate, however, is not a cholinesterase inhibitor and the mechanisms of toxic action have not been fully elucidated. A reduced respiratory control ratio, enhanced ATPase activity and stimulated oxygen uptake rate were observed in liver mitochondria obtained from rats given glyphosate. Based on these results, it was suggested tha these toxicological effects may be primarily due to the uncoupling of oxidative phosphorylation (Olorunsogo *et al*., 1979).

2.4 KINETICS

- **Absorption**

Organophosphorous pesticides are absorbed by the skin as well as through the respiratory and gastrointestinal tracts. **Oral exposure**: when 32 p – dimethiote was given orally to volunteers, it was absorbed and excreted rapidly: 76 to 100% of the radioactivity appeared in the urine in 24 hours (Edson *et al*., 1967).

Inhalation exposure: exposure by respiratory and dermal routes were compared in workers spraying parathion, who either breathed a pure air supply but did not wear protective clothing, or who wore total protective clothing but did not have any respiratory protection (Durham *et al*., 1972). Total urinary output of 4 – nitrophenyl as derived from the dermal source, was 1.2 % in one test and 12 % in another. Since the total exposure by the dermal and respiratory routes were in the proportion of 1000 : 1 and the efficiency of dermal absorption was 1 to 2 %, it follows that the efficiency of dermal absorption by the respiratory route was more than 20 % and could well have been complete (Durham *et al*., 1972).

Dermal exposure: absorption by the skin tends to be slow, but because the pesticides are difficult to remove, dermal absorption is frequently prolonged. Uptake of active ingredients through the skin from powdered and granulated formulations may be relatively inefficient; the presence of aqueous dispersing agents or organic solvents in a spray concentrate or formulation may greatly enhance uptake. On the basis of radioautographic studies in man and animals, it appears that skin absorption of parathion is transepidermal (Fredrickson, 1961). The rate of dermal absorption of parathion in the rabbit is 0.059 mg/min/cm^2 (Nabb *et al*., 1966).

- **Distribution by Route of Exposure**

The intrinsically reactive chemical nature of organophosphorous pesticides means that any of them that enters the body is immediately liable to a number of biotransformations and reactions with tissue constituents, so that the tracing of radiolabelled material alone does not give any clue to the unchanged parent compound. In view of the inherent instability of the organophosphorous pesticides, storage in human tissue is not expected to be prolonged. Experimental animal studies indicated rapid excretion of these compounds. However,some organophosphorous pesticides are very lipophilic and may be taken into, and then released from fat depots over a period of many days (Ecobichon *et al*., 1977; Minton and Murray, 1988).

The lipophilic diethyl pesticides including azinophos – ethyl, bromophos – ethyl, chlorpyrifos, coumaphos, diazinon, parathion, phosalone and sulfotep may remain in the body for many days or weeks in severe cases and may promote a recurrence of clinical

effects after an initial period of apparent recovery (Ecobichon *et al*., 1977; Minton and Murray, 1988).

- **Biological half – life by Route of Exposure**

The half – life of most organophosphorous pesticides and their inhibitory metabolites *in vivo* is comparatively short (WHO, 1986). For example the serum half – life of malathion was 2.89 hours in a 24 year – old white man who, in a suicide attempt, injected approximately 3 ml of 50% malathion intravenously into his right fore – arm (Lyon *et al*., 1987). A few organophosphorous pesticides, however, are lipophilic and may remain in the body for many days or weeks. For example, Leptophos tends to persist in the fat of hens and pharmacokinetic studies using radiolabelled leptophos showed elimination half – life of 17 – days (Abou – Donia and Graham, 1978).

- **Metabolism of Organophosphates**

Metabolism occurs principally by oxidation and hydrolysis by esterases and also by reaction with glutathione. Demethylation and glucuronidation may also occur. Oxidation of organophosphorous pesticides may result in more or less toxic products. The glutathione transferase reactions produce products that are in most cases of low toxicity, hydrolytic and transferase reactions affect both the thioates and their oxons. Numerous conjugation reactions follow the primary metabolic processes, and elimination of the phosphorus – containing residue may be via the urine or faeces (Baselt, 1982).

Parathion, for example, must be activated by an oxidative conversion via liver cytochrome P450 microsomal enzymes to paraoxon, a potent cholinesterase inhibitor. Both compounds are rapidly hydrolysed by plasma and tissue esterases, to diethylthiophosphoric acid, diethyl – phosphoric acid, and p – nitrophenol (Baselt, 1982).

- **Elimination and Excretion**

There is evidence of prolonged storage of organophosphorous pesticides compounds in the body, but the process of elimination can be subdivided roughly according to the speed of the reactions involved. Most organophosphorous pesticides are degraded quickly by the metabolic reactions described. The elimination of the products, mostly in the urine with lesser, amounts in the faeces and expired air, is not delayed, so that rates of excretion usually reach a peak within two days and decline quite rapidly (WHO, 1986).

Experimental animal studies have shown that most of a radiolabelled dose of organophosphorous pesticide is rapidly excreted in expired air, urine and faeces thus, it was reported that from 67 to 100 % of the administered radioactivity was recovered within 1 week in the combined urine and faeces of cows, rats and a goat that were given various doses of 32 p – dichlorvos (Blair *et al*., 1975).

2.5 FREE RADICALS

The first organic free radical identified was triphenylmethyl radical, by Moses Gomberg in 1900 at the University of Michigan. A free radical is an atom, molecule, or compound that

is highly unstable because of its atomic or molecular structure (i.e, the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair with other molecules, atoms, or even individual electrons to create a stable compound e.g H_2O_2 and $^{\cdot}OH$. To achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals (Adachi and Ishii, 2002).

2.5.1 Reactions Involving Free Radicals

Free radicals are highly unstable molecules that attempt to achieve a more stable state by reacting with other atoms or molecules in the cell. The four primary types of chemical reactions that free radicals undergo are:

- Hydrogen abstraction: A radical interacts with another molecule that has a free hydrogen atom (i.e, a hydrogen donor). As a result, the radical binds to the hydrogen atom and becomes stable, whereas the hydrogen donor is converted to a free radical.
- Addition: A radical binds to another originally stable molecule, converting the combined molecule into a radical.
- Termination: Two radicals react with each other to form a stable compound.
- Disproportionation: Two identical radicals react with each other, with one of the radicals donating an electron to the other so that two different molecules are formed, each of which is stable (Adachi and Ishii, 2002).

2.5.2 Free Radical Formation

One chemical element frequently involved in free radical formation is oxygen. Molecular oxygen (O_2) is essential for cell function because it plays a pivotal role in a series of biochemical reactions occurring in the respiratory chain, which is responsible for most of the production of adenosine triphosphate (ATP) which provides the energy required for a multitude of cellular reactions and functions (Bondy, 1992; Halliwell, 1999).

In the respiratory chain, which takes place in cell membrane – enhanced cell structures called mitochondria, an electron and a proton (H^+) are removed from a helper molecule (i.e cofactor) called reduced nicotinamide adenine dinucleotide (NADH). Reduced nicotinamide adenine dinucleotide is generated in the fluid filling the cell (i.e, cytosol) and then moves to the mitochondria). The electron is transferred to the first component of the respiratory chain, and the proton is released into the surrounding fluid. Chemically speaking, NADH is oxidized to NAD^+ in this reaction, whereas the respiratory chain component that accepts the electron is reduced. (Oxidation reactions are those reactions that add oxygen or remove hydrogen or electrons from a molecule while reactions which involve removal of oxygen or addition of hydrogen or electron to a molecule are called reduction reactions) are called reductions). The NAD^+ subsequently can be used again to accept new hydrogen atoms that are generated during the metabolism of sugars (e.g, glucose) and other nutrients. The reduced respiratory chain component, in turn, passes the electron on to other molecules in the respiratory chain until it is finally transferred to O_2, which then interacts with protons in cells to generate water. This series of electron transfer reactions generates sufficient energy to

produce several molecules of ATP for each electron that passes through the respiratory chain (Bailey and Cunningham, 2002).

Molecular oxygen can accept a total of four electrons, one at a time, and the corresponding number of protons to generate two molecules of water. During this process, different oxygen radicals are successively formed as intermediate products, including superoxide ($O_2^{\cdot -}$); peroxide ($O_2^{=}$), which normally exists in cells as hydrogen peroxide (H_2O_2); and the hydroxyl radical ($^{\cdot}OH$). Superoxide, peroxide, and the hydroxyl radical are considered as the primary ROS (reactive oxygen species) and have sparked major research activities on the role of free radicals in biology and medicine. Superoxide can react with itself to produce H_2O_2. Thus, systems producing superoxide also will result in formation of H_2O_2. Technically, H_2O_2 is not a free radical, but it is commonly included among the ROS. However, because they are unstable and rapidly react with additional electrons and protons, most of these ROS are converted to water before they can damage cells. It has been estimated that only about 2 to 3 percent of O_2 consumed by the respiratory chain is converted to ROS (Chance *et al*., 1979).

Nevertheless, the toxic effects of oxygen in biological systems such as the breakdown (i.e, oxidation) of lipids, inactivation of enzymes, introduction of changes (i.e, mutations) in the DNA, and destruction of cell membranes and, ultimately, cells are attributable to the reduction of O_2 to ROS (Toykuni, 1999: de Groot, 1994; Nakazawa *et al*., 1996).

2.6 OXIDATIVE STRESS: WHAT IS IT?

Oxidative stress describes cell damage caused by an overabundance of oxidants, including reactive oxygen species (ROS e.g oxygen ions, free radicals, and peroxides). Reactive oxygen species are harmful in excess but some level of is necessary for important cellular functions. Some cells produce ROS to kill invading microbes, and ROS are involved in cell signaling. Also, oxidative stress plays a role in cellular process, such as aging and apoptosis (Decoursey and Ligeti, 2005). The ROS produced in the cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (OH) and the superoxide anion (O^-_2) (Valko *et al*., 2007). The hydroxyl radical is particularly unstable and will react rapidly and non – significantly with most biological molecules. This species is produced from hydrogen peroxide in metal – catalyzed redox reactions such as the Fenton reaction (Stohs and Bagchi, 1995). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or oxidizing DNA or proteins (Sies, 1997).

2.7 WHAT IS AN ANTIOXIDANT?

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules (Sies, 1997). Organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Sies, (1997); Vertuani *et al*. (2004)). In general, antioxidants prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Sies, 1997; Davies, 1995). However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, 2006).

2.7.1 Types of Antioxidants.

Antioxidants are classified in to two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water – soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid – soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet (Vertuani *et al*., 2004). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors (Miller and Britigan, 1997).

The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another (Chaudiere and Ferrari – Illiou, 1999; Sies, 1993). The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system (Vertuani *et al*., 2004). The amount of protection provided by any one antioxidant will also depend on its concentration, reactivity towards the particular ROS being considered, and the status of the antioxidant with which it interacts (Vertuani *et al*., 2004). Some compounds contribute to antioxidant defence by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron – binding proteins such as transferrin and ferritin (Imlay, 2003). Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements

have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes (Imlay, 2003).

Table 2.2: Antioxidant metabolites and their solubility

Antioxidant metabolite	Solubility
Ascorbic acid (vitamin C)	Water
Glutathione	Water
Lipoic acid	Water
Uric acid	Water
Carotenes	Lipid
α – Tocopherol (vitamin E)	Lipid
Ubiquinol (co – enzyme Q)	Lipid

(Sies, 1997).

Natural antioxidants include such antioxidants as ascorbic acid (vitamin C), α – Tocopherol (vitamin E), Ubiqinol (co – enzyme Q), lipoic acid, uric acid, carotenes, glutathione, carvedilol, melatonin and quercetin (Meister, 1994). Commonly used synthetic antioxidants include ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole (Singh *et al*., 2002).

2.8 EFFECT OF ORGANOPHOSPHATES ON HAEMATOLOGY IN ANIMALS AND THE ROLE OF ANTIOXIDANTS

A short – term alteration in haematological parameters in wistar rats exposed to acute Chlorpyrifos (CPF) toxicity was reported by Ambali *et al*. (2010a) as follows: The packed cell volume (PCV) in all the groups were not significantly different from each other. However, rats in the CPF group had 9.4 % decreases in their PCV compared to the control group. On the other hand, the PCV of vitamin C + CPF group dropped by only 1.7 % compared to the soya oil group. On the haemoglobin (Hb) concentration, there was no significant difference in the Hb concentration of rats in all the groups. However, rats in the CPF group showed a 10 % drop in the Hb concentration compared to 2.1 % decrease recorded in the Vitamin C + CPF group, relative to the soya oil group. The white blood cell

(WBC) counts showed no significant changes in the rats from all the groups. However, rats in the CPF group had a 7.8 % increase in WBC concentration relative to th soya oil group. On the other hand, the vitamin C + CPF group had a 14.1 % decrease in WBC concentration compared to the soya oil group. Therefore, the parameters indicative of anaemia observed in rats exposed to CPF only may be due to the abilityof the organophosphate (OP) compound to decrease tissue iron concentration (Goel *et al*., 2006), interfere with Hb biosynthesis and induce shortening of red blood cell (rbc) lifespan

(Ray, 1992) or even increase erythrocyte fragility (Ambali *et al*., 2010d). Pretreatment with vitamin C improved the rbc parameters depressed by CPF. This may be due to the ability of the antioxidant vitamin to improve the absorption of iron from the gut (Iqbal *et al*., 2004; Wardlaw, 1999) by facilitating the reduction of oxidized iron to its reduced form (Sayers *et al*., 1973). The apparent increase in WBC reported by Ambali *et al*. (2010a) contradicted the result of the previous studies which showed that repeated CPF exposure causes leucopaenia (Ambali *et al*., 2007; Goel *et al*., 2006). The reason for apparent increase in the WBC and its implication is not known especially as it relates to the immune system and deserves further studies (Ambali *et al*., 2010a).

The lower values of PCV, Hb, and RBC observed in the CPF group as reported by Ambali *et al*. (2010a) agreed with those obtained in earlier studies (Barna – Lloyd *et al.,*1991; Goel *et al*., 2006; Ambali, 2009). This indicates that repeated CPF exposure causes anaemia. The lack of significant change in the MCV in all the groups showed that CPF does not alter erythrocyte size. However, anisocytosis in the CPF group agreed with the observation of Goel *et al*. (2006). The reason for the anaemia in the CPF group is not known. It may however, be related to disruption of erythropoiesis or an increase in RBC destruction

(Vural *et al*., 1986). Goel *et al*. (2006) observed a decrease in serum iron concentration which eventually results in reduced haemoglobin production. Repeated CPF exposure increases erythrocyte fragility due to increased

Lipo – peroxidative changes in the erythrocyte membrane (Ambali *et al*, 2009).

The significant improvement in the PCV, Hb, and RBC concentrations and anisocytosis in rats co – administered with zinc and CPF suggests the ameliorative effect of the trace element. The ameliorative effect of zinc on the indices of anaemia agreed with the observation of Goel *et al*. (2006).The positive association between the improvement in anaemia indices and amelioration of the erythrocyte lipo – peroxidative changes demonstrated that oxidative damage probably plays an important role in CPF – induced anaemia (Ambali *et al*., 2010a). The ameliorative effect of zinc on CPF – induced anaemia may have arisen from its antioxidant properties (Powell, 2000). Cellular zinc exists in only one redox state (II); thus, it cannot undergo redox reactions that are commonly responsible for the generation of ROS (Zhou *et al*., 2007). Apart from its direct antioxidant effect via occupation of iron and copper binding sites on lipids, proteins and DNA (Deoxyribonucleic acid) thereby preventing hydroxyl radical formation near these structures (Powell, 2000; Prasad and Kucuk, 2002), zinc also plays a structural role in the maintenance of the integrity of Cu – Zn superoxide dismutase as a co – factor (Caudry *et al*.,1992; Sahin and Kucuk, 2003) and in the regulation of glutathione that is vital to cellular antioxidant defense (Parrat *et al*., 1997). In addition, zinc protects sulphyhydryl group against oxidation thereby preventing protein from oxidation, hence stabilizing cellular thiol pools (Kraus *et al*., 1997; Verhaegh *et al*., 1998). Furthermore, zinc regulates metallothionein, a highly conserved ion molecular weight cystein rich metal chelating

antioxidant protein, which has been demonstrated to share a lot of features with glutathione (Quesada *et al*., 1996; Zhou *et al*., 2007).

Goel *et al*. (2005) have shown that CPF intoxication causes reduction in metallothionein level, a feature that was ascribed to zinc deficiency. Zinc deficiency has been associated with increased erythrocyte fragility (Kraus *et al*., 1997; O'Dell, 2000) apparently due to the alteration in erythrocyte membrane integrity. A decrease in membrane zinc content could cause subtle changes in membrane rheology and leave vacant potential binding sites for redox – active metals such as iron and copper (Drisscoll and Bettger. 1992). This can promote membrane – lipid oxidation (Verstraeten *et al*., 2004) and alterations in membrane lipid composition (Driscoll and Bettger,1992).

Repeated CPF exposure has been shown by Ambali *et al*. (2010a) to cause lymphopenic leucopenia. This finding agreed with that obtained in earlier studies (Ambali *et al*., 2007; Ambali, 2009). The lymphopenia observed in the CPF – exposed rats may be due to either decreased production and/or increased rate of removal due to rapid destruction (Ambali *et al*., 2010a). Ambali *et al*. (2010a) did not, however, observe significant changes in the neutrophils content of blood even though Goel *et al*. (2006) observed neutrophilia following CPF exposure. The high neutrophils:lymphocyte ratio in the CPF group reported by Ambali *et al*. (2010a) did, however, corroborate with the high neutrophil count observed in the earlier study (Goel *et al*., 2006). Indicating that the animals exposed to this chemical were under stress (Ambali *et al*., 2010a).

Furthermore, the protective effect of zinc on WBC may be attributed to their antioxidant properties either as a vital component of enzymatic antioxidant Cu – Zn SOD or due to

their ability to antagonize the catalytic properties either of the redox – active transition metal iron and copper with respect to the promotion of hydroxyl radicals formation from hydrogen peroxide and superoxide (Powell, 2000). Zinc also causes inhibition of both endogenous as well as induced lipid peroxidation thereby resulting in stabilization of biomembranes (Dhawan *et al*., 1992; Srivastava *et al*., 1993). The significant elevation of PCV, Hb concentration and Rbc counts in mice administered CPF (only) may be due to the mild diarrhoea and the resultant haemoconcentration (Ambali *et al*., 2010c). However, Vitamins C + E pretreatment significantly suppressed the adverse haematological effect by CPF. The leucopenia observed in the CPF group showed its immunotoxic potentials (Ambali *et al*., 2010c). The neutropenia in the CPF group may be related to the essential role played by neutrophils in free radical mediated injury by inducing extracellular release of superoxide and other free redicals (McCord *et al*., 1994). This also leads to neutrophil destruction, resulting in their decrease in the peripherial circulation. Pretreatment with combination of vitamins C and E significantly improved the concentration of leucocytes in the circulation, indicating that oxidative stress plays an essential role in the leucopenia induced by CPF administration (Ambali *et al*., 2010c). Vitamin E is an important intracellular antioxidant in the cytomembranes responsible for maintenance of cellular integrity (Baker *et al*., 1996). Therefore, the membrane stabilization by vitamin E may have played a significant role in the improvement of the cellular integrity of the neutrophils, preventing the release of the cell – damaging free radicals (Ambali *et al*., 2010c). Similarly, vitamin C may have played a significant role by scavenging for free radicals in the extracellular medium, and regeneration of active vitamin E (Ambali *et al*., 2010c).

Diazinon exposure was associated with significant decreases in Rbc counts and blood Hb (Mana *et al*., 2007). A similar trend has been observed in mice treated with Nuvacron and Furadan (Gupta *et al*., 1982). Shalby (1998) found that treating rats with chlorpyrifos insecticide reduced Hb and Rbc counts. This reduction in Hb content was suggested to be due to the adverse effects of Nuvacron and Furadan on the bone marrow (Seiverd, 1972).

Haematological studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets or those exhibiting anaemia all posses' reduced haematocrit (PCV) values (Gaitlin and Wilson, 1986). The haematological changes during the chronic toxicosis of edifenphos showed a significant decrease mostly during the entire period in Hb, Rbc count and PCV (Gaafar *et al*., 2010) This revealed the prominent anaemia inducing effect of edifenphos confirmed by the results of the blood indicies, which, in turn, revealed the haemolytic effect of edifenphos. This may explain the chocolate discoloration of parenchymatous organs, as Hb may be converted in to methaemoglobin with resultant hemolysis and reduced blood oxygen carrying capacity which accumulates with the irritant effect of edifenphos causing respiratory distress to the fish (Gaafar *et al*., 2010). The severity of the anaemia is also magnified by the hypoproteinaemic effect showed by edifenphos. Consequently, the haemolytic and destructive effects of the pesticides on blood cells are supported by El – Boushy (1994) and Robert (2001).

In previous haematological studies on nutritional effects (Rehulka, 2000; Sniezsko, 1961; Haley and Weisser, 1985) It is evident that erythrocytes reflect the state of the organism over a prolonged period of time and high concentration of pesticides or long term exposure of fish to their sublethal concentration usually decreases erythrocyte indices.

Haematological response of catfish (Cultured and wild) to the acute exposure to diazinon based organophosphorous pesticide revealed a significant decrease of erythrocyte count, haematocrit value and Hb content compared to the control groups (Adedeji *et al*., 2009). Decreased erythrocyte count and Hb content in fresh water fish *Cyprinus carpio,* after acute exposure to diazinon, was also reported by Svobodova *et al*. (2001). Other effective substances of organophosphorous pesticides also induce changes with evidence of decreased haematopoiesis followed by anaemia induction in fish and changes in erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Beharji and Rajendranath, 1990).

Significant decrease of white blood cell count and absolute lymphopenia and granulocytosis characterized the WBC profile of common carp after acute exposure to diazinon – based pesticide (Adedeji *et al*., 2009). Lymphopenia as a consequence of methylparathion – based pesticide was reported by Nath and Banerjee (1996) in *Heteropneustes fossilis*. A decreased nonspecific immunity in fish can be expected after acute exposure to organophosphorous pesticides due to decreased WBC count, lymphopenia and granulocytosis. Lymphopenia and granulocytosis have been reported after exposure to many pollutants (Svobodova *et al*., 1996).

In another study, Thaker (1988) reported no significant changes in Hb and TLC in white leghorn chicks orally administered malathion and endosulfan. Administration of chlorpyrifos at 0.8 mg/kg body weight did not produce any significant changes in the concentration of Hb, total leucocyte count (TLC) and differential leucocyte count (DLC) in broiler chickens on day 24 and day 45 (Kammon *et al*., 2011). No significant changes in

Hb, TLC, and DLC were observed in white leghorn cockerels fed Ekalux (Quinalphos) insecticide for 20 days (Mohiuddin and Ahmed, 1986).

2.9 EFFECT OF ORGANOPHOSPHATES ON SERUM – BIOCHEMICAL PARAMETERS IN ANIMALS AND THE ROLE OF ANTIOXIDANTS

Ambali *et al*. (2010b) reported reduced total protein (TP) observed in the CPF treated group which agreed with the previous findings (Ambali, 2009; Szabo *et al*., 1988). The relatively lowered TP in the CPF group was apparently due to low globulin (Ambali *et al*., 2010b). This contradicts previous findings where CPF – induced hypoproteinaemia has been attributed to hypoalbuminaemia (Ambali, 2009; Goel *et al*., 2006). Indeed, high albumin concentration was recorded in the group exposed to CPF (Ambali *et al*., 2010b). However, Szabo *et al*. (1988) observed hypoglobulinaemia following repeated CPF exposure in rats. The reason for the apparent reduction in globulin concentration following acute CPF exposure is not known for certain, especially in the light of apparent leucocytosis observed in the CPF group (Abali *et al*., 2010b). This may however be due to the ability of CPF to induce cytotoxic damage to the cells of the immune system via the induction of necrosis or apoptosis (Corcoran *et al*., 1994; Rabideau, 2001) resulting in the destruction of the source of these immunoglobulins. On the other hand, pretreatment with vitamin C was shown to improve the total protein and the globulin concentrations. This shows that oxidative stress plays an important role in the alteration of serum proteins concentration observed in CPF poisoning (Ambali *et al.*, 2010b). The vitamin may have protected the liver and other cells of the immune system from CPF – induced oxidative damage. Besides, vitamin C stimulates the immune system by enhancing T – cell proliferation, which ultimately assists the B – cells to synthesize immunoglobulin (Campbell *et al*., 1999; Naidu, 2003).

Previous study by Ambali *et al*. (2010b) showed that single high dose level of CPF did not significantly alter the serum concentration of Na^+, K^+, and Cl^-. This is in agreement with the findings of Ambali *et al*. (2007) and Ambali (2009). Of note however, is that exposure to CPF was shown to cause about 11 % increase in serum K^+ concentration compared to the soya oil group. This shows that acute exposure to large dose CPF may have caused oxidative damage to the muscle and pretreatment with vitamin C apparently restored the K^+ concentration probably due to the reduced lipoperoxidative damage to the muscle (Ambali *et al*., 2010b). A slight (2.7%) increase in urea concentration in rats exposed to CPF only was reported by Ambali *et al*. (2010c). This agrees with the previous findings by Ambali *et al*. (2007) and Ambali, (2009). This apparent increase in urea revealed the ability of the acute large dose CPF exposure to cause short – term low – grade pathological changes in the kidneys. The apparent normalization of the urea concentration observed in vitamin C pretreated group revealed the role of oxidative stress in these pathological changes (Ambali *et al*., 2010b).

The significant decrease in the activity of ALT observed in the CPF group reported by Ambali *et al*. (2010b) agreed with those recorded in previous studies (Ambali *et al*., 2007; Barna – Lloynd *et al.*, 1990; Szabo *et al*., 1988). The reason for the low level of serum ALT is not known and besides, the toxicological significance of this remains obscure. However, pretreatment with Vitamin C was shown to partly restore ALT levels although it was still relatively low compared to the soya oil group (Ambali *et al*., 2010b).

Ambali *et al*. (2010b) showed an apparent elevation (19.6 %) in the AST activity of rats exposed to CPF only compared to those of the soya oil group which indicated some level of damage to any or all of the organs producing this enzyme, such as liver, cardiac and

skeletal muscles. This finding agreed with those obtained in previous study (Ambali, 2009; Gomes *et al*., 1999; Yoshida *et al*., 1985) Indeed, it has been revealed that CPF causes damage to the liver (Ambali, 2009; Goel *et al*., 2005) and the cardiac muscles (Ambali, 2009), probably through the induction of oxidative stress. The oxidative damage induced by CPF may have been prevented by pretreatment with vitamin C which apparently brought about restoration of the AST activity in rats in the vitamin C + CPF group (Ambali *et al*., 2010b)

Similarly, apparent increase in serum ALP in rats exposed to CPF was reported by Ambali *et al*. (2010b). This finding agreed with previous works (Ambali *et al*., 2007; Ambali, 2009; Goel *et al*., 2005). This finding shows that acute CPF exposure causes short – term pathological lesions in any or all organs involved in the synthesis and/or release of ALP such as the liver, kidneys, bones, muscles and intestinal mucosa probably due to oxidative damage (Ambali *et al*., 2010b). The apparent restoration of the ALP activity in rats pretreated with vitamin C further underscore the role of oxidative damage in the apparent increase in ALP activity. Pretreatment with vitamin C apparently normalized the ALP activity restoring it to almost the values obtained in the soya oil group (Ambali *et al*., 2010b).

Increase in the levels of aminotransferases (ALT and AST) and the level of ALP as well as the decrease in the levels of total protein and albumin in serum, are the major diagnostic symptoms of liver diseases (Chatterjea and Shinde, 2005). The decrease in the serum albumin may also indicate to the renal inability to keeps it in; therefore it is excreated with urine (Albumiurea) (Vosilenko and Grebener, 1990). The increase in the uric acid and creatinine in the serum are the major symptoms of glomerular filtration damage (Chatterjea

and Shinde, 2005). Hepatotoxic as well as pancreatic B – cells of Dimethoate and Diazinon was reported by Elias (2010). This result is in agreement with the results of Ansaria and Kumar (1988), who found that, Diazinon reduced the total protein level in Zebra fish (Matin *et al*., 1990), who observed thet Diazinon administration to rats reduced carbohydrate and protein metabolism, (Begum and Vijavaraghaven, 1995), who showed that Dimethoate inhibited carbohydrate and protein metabolism, and affected the aminotransferases in rats, (Attia and Nasr, 2009), who noticed increase in serum aminotransferases, ALP and decrease in total protein and albumin in rats serum after oral administration of Dimethoate and (Kossman *et al*., 1997), who assured the nephrotoxic effect of pesticides.

Serum biochemistry revealed a significant increase in serum ALT, AST and ALP at the beginning of the experiment (Gaafar *et al*., 2010). These findings supported the hypothesis that the increased serum transaminases (ALT and AST) may reflect hepatic toxicity which leads to extensive liberation of the enzymes in to the blood circulation (Daabes *et al*., 1992). Then after that, the enzymes suffered from significant decrease in their levels. Alkaline phosphatase level also showed significant increase then significant decrease towards the end of the experiment (Gaafar *et al*., 2010). The decrease in the activity of AST, ALT nad ALP in fish exposed to pesticides was also reported by different authors (El – Boushy, 1994; Begum, 2004). Agius and Coushman (1986) linked the increased activity of ALP in fish to the increased catabolic tissue breakdown in melanomacrophage centres.

Saeed (1983) attributed the decrease in liver transaminases activity to the decrease in protein content in serum and tissues due to the resultant hepatic necrosis of fish exposed to pesticides. Also the severe hepatic necrosis leads to lack of cells from which the enzymes

are produced. These findings indicated that decrease in liver function was occurred which may lead to major dangerous sequelae in body metabolism.

Total serum proteins showed general significant decrease after exposure to edifenphos. This may be due to liver damage where most of the plasma protein synthesis usually occurs in the liver (Gaafar *et al*., 2010) this result agreed with that of Singh *et al*. (1998) (Gaafar *et al*., 2010) reported harmful effects on the kidney exerted by edifenphos in which there was a significant increase in creatinine after the 5^{th} week of exposure and also a significant increase in blood urea nitrogen levels throughout the experimental period.

Chlorpyrifos did not significantly influence the activity of serum ALP, but this enzyme was slightly elevated in chlorpyrifos treated group compared to the control on day 45 of age (Kammon *et al*., 2011) also reported that chlorpyrifos did not produce significant changes in serum levels of glucose, cholesterol, creatinine, total protein, albumin, uric acid and the activity of creatinine kinase. In acute hepatic injury caused by chlorpyrifos, ALT and AST were significantly elevated (Kammon *et al*., 2011). Whereas in chronic disease; ALT and AST may be normal or only slightly elevated (Cornelius, 1989).

In subacute toxicity test, the effects of sublethal glyphosate concentrations (2.5, 5.0 and 10.0 mg/l) on activity in the serum and organs were performed.statistically significant increase in alkaline phosphatase activity in liver (all three concentrations tested) was obtained. Also statistically significant increase in alkaline phosphatase activity in heart of fish exposed to 10.0 mg/l glyphosate concentration was obtained (Neskovic *et al.*, 1996). Slightly lower effect in the glutamic – oxaloacetic transaminase activity was noted. In the fish exposed to 2.5 and 5.0 mg/l glyphosate concentrations, statistically significant increase

in glutamic – oxaloacetic transaminase activity in liver and kidneys (glyphosate concentrations 2.5 mg/l) and in serum (glyphosate concentrations 5.0 and 10.0 mg/l). In all other cases, no statistically significant changes in the glutamic – oxaloacetic transaminase and glutamic – pyruvic transaminase activity were noted, in relation to the control (Neskovic *et al*., 1996). Benedetti *et al*. (2004) reported a two – fold increase in AST in glyphosate – treated rats at the end of sub – chronic treatment, the serum AST elevated to about one and half – fold in relation to the control at the end of the treatment. Although low, this difference was significant due to automatic system used to measure serum ALT and AST, indicating the hepatic toxicity induced by glyphosate.

Albino male rats were intraperitoneally treated with sub lethal concentration of Roundup® (269.9 mg/kg) or glyphosate (134.95 mg/kg) each 2 days during 2 weeks. Hepatotoxicity was monitored by quantitative analysis of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, total protein, albumin, triglyceride and cholesterol. Creatinine and urea were used as the biochemical markers of kidney damages. Treatment of animals with Roundup® induced leakage of hepatic intracellular enzymes, ALT, AST, and ALP suggesting irreversible damage in hepatocytes starting from the first week. It was found that the effects were different on the enzymes in Roundup® and glyphosate – treated groups i.e the results suggested that excessive antioxidant disruptor and oxidative stress is induced with Roundup® than glyphosate (Nahla, 2009). There were no statistical significant changes of serum enzyme activities and lipid profile; however AST/ALT value of animals following sub-acute and sub-chronic exposure to glyphosate were higher than the control (Caglar and Kolankaya, 2008).

2.10 HISTOPATHOLOGICAL EFFECT OF ORGANOPHOSPHATES IN ANIMALS AND THE ROLE OF ANTIOXIDANTS

Zinc treatment protected hepatocytes from marked distruptions in the membranous organelles and narrowing/blocking of biliary channels (Goel and Dhawan, 2001). Abdel – Rahman *et al*. (2004) reported that malathion caused neuronal degeneration in hippocampus of rats exposed to this pesticide subchronically, 30 days to 44.4 mg/kg of malathion by dermal route. Degeneration of neurons in the dentate gyrus region and the attenuated effect of the neuronal alterations following zinc administration were reported by Brocardo *et al*. (2007).

Histhopathological changes in liver, stomach, intestine, kidney and gills of fresh water teleost fish *Ophiocephalus punctatus* following exposure to sublethal concentrations of diazinon (0.2 and 0.4 mg/litre) were fibrosis of liver with small degenerative nuclei, and invasion of a number of phagocytes with erosion of the mucosa, in the intestine, few villi were necrosed and nuclei were reduced in volume, in kidney, shrinkage of the glomerular network and necrosis of proximal tubules, the distal and collecting tubules were ruptured and the nuclei were degenerated and pyknotic, the only visible change in the stomach was erosion of the mucuosa. Degeneration and thinning of epithelial linning of the lamellae separation of the vascular layer from the epithelium, and in some cases fusion of the epithelium of the adjacent villi were the alterations in the gills (Sastry and Sharma, 1981)

Infiltration of mononuclear cells at parenchymal tissue, sinusoidal dilatation, focal necrotic areas, granular degeneration and pyknotic nucei in hepatocytes following exposure to methidathion were shown to be significantly decreased following administration of

vitamins E and C (Gokalp *et al*., 2003). Joshi *et al*. (2007) reported mild to severe degenerative changes in seminiferous tubules at various dose levels of chlorpyrifos (7.5, 12.5 and 17.5 mg/kg b.wt/ day in albino rats exposed to this organophosphate for 30 days might be useful in controlling the toxic effects of insecticides and chemicals (Packer, 1992; Meydani, 2001).

Histopathologically, proventriculus of chickens administered CPF showed mild necrosis of glandular cells and accumulation of exfoliated cells in the lumen of proventricular glands on day 24. There was mild degeneration of the tips of the mucosal papillae, moderate necrosis of glandular cells and accumulation of desquamated cells in the lumen of the proventricular glands on day 45 (Kammon *et al*., 2011).

Mild mucous degeneration and mild necrosis associated with sloughing of epithelial cells were observed in the intestine on day 24. Lesion observed on day 45 comprised of mild mucous degeneration and moderate necrosis of villi associated with sloughing of the epithelial cells (Kammon *et al*., 2011). The lesions observed in the intestine suggested that CPF has some irritant effects on the epithelial membrane (Kammon *et al*., 2011). Enteritis has been observed in a dog with oral ingestion of dichlorvos insecticide (Snow, 1973).

Chlorpyrifos produced mild dilatation of hepatic sinusoids, moderate vacuolar degeneration and fatty changes and moderate coagulative necrosis on day 24. In addition, CPF produced mild proliferation of bile duct epithelium on day 45 (Kammon *et al*., 2011). Some of the observed lesions by Kammon *et al*. (2011) are consistant with several workers, who reported congestion, vacuolar degeneration and fatty changes, focal to extensive necrosis, hyperplasia of kuffer cells, dilation of sinusoids, nuclear aberrations, cytoplasmic

degranulation and picnotic nuclei (Gupta and Chandra, 1977; Varshneya *et al*., 1986; Chaudhary *et al*., 2003).

In pancrease, Kammon *et al*. (2011) reported mild degeneration of glandular acini, mild necrosis of glandular cells, mild degeneration of beta islets on day 24 and mild proliferation of interlobular duct of pancreas on day 45. Chronic oral administration of CPF produced mild congestion and haemorrhage, mild focal coagulative necrosis and sloughing of tubular cell in chickens on day 24. On day 45, CPF produced moderate severity of similar lesions to that seen on day 24 (Kammon *et al*., 201). These lesions are similar to earlier studies, which revealed marked congested and focal degenerative changes in the lining of distal convulated and collecting ducts, leading to nephrosis in kidney of rats after repeated oral administration of endosulfan (Gupta and Chandra, 1977). Majumder *et al*. (1994) observed larger size of glomeruli, glomerular and tubular necrosis in broiler chicks following subacute toxicity of fenvalerate. This is reasonable since the tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part, because they have complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins. In addition, the tubules are exposed to toxic chemicals during their excretion and elimination by the kidneys (Tisher and Brenner, 1989). The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of cells (Shimizu *et al*., 1996). One possible mechanism for the tubular lesions observed was the direct toxic effect on the cell function (Kammon *et al*., 2011). Other possible mechanisms for tubular lesions may involve reactive free radicals or oxidative stress, or both (Alden and Frith, 1992).

Brain of chickens administered CPF showed mild neuronal degeneration and vacuolation and mild degeneration in purkinge cells on day 24 and 45 (Kammon *et al*., 2011). In heart muscles, there was mild granular degeneration of myocytes on day 24. On day 45, there were moderate granular degeneration and loss of striations with moderate infiltration of mixed inflammatory cells composed mainly of mononuclear cells and heterophils (Kammon *et al*., 2011). Bhattacharya *et al*. (1993) reported coagulative necrosis and fragmentation in cardiac muscle, coronary vessels which were filled with Rbc and the presence of mononuclear cells in the spaces between the myofibrils.

Histopathological changes in gills, liver and kidney were examined and compared with control fish that had normal morphological structure. On the gills of fish exposed to 5.0 mg/l glyphosate concentration, epithelial hyperplasia and subepithelial oedema were found. Similar changes remained at 10.0 mg/l glyphosate, but were more pronounced and followed with leucocyte infiltration, slight hypertrophy of chloride cells, as well as lifting and rupture of the respiratory epithelium on some secondary lamellae. Liver in the control fish had normal structure, while changes in liver structure appeared only at 10.0 mg/l glyphosate concentration. Congestion of few sinusoids and in some places, signs of early fibrosis was recorded. Kidney structure of fish was not affected by the glyphosate since no histopathological changes were found when exposed to different sublethal glyphosate concentrations (Neskovic *et al*., 1996).

Olurin *et al*. (2006) reported varied morphologic changes in the gill tissue of the treated aquaria. This was more evident in higher concentration of glyphosate compound as well as duration of exposure. At 0.05 % concentration on the 14^{th} day, the gills exhibited marked alterations in the epithelia. The epithelia was no longer continuous, particularly the more

delicate respiratory lamellae. Thus, there was fusion at adjacent secondary lamellae as a result of hyperplasia. Oedema at the secondary lamellae and swelling of the epithelial cells were observed with epithelial detachment. At concentration of 0.1 % at the 14th day interval, the primary and secondary lamellae showed significant reduction in size. The oedematous separation of primary and secondary lamellae led to sloughing at the tip. The pillar cells had altered and blood spaces expanded. At 28th day (0.05 %), following supervening distress, the elongated secondary lamellae appeared club – like. A measure of fusion is equally noticed at the tips. Severe epithelial disintegration occurred after 42nd day (0.05 %) of exposure; this was followed by a necrosis of lamellae epithelial cells (primary and secondary). There was severe hyperplasia with profound oedematous changes characterized by epithelial detachment.

Histomorphology of liver specimens exposed to glyphosate compound showed diffuse changes in the hepatic parenchyma. More significantly was the enlargement of hepatocytes in proportion to the increase in size of the nuclei. At day 14th (0.05 %), the specimen was characterized by an increase in density of connective tissue with imminent congestion at the sinusoidal spaces. There were large vacuoles within the cytoplasm resulting from cell membrane degeneration. Many of the nuclei had become pyknotic with gradual process of cytolysis. At 0.1 % concentration, the effect became more profound around the hepatic parenchyma. The cytoplasm was highly vacuolated while the nuclei continued to be pyknotic. Focal necrosis was noticed in many regions with increase in cytolysis. Exposure on the 28th day showed similar appearance with more lethal effect. However, following exposure on the 42nd day, haemorrhage occurred that led to extensive necrosis of hepatic cells (Olurin *et al*., 2006).

Ayoola (2008) reported histopathological changes observed in the gill, liver, kidney and brain of *Clarias gariepinus* subjected to different concentration of glyphosate for 96 hours as follows:

Gills: No recognizable changes were observed in the gills of the control fish. Each gill consisted of a primary filament and secondary lamellae. At different concentrations of glyphosate, there were cellular infiltrations, swollen tip of the gill filament, congestion, severe gill damage and heterophilic infiltration.

Liver: The histology of control fish liver revealed normal typical parenchymatous appearance whereas there were glycogen vacuolation, fatty infiltration, hemosiderosis and congested central vein at the concentration of 1.9 – 9 mg/l; severe infiltration of leucocytes, pyknotic and hepatic necrosis were also observed at 21 and 45 mg/l concentration with severe necrosis, haemorrhage and vacuolation.

Kidney: No recognizable changes were observed in the kidney of the control fish. The kidney tissue from *Clarias gariepinus* exposed to different concentrations of glyphosate showed necrosis, degenerated kidney tubules, pyknosis, exfoliated and swollen with pyknotic nuclei.

Brain: No significant lesion was seen in the brain of the control fish and morphological structure was normal. The histological observation on the brain showed that there was discoloration on the brain of fish exposed to different concentration of glyphosate. Fish exposed to concentration of 1.9 – 45 mg/l showed mononuclear infiltration, neuronal degeneration and severe spongiosis.

Light microscopic study of the gills of Nile tilapia exposed to 36 ppm 0f Roudnup® (96 h LC_{50} value) for 24, 48, 72, and 96 hours showed several pathological changes and their frequencies increased with increasing time of exposure. After 24 h of Roundup® exposure, filament cell proliferation appeared regular, similar to that of the control. After 48 h of exposure, the thickness of the primary lamellar epithelium and leucocyte infiltration was observed. After 72 h of exposure, the thickness primary lamellar epithelium was still irregular. Groups of erythrocytes were observed at the secondary lamellae. Similar changes remained after 96 h of exposure, with more pronounced thickness of the primary lamellar epithelium. Clubbing of lamellae was concentrated at the tips of the secondary lamellae (Wannee *et al*., 2002). Liver: After 24 h of exposure the hepatocytes began to swell and slight infiltration of leucocytes were observed. After 48 h of exposure, the hepatocytes were still swelling. They showed extensive pyknosis, and involution exhibiting darkly stained specks of necrotic nuclei, as well as mild infiltration of leucocytes. There was some degeneration of the cell membrane and vacuolation in the cytoplasm. After 72 h of exposure, there were large vacuoles in the cytoplasm, the nuclei continued to be pyknotic, and moderate infiltration of leucocytes was observed. After 96 h of exposure, more notable detrimental changes were observed. There was a severe infiltration of leucocytes. The hepatocytes showed extensive pyknotic nuclei and large vacuoles in many areas (Wannee *et al*., 2002).

Kidney: After 24 h of exposure, the fish had renal lesions of varying degrees. Some glomeruli were collapsed or distorted. The epithelial cells of the first proximal tubule contained small vacuoles. Some of the cells were necrotic with small pyknotic nuclei. After 48 h of exposure, some of the primary epithelial cells still had small vacuoles. Some

exhibited small hyaline droplets. After 72 h of exposure, they had extensive vacuoles and were swollen with pyknotic nuclei. After 96 h of exposure, the epithelium of many of the tubules had become exfoliated. The cytoplasm was pale and the cells appeared swollen (Wannee *et al*., 2002).

Benedetti *et al*. (2004) reported local increase in the number of Kupffer cells in hepatic sinusoid in all animals treated with glyphosate at 487 mg/kg. Kupffer cells did not increase in animals treated with lower doses of glyphosate. This was attributed to local proliferation of these cells since no apparent infiltration of perivascular kuffer cells or lymphocytes were observed on portal tract. There was increase in connective tissue deposition in all glyphosate treated animals with 487 mg//kg. This was not observed in animals treated with lower doses of glyphosate. These data suggest that in glyphosate – treated animals, hepatic modulation may occur where collagen or other extracellular matrix proteins were induced. Studies on carp (*Cyprinus carpio*) exposed by emersion in Roundup® super (205 mg glyphosate/l or 410 mg of glyphosate/l) in concentrations of 40 to 20 fold lower than those used in practice revealed that the herbicide caused the appearance of myelin – like structures in hepatocytes, swelling of mitochondria and disappearance of internal membrane of mitochondria at both exposure concentrations, meaning that Roundup® was harmful to carp when used at recommended application concentrations (Szarek *et al*., 2000).

2.11 EFFECT OF ZINC SUPPLEMENTATION ON OXIDATIVE STRESS IN ANIMALS

Alteration in the values of packed cell volume (PCV), haemoglobin (Hb), red blood cells, platelets, absolute and differential leucocyte counts and malonaldehyde concentration induced by chlorpyrifos (CPF) in Wistar rats were ameliorated by co – administration with Zinc which indicated that the attenuations of CPF – evoked alterations in haematological parameters by Zinc may be partly mediated through its antioxidant properties (Ambali *et al*., 2010). Arsenic treatment in rats resulted in a significant increase in lipid peroxidation (LPO); however, glutathione (GSH) levels and the activities of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) were found to be significantly decreased following arsenic treatment. Furthermore, arsenic treatment resulted in a significant decrease in hepatic Zinc levels. Histological studies showed well – differentiated signs of focal hepatitis, lobular inflammation, prominent hepatocytes degeneration, and severe periportal necrosis. Administration of Zinc to arsenic – treated rats significantly decreased the level of LPO, but increased the level of GSH compared with the arsenic – treated rats. Furthermore, the Zinc level and activities of SOD, GPx, GR, and CAT were found to be significantly increased following Zinc treatment. The administration of Zinc to arsenic – treated rats caused signs of improvement in liver histoarchitecture, but a few focal areas of degeneration and necrosis were still occasionally seen, hence, these suggested that Zinc can be beneficial against arsenic – induced Hepatotoxicity in rats (Kumar *et al*., 2010).

Sidhu *et al*. (2004) reported that a significant inhibition in the levels of SOD activity and reduced GSH were observed following protein deficiency in normal rats. Zinc treatment to protein deficient animals lowered lipid peroxidation and catalase, GPx and GST activities, and also GSH and SOD activity. The concentration of Zinc returned to normal level when

Zinc was administered. Zinc supplementation in cadmium – exposed rats restored the activities of GPx, CUZn – SOD, and Mn – SOD in the testes to normal levels. Moreover, Zinc administration was capable of reducing the elevated levels of malonaldehyde in the testes. Interestingly, Zinc supplementation attenuated DNA oxidation induced by cadmium in the gonad and restored the testosterone level and sperm count to normal levels. Zinc administration minimized oxidative damage and reversed the impairment of spermatogenesis and testosterone production induced by cadmium in the rat testes (Amara *et al*., 2008). Mononuclear cell infiltration, apoptosis of some hepatocytes, focal necrosis, congestion and swelling of hepacytes in liver tissue were the histopathological lesions observed by Caglar and Kolankaya, (2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Sixty male and female Wistar rats, 160 – 200 g body weight, were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were housed in the Animal Room of the Department of Veterinary Pathology and Microbiolog, Ahmadu Bello University, Zaria, for two weeks before the commencement of the experiment which lasted for eight weeks. The rats were fed appropriately using standard rat chow (Growers mash, maize offal and ground nut cake in the ratio of 4 : 2 : 1 respectively) and water was provided *ad libitum.*

3.2 CHEMICAL SOURCE

Glyphosate (Bushfire®, Monsanto Europe S. A) which contains 360 g glyphosate / Litre in the form of 441 g / Litre Isopropylamine salt and zinc chloride with the following specification: M.W. 136.29, Cat no_ 45114, Lot no_ 11601(BDH Chemical Ltd Poole England) is a white deliquescent granule with minimum assay 98.0 %, maximum limits of impurities: Acid – insoluble matter 0.005 %, Zinc oxide (ZnO) 1.2 %, Sulphate (SO_4) 0.002 %, Cadmium (Cd) 0.0005 %, Calcium (Ca) 0.001 %, Copper (Cu) 0.0005 %, Iron (Fe) 0.001 %, Lead (Pb) 0.001 %, Magnesium (Mg) 0.001 %, Potassium (K) 0.001 % and Sodium 0.001 % were purchased from a reputable chemical store in Zaria.

3.3 EXPERIMENTAL DESIGN

3.3.1 Determination of Median Lethal Dose (LD_{50})

The median lethal dose (LD_{50}) was determined through a two phase approach as described by Lorke (1983).The first phase involved 9 Wistar rats divided in to 3 groups of 3 Wistar rats each. Each group was administered glyphosate at doses of 4000 mg / kg, 5000 mg / kg and 6000 mg / kg body weight *per os* respectively. Signs of toxicity and death were observed over a period of 48 hours. The doses of the second phase which depended on the results obtained from phase 1, consisted of three animals divided in to three groups of one animal each, administered with glyphosate at 3500 mg / kg, 4000 mg / kg and 4500 mg / kg

respectively. The LD_{50} was then evaluated to be 3750 mg / kg by calculating the average of the highest dose that killed the rats and the lowest dose that did not kill the rats.

3.3.2 Subchronic Toxicity Study

Forty eight adult male and female Wistar rats were randomized into six groups of eight rats each as described below:

Group I (DW): served as the control and were administered 2ml / kg of distilled water daily.

Group II (Z): were administered Zinc at the dose rate of 50mg / kg body weight (Ambali *et al*. 2010a).

Group III (G): were administered glyphosate (10% of the LD_{50}), 375 mg / kg body weight.

Group IV (Z + G): Were administered Zinc at 50mg / kg + glyphosate (10% of the LD_{50}), 375 mg / kg body weight.

Group V (GC): were administered glyphosate at the concentration of 1:50 glyphosate and distilled water respectively, 14.4 mg / kg body weight.

Group VI (Z + G): were administered Zinc at 50 mg / kg + glyphosate at the concentration of 1:50 glyphosate and distilled water respectively, 14.4 mg / kg body weight.

The dose regimens were administered *per os* by gavage once daily for a period of 8 weeks. The rats were monitored for clinical signs and death.

3.3.3 Haematological Analysis

At the end of the study period, the rats were sacrificed by severing the jugular vein after light chloroform anesthesia. Blood samples (3ml each) were collected into heparinized sample bottles and assayed for packed cell volume, haemoglobin concentration, total white blood cell count , absolute and relative leucocytes count using an auto analyzer (Abbot Haematological analyzer®, cell-Dyn 1700; Abbot Park, Illinois, U.S.A).

3.3.4 Serum-Biochemical Analysis

Another set of blood sample (5ml each) was collected from each rat into clean test tubes. The blood samples were incubated for 30 minutes, and thereafter centrifuged at 800 x g for 10 minutes to obtain the sera. The sera were analysed for aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. Other parameters measured included total serum proteins and albumin, electrolytes, blood urea nitrogen, urea, uric acid and creatinine, but the globulin concentrations were calculated by subtracting the albumin concentrations from the total proteins. They were determined using Bayer Express plus Clinical Chemistry Autoanalyser (Bayer® Germany).

3.3.5 Histopathological Examination

The sections of the brain, liver, spleen, pancreas, kidney, stomach, heart, testis, and uterus were taken for histopathological preparation and examination. The samples collected were fixed in 10 % buffered neutral formalin. They were processed for histopathological assessment using the method outlined by Baker *et al*. (2000) and viewed under light microscope.

3.3.6 Statistical Analysis

Data obtained were expressed as mean ± SEM. They were subjected to one-way Analysis of Variance followed by Tukeys' post hoc test using Graph Pad Prism Version 4.0 Values of $p < 0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 CLINICAL SIGNS

The rats administered with distilled water and Zinc ($Zncl_2$) and Zinc + glyphosate at the two different concentrations (10% of the LD_{50} and 1:50 glyphosate and water respectively) did not show any sign of toxicity. The toxic signs observed in the rats administered with glyphosate in G group included respiratory distress, diarrhoea, rough hair coat and death of one rat in the group.

4.2 HAEMATOLOGY

4.2.1 Effect of the Treatments on Packed Cell Volume (PCV)

There were no significant changes ($p > 0.05$) in the Packed Cell Volume (PCV) between the groups. However, there was a relatively high (5.9%) PCV in the group administered with glyphosate at 1:50 glyphosate and water respectively (GC) group when compared to the group administered with distilled water (DW) group. On the other hand, there was a relatively low PCV in the (Z + G) group pretreated with zinc at 50 mg / kg body weight and glyphosate at 10 % of the LD_{50} (7.6%), (Z + GC) the group pretreated with zinc and glyphosate at 1:50 glyphosate and water respectively (9.0%), (Z) the group administered with zinc at the rate of 50 mg / kg body weight (10.0%) or (G) the group administered with glyphosate at 10 % of the LD_{50} (11.5%) group when compared to DW group (Figure 4.1).

4.2.2 Effect of the Treatments on Haemoglobin (Hb) Concentration

The effect of the treatments on haemoglobin concentration is shown in Figure 4.2. There were no significant changes ($p > 0.05$) in the Hb concentration in all the groups. However, there was a relatively high (5.8%) Hb concentration in GC group when compared to DW group, on the other hand, there was a relatively low Hb concentration in the Z + G (7.6%), Z + GC (8.9%), Z (10.0%) and G (11.4%) groups, respectively when compared to DW group.

4.2.3 Effect of the Treatments on Total White Blood Cell (WBC) Counts

The effect of the treatments on WBC counts in all the groups was not significantly different from each other. However, there was a relatively low (5.5%) in the WBC count in the Z group when compared to DW group. On the other hand, the WBC count was relatively high in the Z + GC (9.5%), Z + G (4.4%), GC (3.1%) and G (0.6%) groups, respectively when compared to those recorded in the DW group (Figure 4.3).

4.2.4 Effect of the Treatments on Differential Leucocyte Counts (DLC)

The effect of the treatments on DLC is shown in Figure 4.4. There were no significant changes in the neutrophils count of the groups. However, there was relatively high neutrophils count in the Z + GC (28.3%), GC (22.1%), G (12.8%), Z (10.9%) and Z + G (3.7%) groups, respectively when compared to those recorded in the DW group.

The differences in the lymphocyte count between the groups were not significant ($p > 0.05$). However, a relatively low lymphocyte count was recorded in the Z + GC (34.30%),

GC (29.40%), Z + G (29.00%), Z (28.30%) or G (17.80%) group (Figure 4.4) when compared to those recorded in the DW group.

4.3 BLOOD CHEMISTRY

4.3.1 Effect of the Treatments on Serum Protein, Albumin, and Globulin Concentrations

The effect of the treatments on serum protein, albumin and globulin concentrations is shown in Figure 4.5. There was no significant change in serum protein concentration between the groups. However, there was a relatively low total protein concentration (7.6%) in Z + GC group when compared to DW group. On the other hand, there was a relative high total protein concentration in Z (11.3%), Z + G (0.7%), G (0.5%) and GC (0.3%) groups, respectively when compared to DW group.

There was a significantly high ($p < 0.05$; 34.7%) albumin concentrations in GC group when compared to DW group. On the other hand, there was a relatively high albumin concentration in Z (19.1%), G (15.2%), Z + G (11.1%) or Z + GC (5.8%) group when compared to DW group.

The globulin concentrations showed no significant change between the groups. However, there was a relatively high (8.5%) globulin concentration Z group when compared to DW. On the other hand, there was a relatively low globulin concentration in Z + G (3.1%), G (3.7%), Z + GC (12.2%) and GC (17.9%) groups, respectively when compared to DW group.

4.3.2 Effect of the Treatments on Serum Enzymes

The activities aspartate aminotransferase (AST) were not significantly different ($p > 0.05$) between the groups. However, there was relatively low AST in Z (5.80%) and Z + G (18.10%) group when compared to DW group. On the other hand, there was a relative decrease in AST activity in DW group compared to those recorded in G (33.5%), GC (13.0%) or Z + GC (9.8%) group.

There were no significant changes in the activities of alanine aminotransferase (ALT) between the groups. However, there was a relatively low (11.9%) ALT activity Z + GC group when compared to DW group. On the other hand, there was a relatively high ALT activity in Z (44.90%), GC (35.50%), Z + G (17.00%) or G (11.60%) group when compared to DW group.

The activity of alkaline phosphatase was significantly higher ($p < 0.05$; 60.60%) in Z + GC group when compared to GC group. On the other hand, there was a relatively low ALP activity in G (31.00%), Z (17.70%) and Z + G (4.20%) groups, respectively when compared to DW group (Figure 4.6).

4.3.3 Effect of the Treatments on Serum Electrolyte Concentrations

There were no significant changes ($p > 0.05$) in the Na^+ concentration among the groups. However, there was a relatively low Na^+ concentration in Z + G (11.70%), Z + GC (10.30%), Z (8.80%), GC (8.50%) and G (6.20%) groups, respectively when compared to DW group.

The difference in the K^+ concentration among all the groups was not significant ($p > 0.05$). However, there was a relatively low K^+ concentration in Z + G (8.10%) group when compared to DW group. On the other hand, there was a relatively high K^+ concentration in G (20.90%), Z + GC (10.60%), GC (8.20%) or Z (0.60%) group when compared to DW group.

There were no significant changes ($p > 0.05$) in the Cl^- concentration among all the groups. However, there was a relatively low Cl^- concentration in Z (8.50%), GC (5.70%) G (5.50%), Z + G (3.10%) and Z + GC (0.50%) groups, respectively when compared to DW group.

The differences in the HCO^-_3 concentration among the groups were not significant ($p > 0.05$). However, there was no change in the HCO^-_3 concentration between GC group and DW group. On the other hand, there was a relatively low HCO^-_3 concentration in Z (9.40%) or Z + G (7.40%) group and a relatively high HCO^-_3 concentration in G (18.70%) or Z + GC (4.70%) group when compared to DW group.

There was a highly significant rise ($p < 0.01$) in Ca^+ concentration in Z + GC group when compared to DW and Z groups respectively. There was also a very highly significant rise ($p < 0.001$) in Z + GC group compared to those recorded in G and GC groups respectively. In addition, there was a significant rise ($p < 0.05$; 27.40%) in Ca^{2+} Z + GC group compared to Z + G group.

There was no significant change ($p > 0.05$) in the PO_4 concentrations in between the groups. However, there was a relatively high PO_4 concentration in GC (10.80%), G

(10.60%), Z + G (6.70%), Z (6.10%) and Z + GC (2.50%) groups, respectively when compared to DW group (Figures 4.7 (a) and (b)).

4.3.4 Effect of the Treatments on Serum Urea Concentration

There was no significant change ($p > 0.05$) in the urea concentration between the groups. However, there was a relatively low urea concentration in Z + GC (20.50%) group when compared to DW group. On the other hand, there was a relatively high urea concentration in Z + G (22.50%), G (11.50%), GC (9.90%) and Z (6.20%) groups, respectively compared to DW group (Figure 4.8).

4.3.5 Effect of the Treatments on Serum Creatinine Concentration

There was no significant change ($p > 0.05$) in creatinine concentration between the groups. However, there was a relatively low creatinine concentration in Z + GC (18.80%) group when compared to DW group. On the other hand, there was a relatively high creatinine concentration in G (15.00%), GC (14.90%), Z (7.90%) and Z + G (5.70%) groups, respectively when compared to DW group (Figure 4.10).

Table 4.1: Determination of Median Lethal Dose (LD_{50}) for Glyphosate – Phase I

Group	Dose mg / kg	No_ dead/ No_ dosed
1	4000	1/3
2	5000	0/3
3	6000	0/3

Table 4.2: Determination of Median Lethal Dose (LD_{50}) for Glyphosate – Phase II

Group	Dose (mg / kg)	No_ dead/ No_ dosed
1	3500	0/1
2	4000	0/1
3	5000	0/1

Thus, based on the method of Lorke (1983), the result of the LD_{50} = 4000 + 3500/2 = 3750 mg / kg.

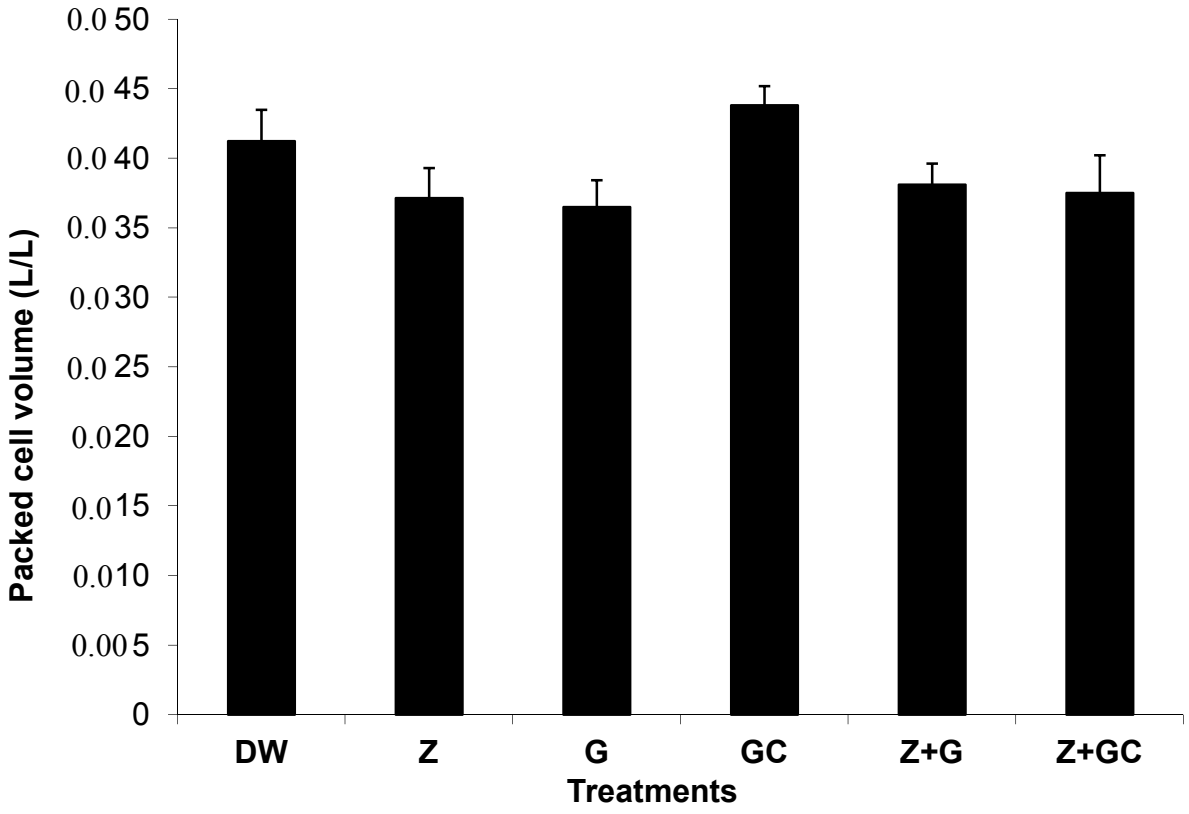


Figure 1: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on packed cell volume in Wistar rats.

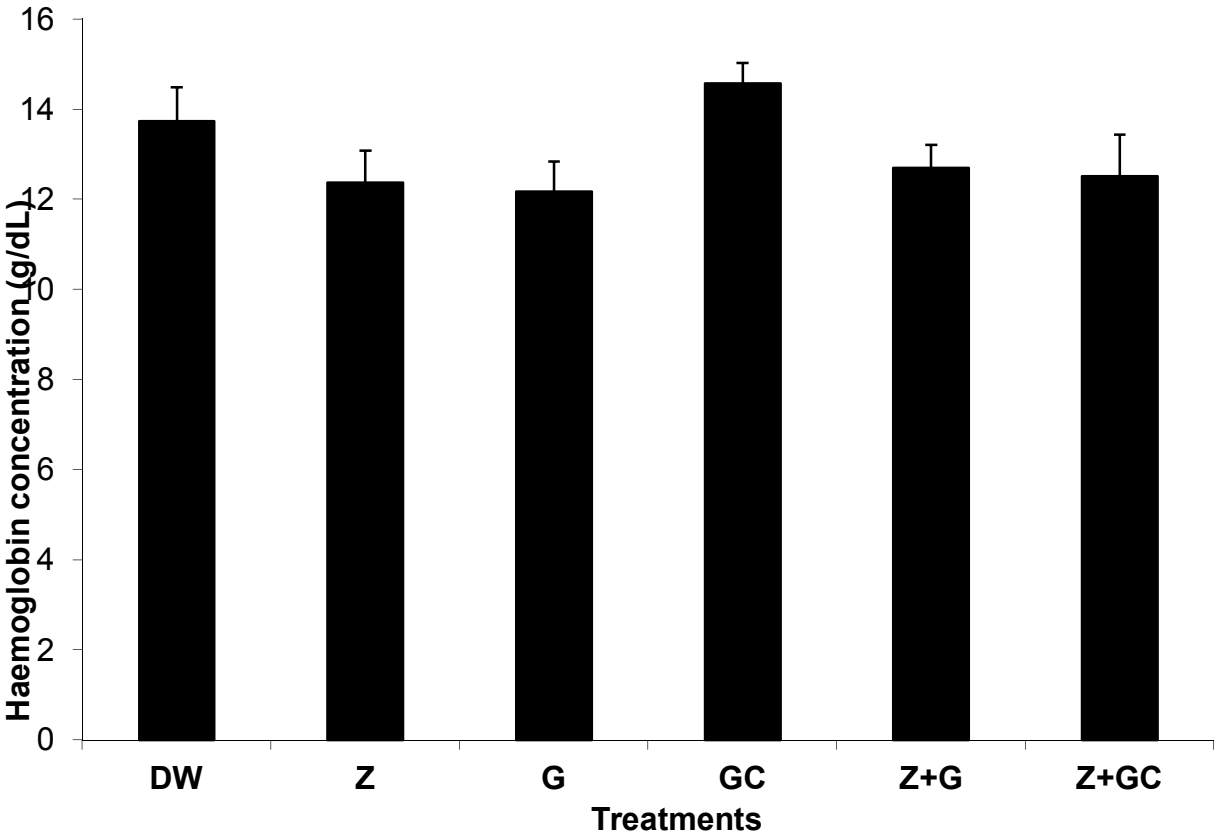


Figure 2: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on haemoglobin concentration in Wistar rats.

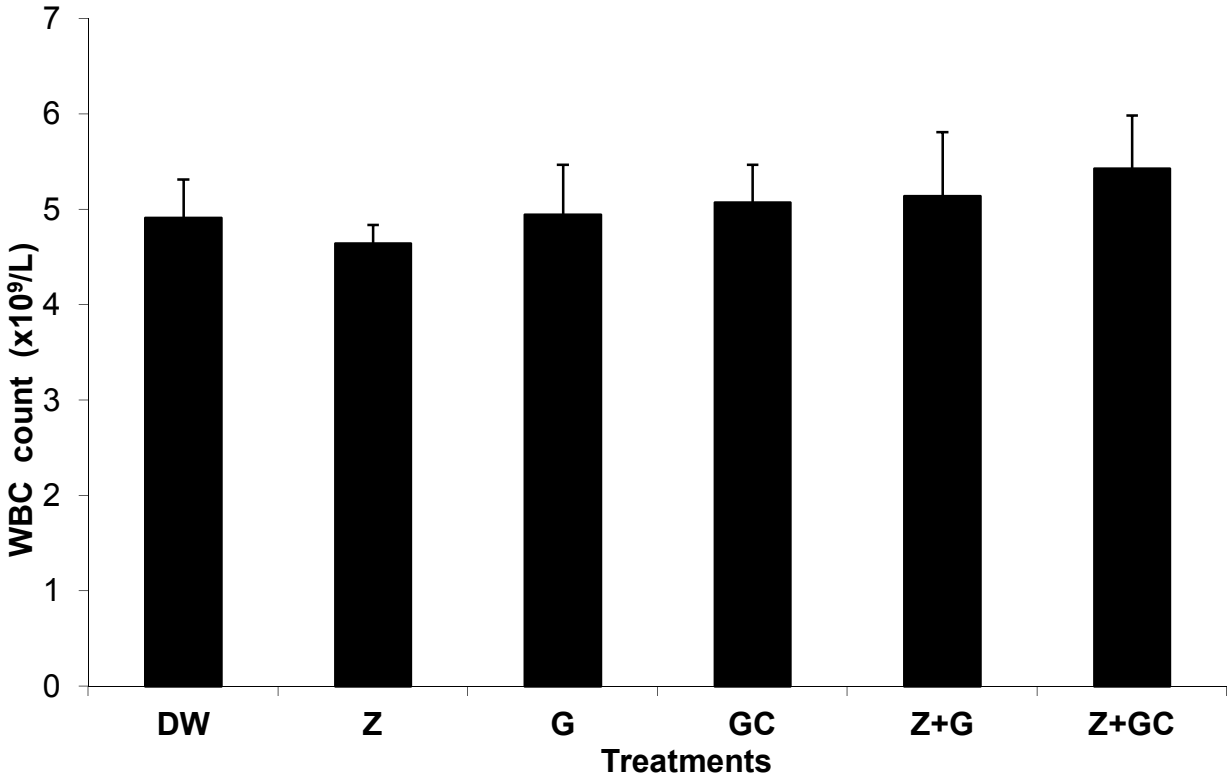


Figure 3: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on total white blood cell count in Wistar rats.

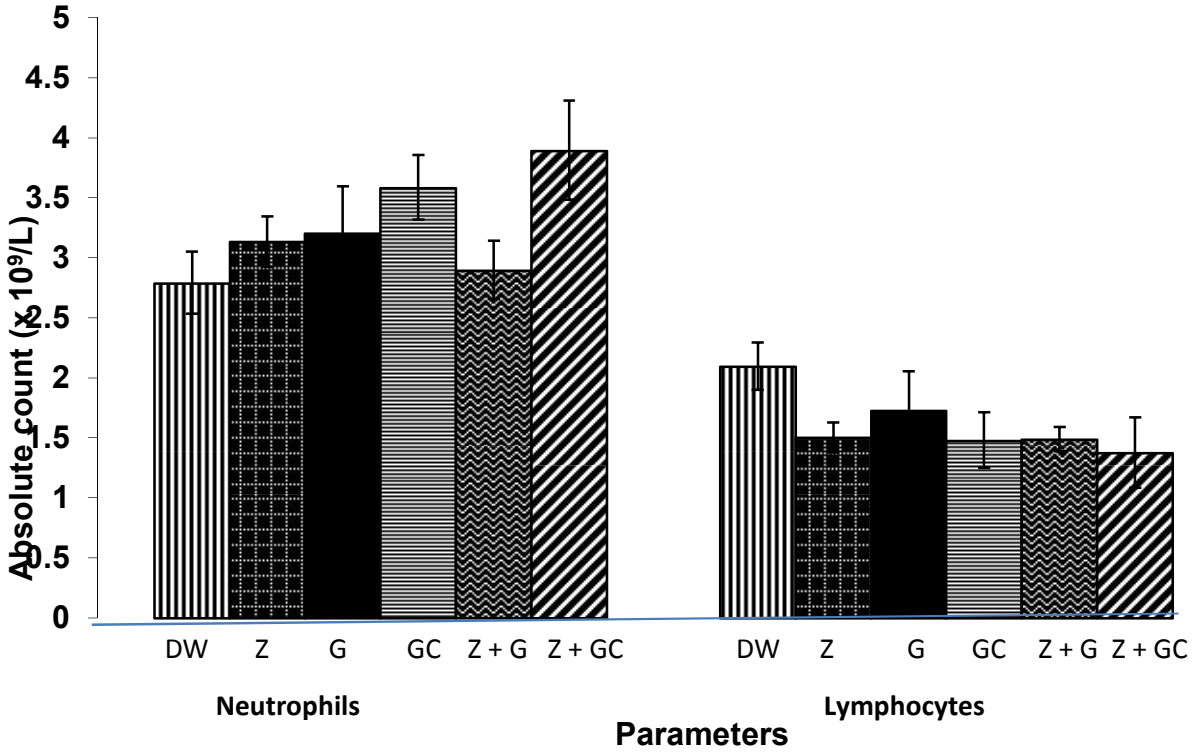


Figure 4: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on differential leucocyte counts in Wistar rats.

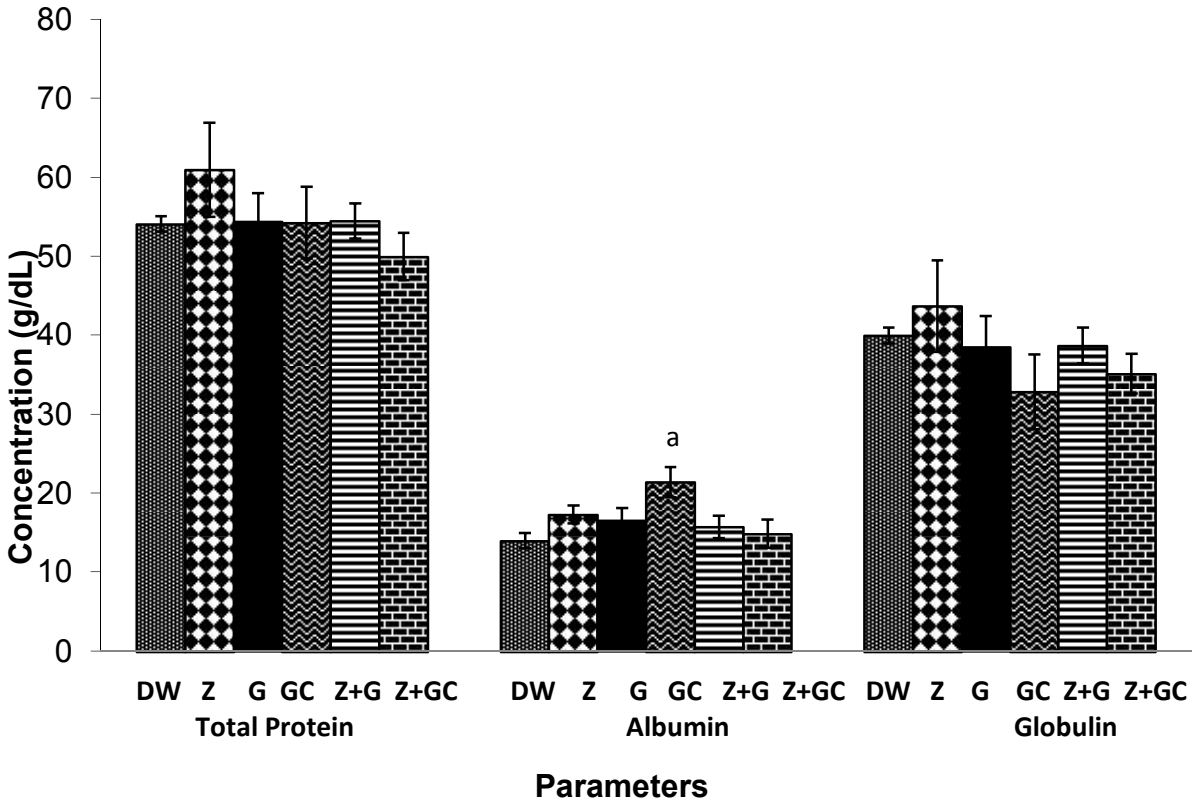


Figure 5: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in total protein, albumin and globulin concentrations in Wistar rats,a = $p < 0.05$ versus DW group.

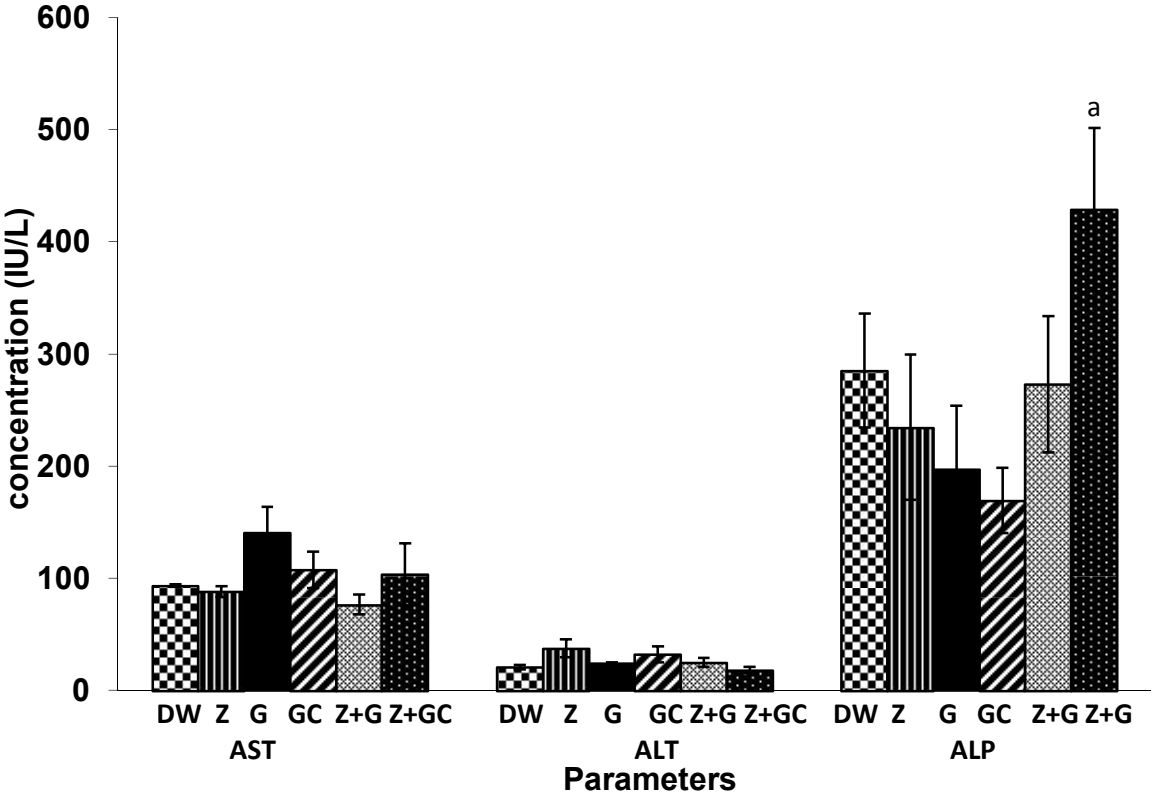


Figure 6: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) changes in serum enzymes activities in Wistar rats, a = $p < 0.05$ versus GC.

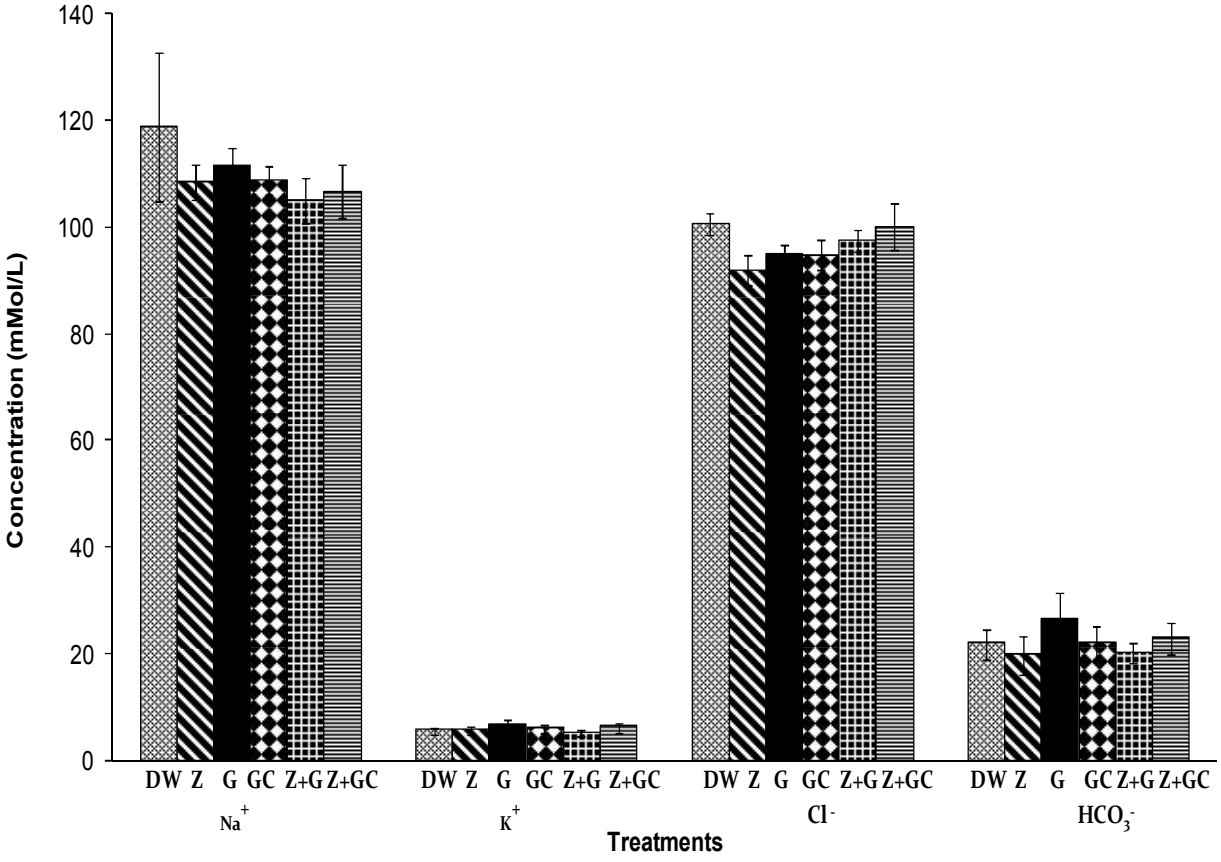


Figure 7a: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in serum electrolytes concentrations in Wistar rats.

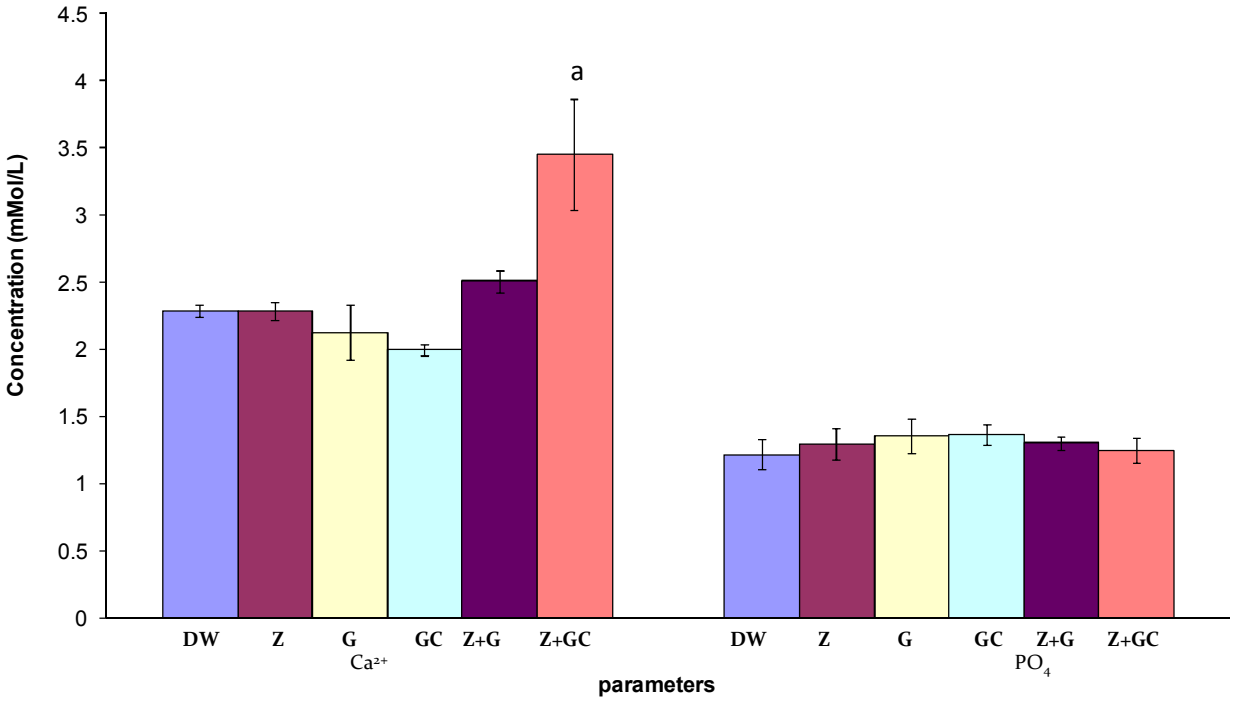


Figure 7b: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) changes in serum electrolytes in Wistar rats.a = $p < 0.01$ versus DW and Z, $p < 0.001$ versus G and GC, $p < 0.05$ versus Z + G.

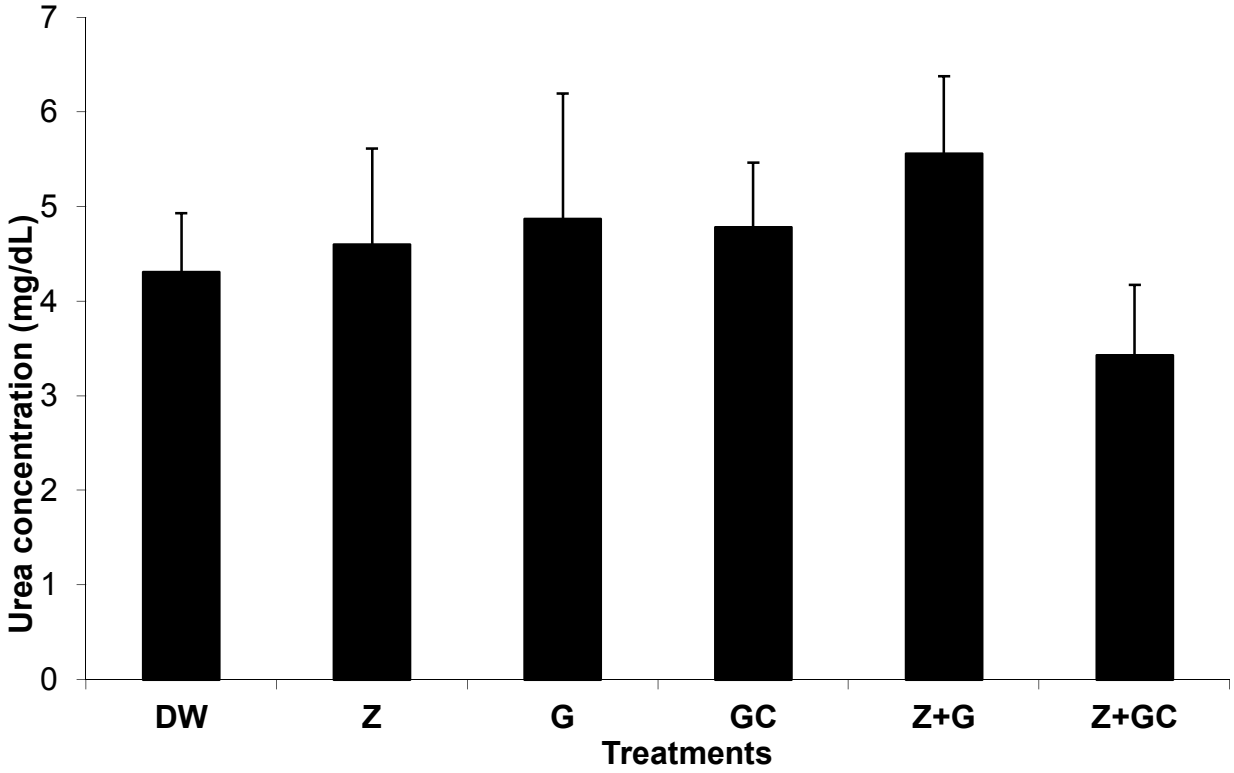


Figure 8: Effects of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on changes in serum urea concentration in Wistar rats.

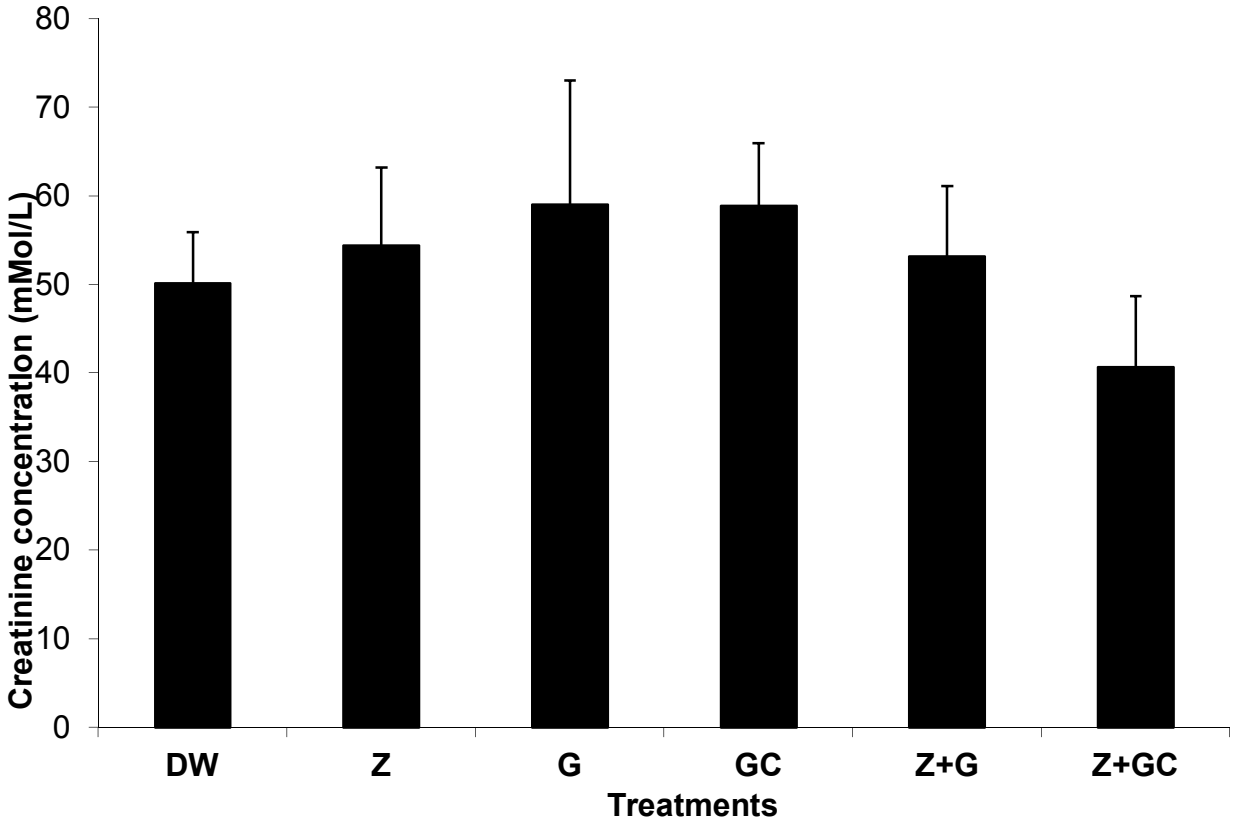


Figure 9: Effect of sub – chronic exposure to distilled water (DW), zinc (Z) and glyphosate at 10% of the LD_{50} (G) and at 1:50 glyphosate and distilled water (GC) on serum creatinine concentration in Wistar rats.

4.4 HISTOPATHOLOGICAL FINDINGS

There was neuronal degeneration in the brains of rats in group III (G) (Plate III).

There were degenerative changes in some hepatocytes especially at the portal areas of the livers of rats in group III (G) (Plate VI). There were splenic cells depopulation (lymphoblast and lymphocytes) in both red and white pulps of the spleens of rats in group III (G) (Plate X); degenerated pancreatic acinar cells in the rats in group III (G) and the pancreases of rats in group IV (Z + G) (Plates XIII and XIV), which were hyperplastic with distention of the interlobular septa by transudate.

There were glomerular degeneration, mononuclear cells infiltration into the interstices and tubular necrosis in the kidneys of rats in group III (G) (Plate XVI).

Degenerated mucosal epithelial cells that involved muscularis mucosa and the glands in the stomachs of rats in group III (G) were observed (Plate XIX).

There were no observable microscopic lesions in the gonads (testes and uteri) of all the six groups; also, there were no observable microscopic lesions in the brains, livers, spleens, pancreas, hearts, kidneys and stomachs of rats in groups I (DW), IV (Z + G) V (GC) and VI (Z + GC). The spleens of the rats in the group II (Z) showed hemosiderosis (Plates IX).The brain of rats in group II (Z) group showed vacuolation (Plate II)

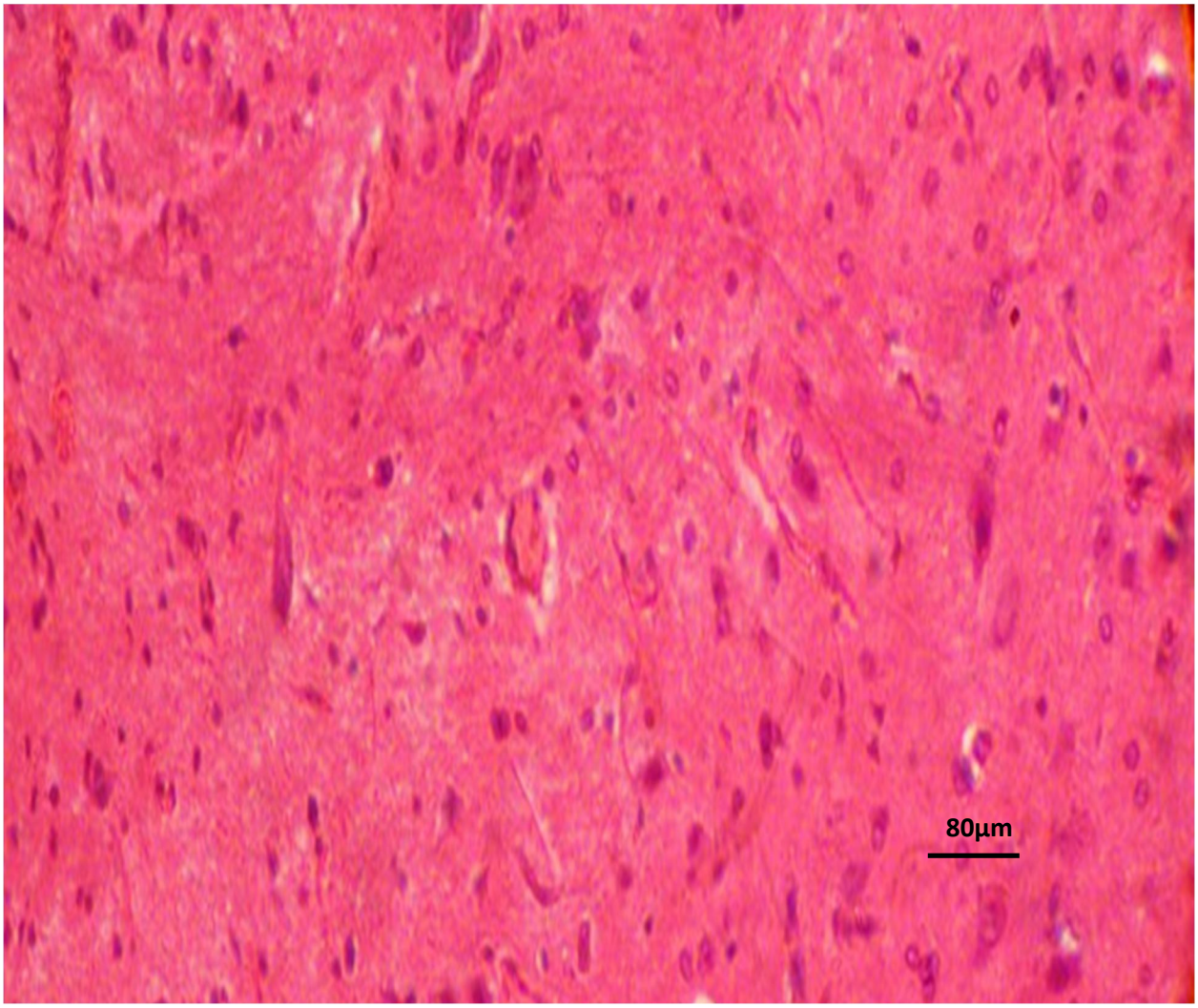


Plate I: Photomicrograph of a section of the brain of a rat exposed to distilled water only. No observable microscopic lesion (H and E Stain).

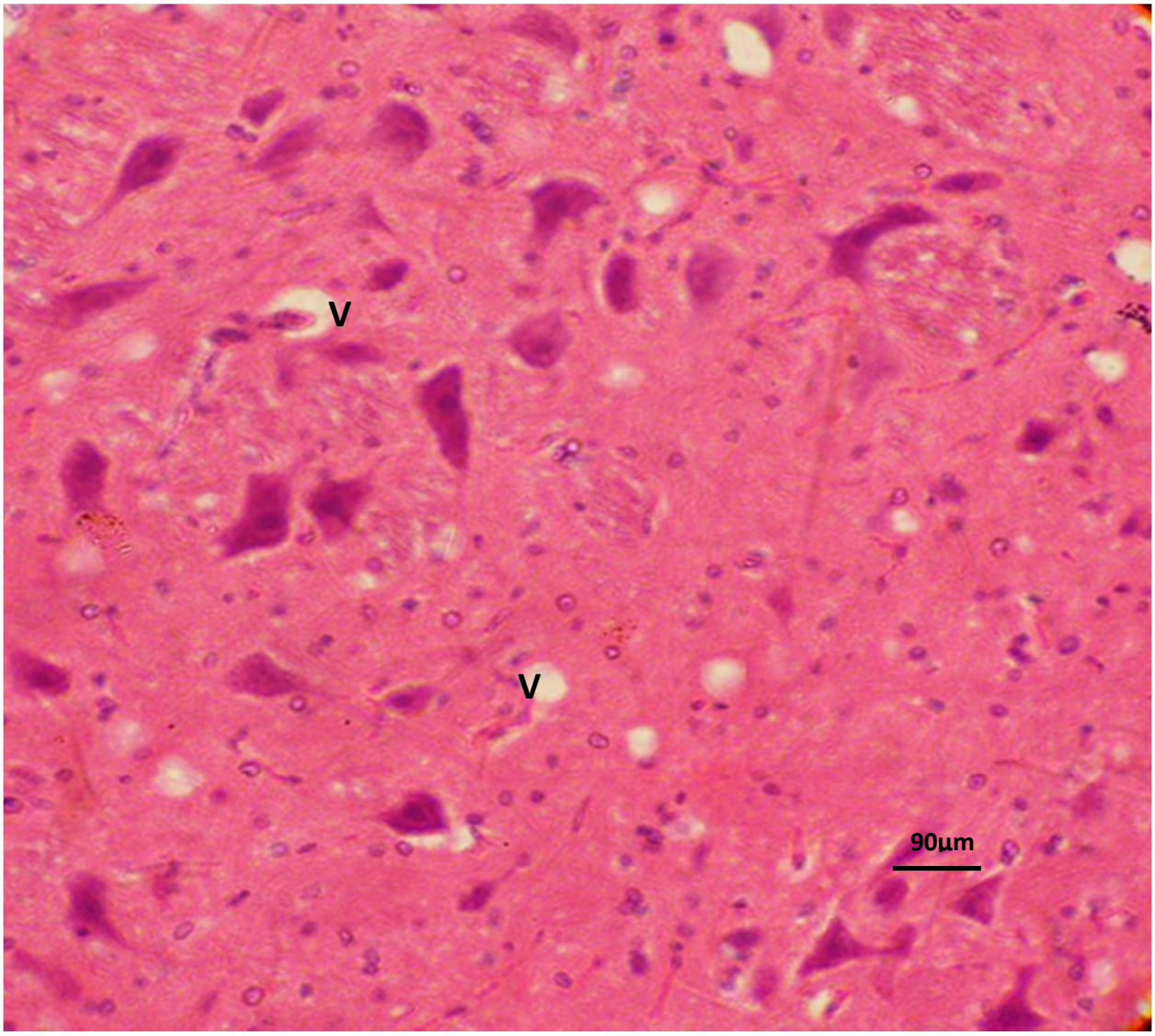


Plate II: Photomicrograph of a section of the brain of a rat exposed to zinc only at 50mg / kg. Note the vacuoles (V) (H and E Stain).

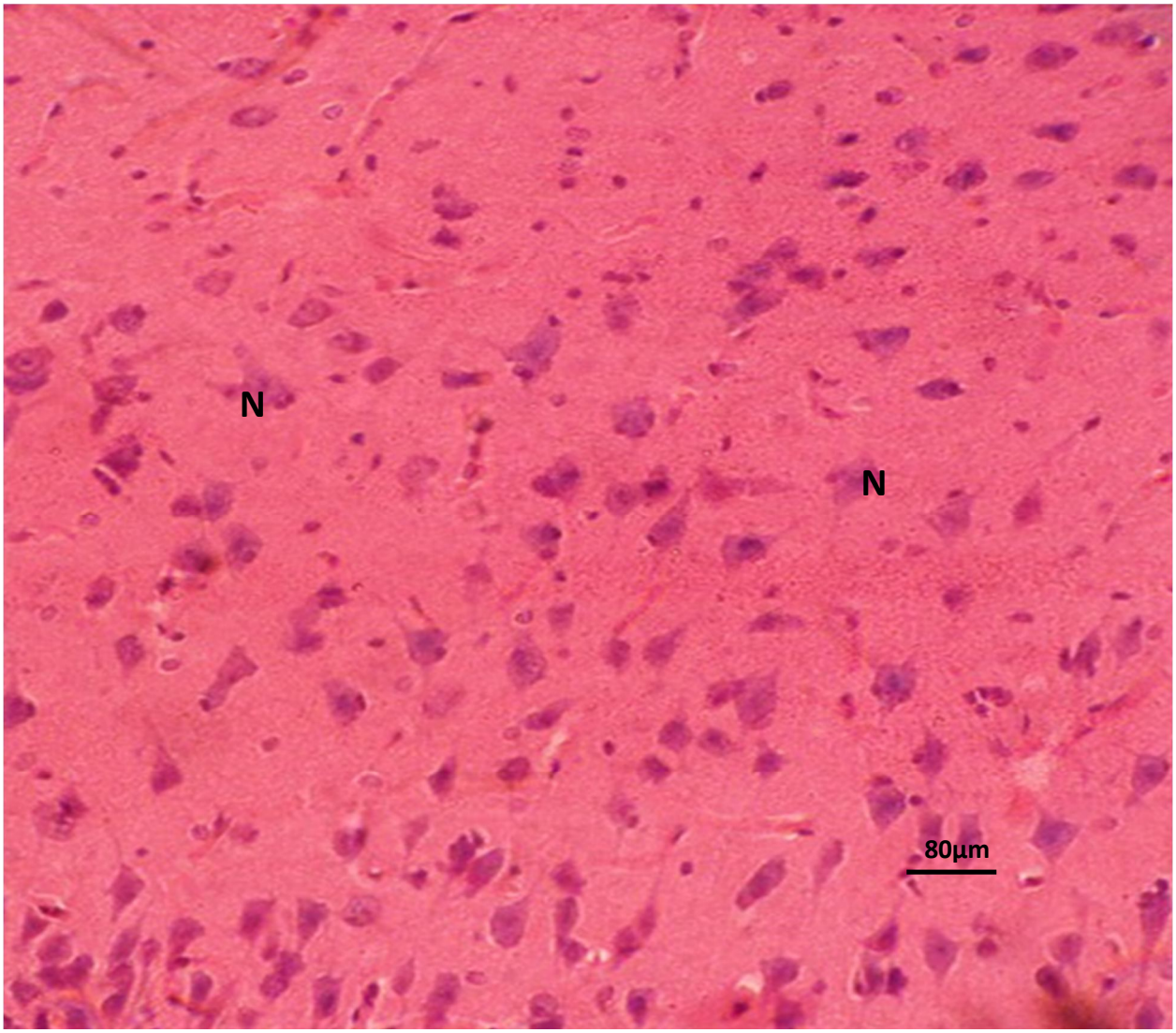


Plate III: Photomicrograph of a section of the brain of a rat exposed to glyphosate only at 375 mg / kg showing neuronal (N) degeneration (H & E Stain).

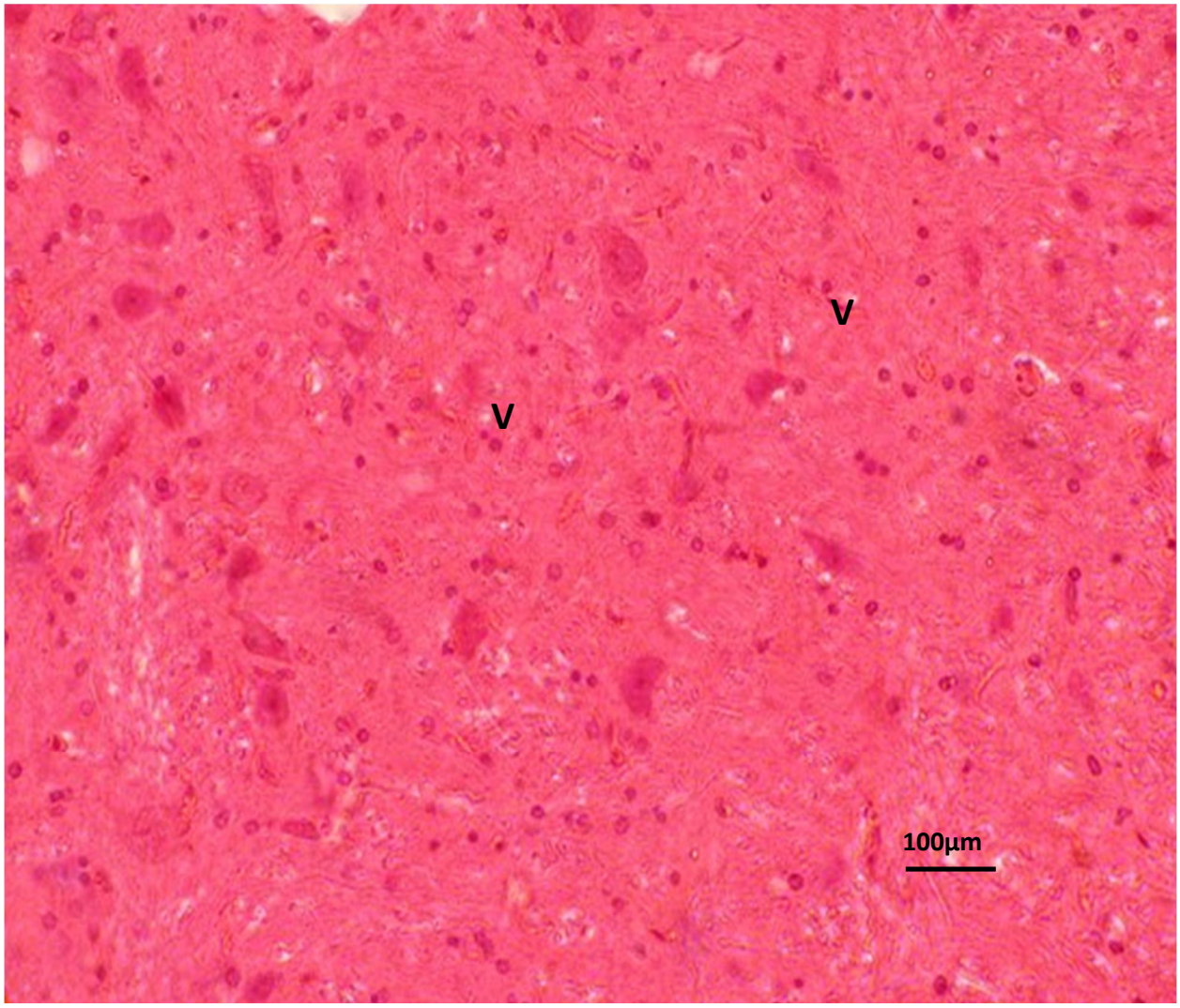


Plate IV: Photomicrograph of a section of the brain of a rat exposed to zinc and glyphosate at 375 mg / kg. Note the reduced vacuolations (V) (H and E Stain).

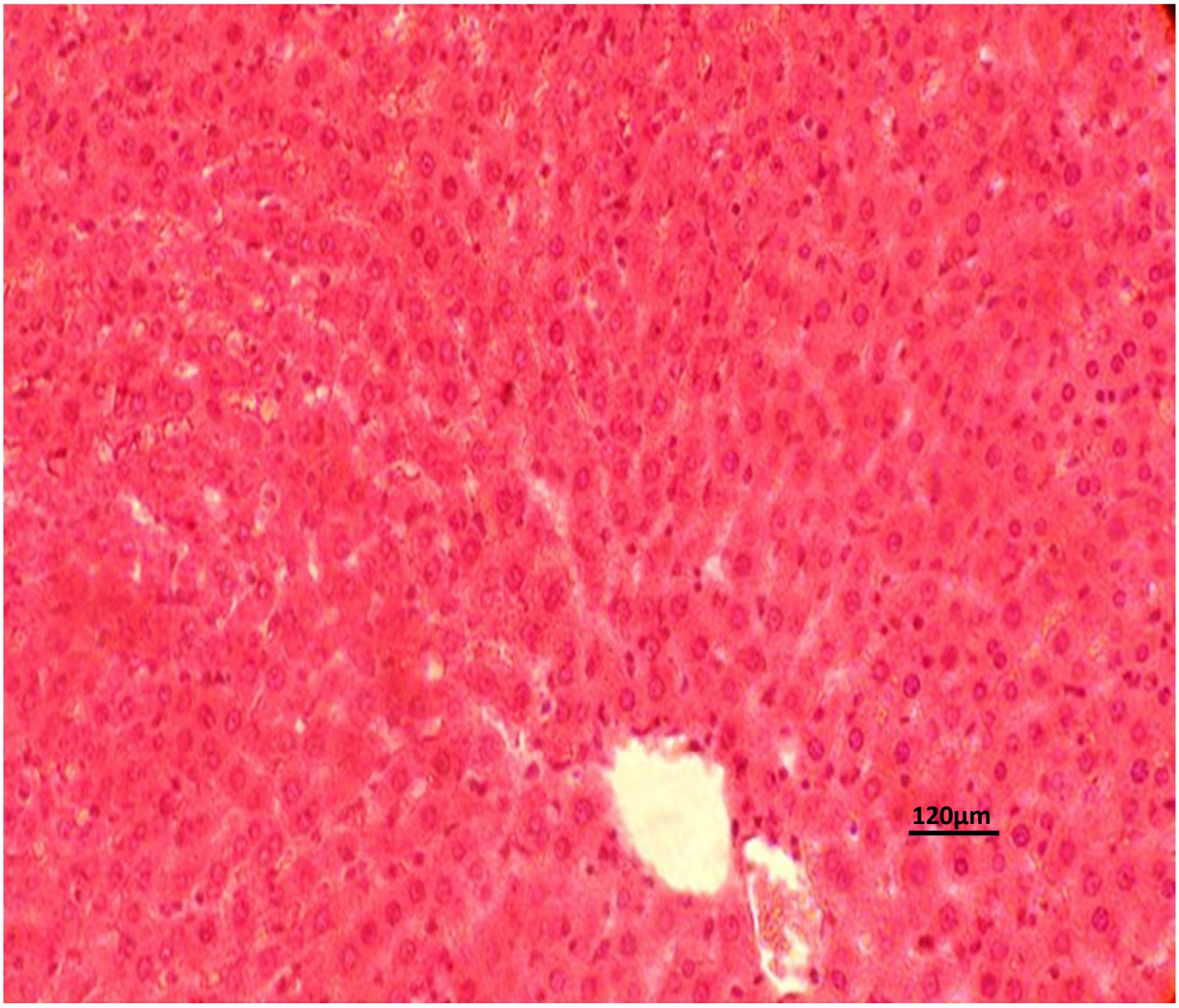


Plate V: Photomicrograph of a section of the liver of a rat exposed to distilled water only. No observable microscopic lesion (H and E Stain).

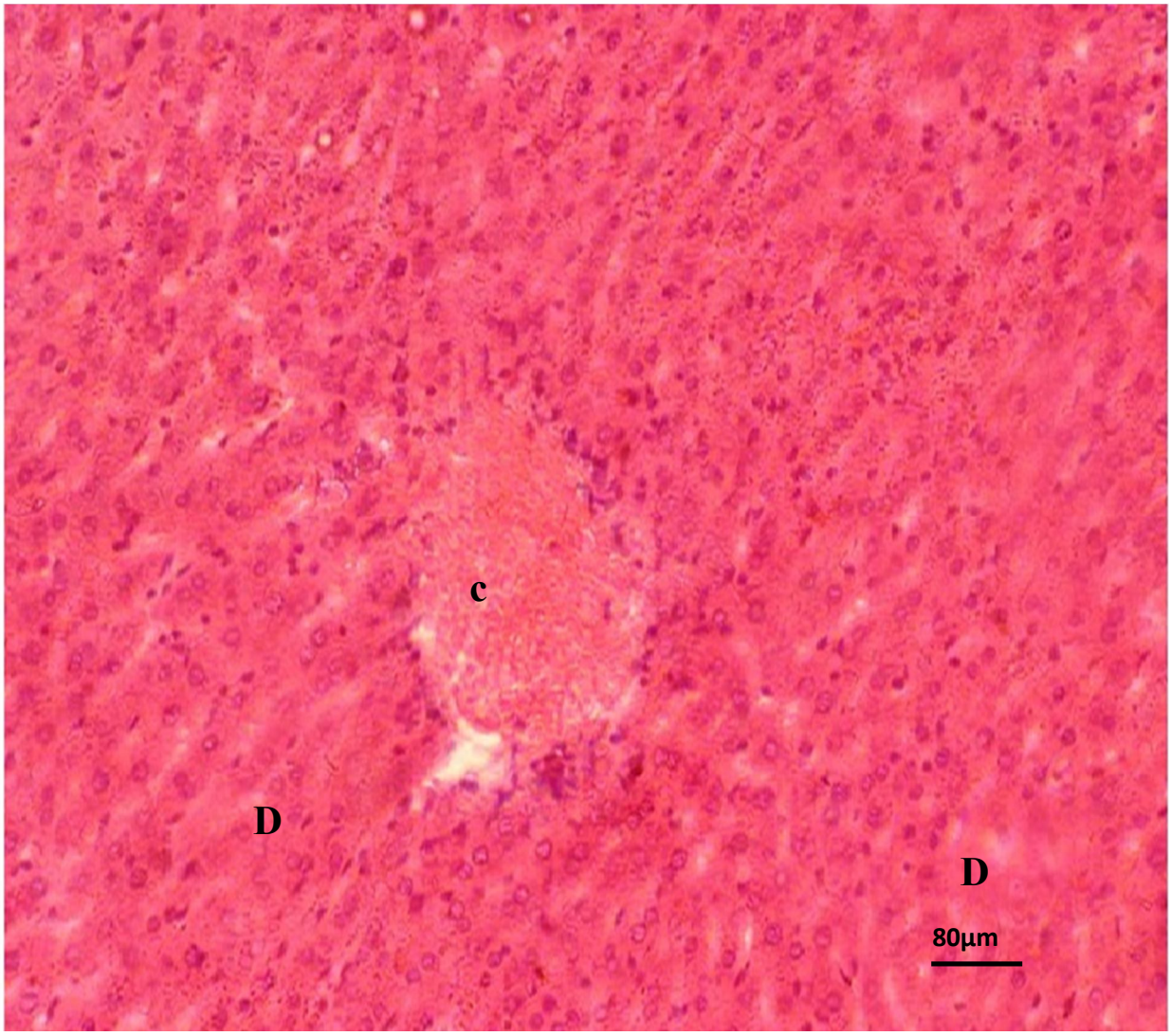


Plate VI: Photomicrograph of a section of a liver of a rat exposed to glyphosate only at 375 mg / kg. Note the degenerated hepatic cells (D) and congested central vein (C) (H and E Stain).

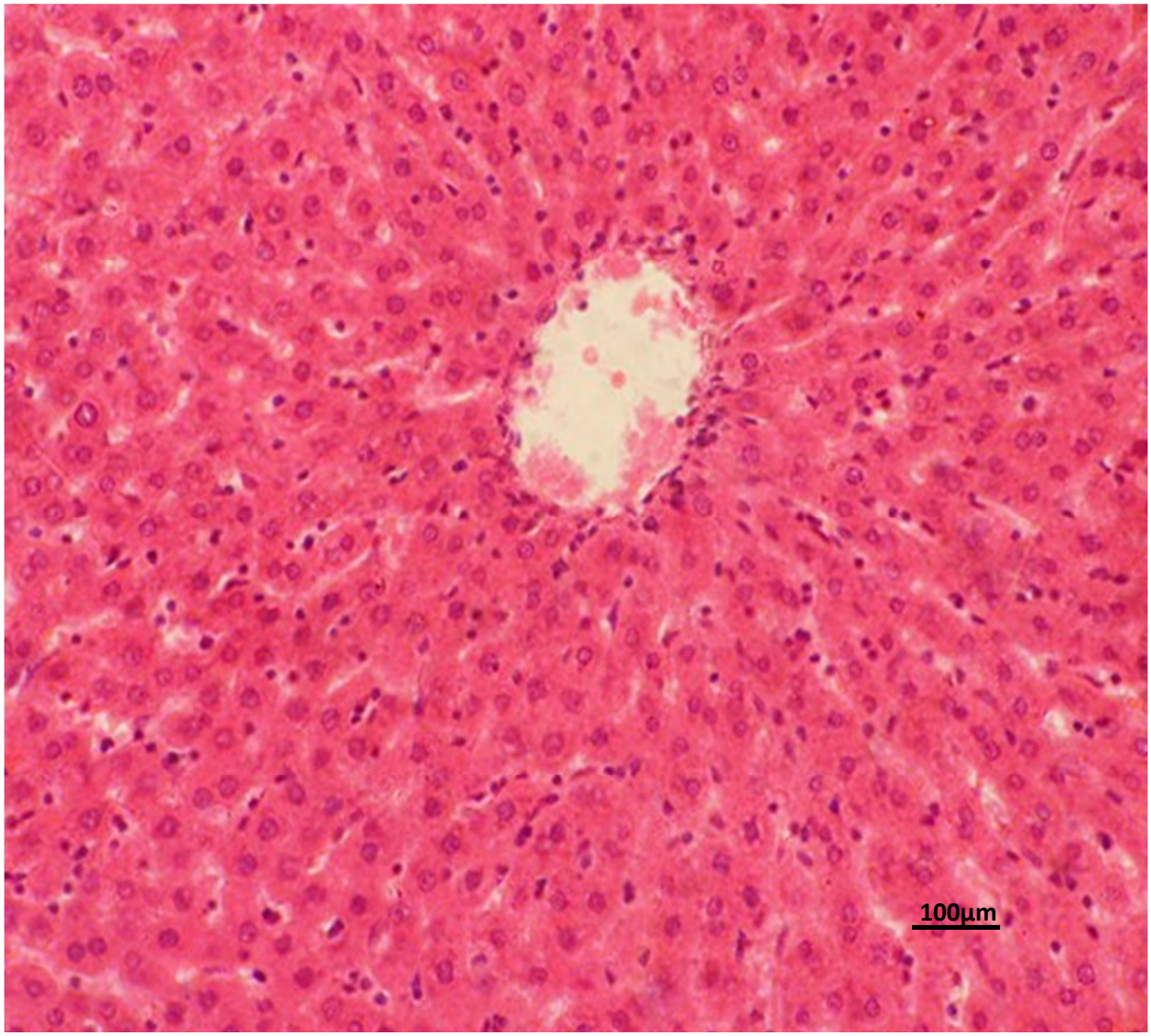


Plate VII: Photomicrograph of a section of the liver of a rat exposed to zinc and glyphosate at 375 mg / kg. Note the relatively preserved architectural morphology of the liver (H and E Stain).

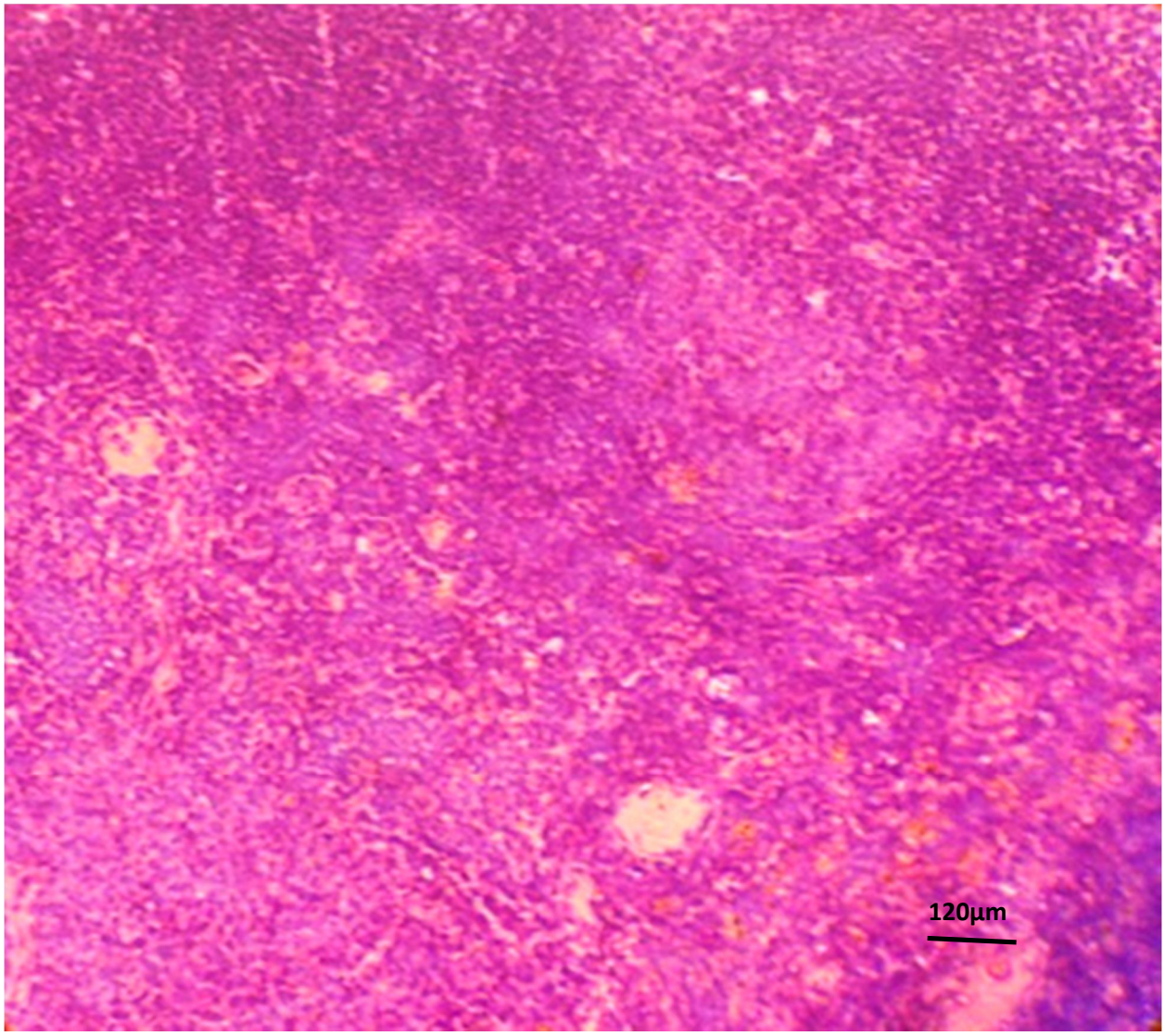


Plate VIII: Photomicrograph of a section of the spleen of a rat exposed to distilled water only. No observable microscopic lesion (H and E Stain).

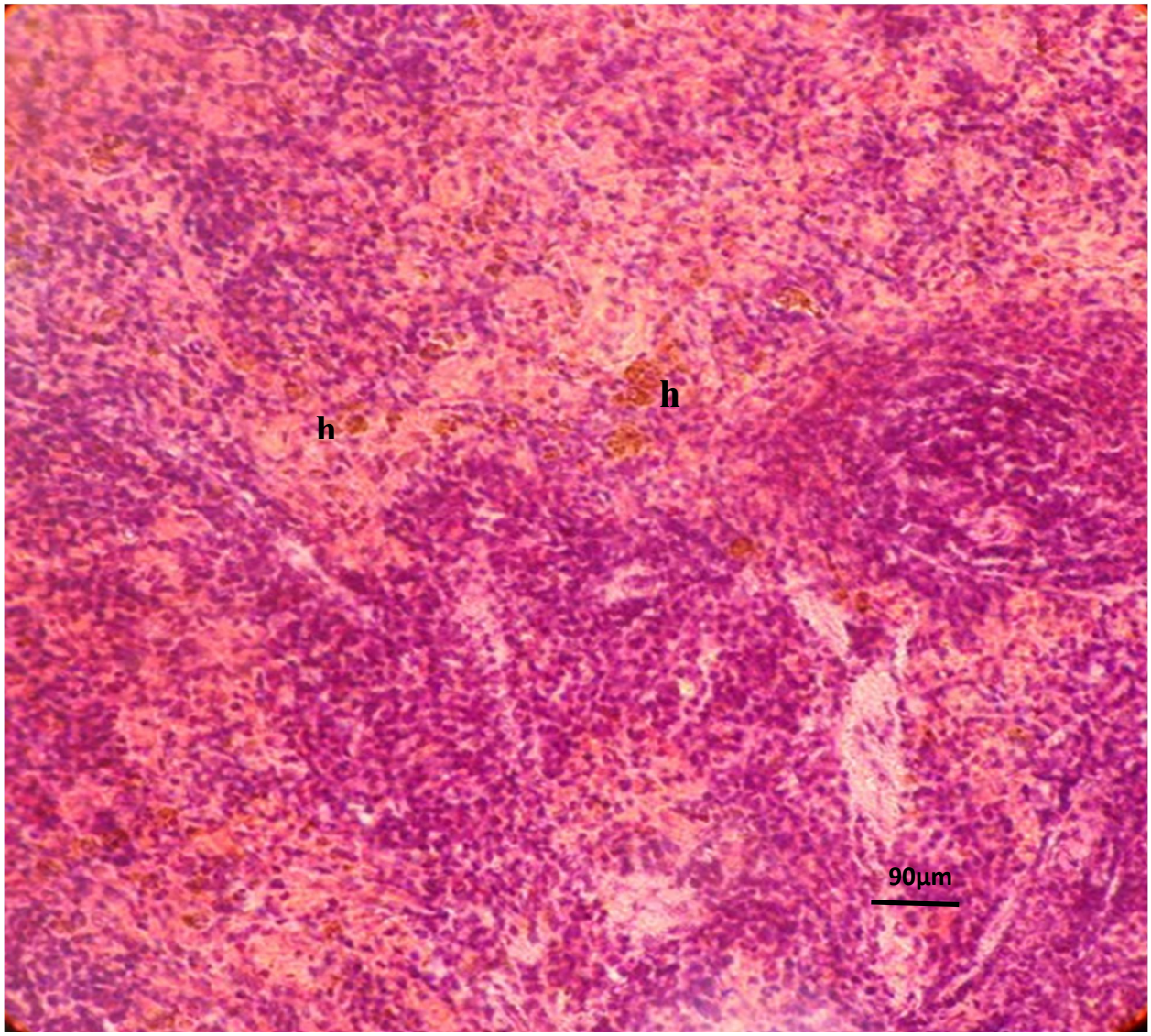


Plate IX: Photomicrograph of a section of the spleen of a rat exposed to zinc only at 50 mg / kg. Note hemosiderosis (h) (H and E Stain).

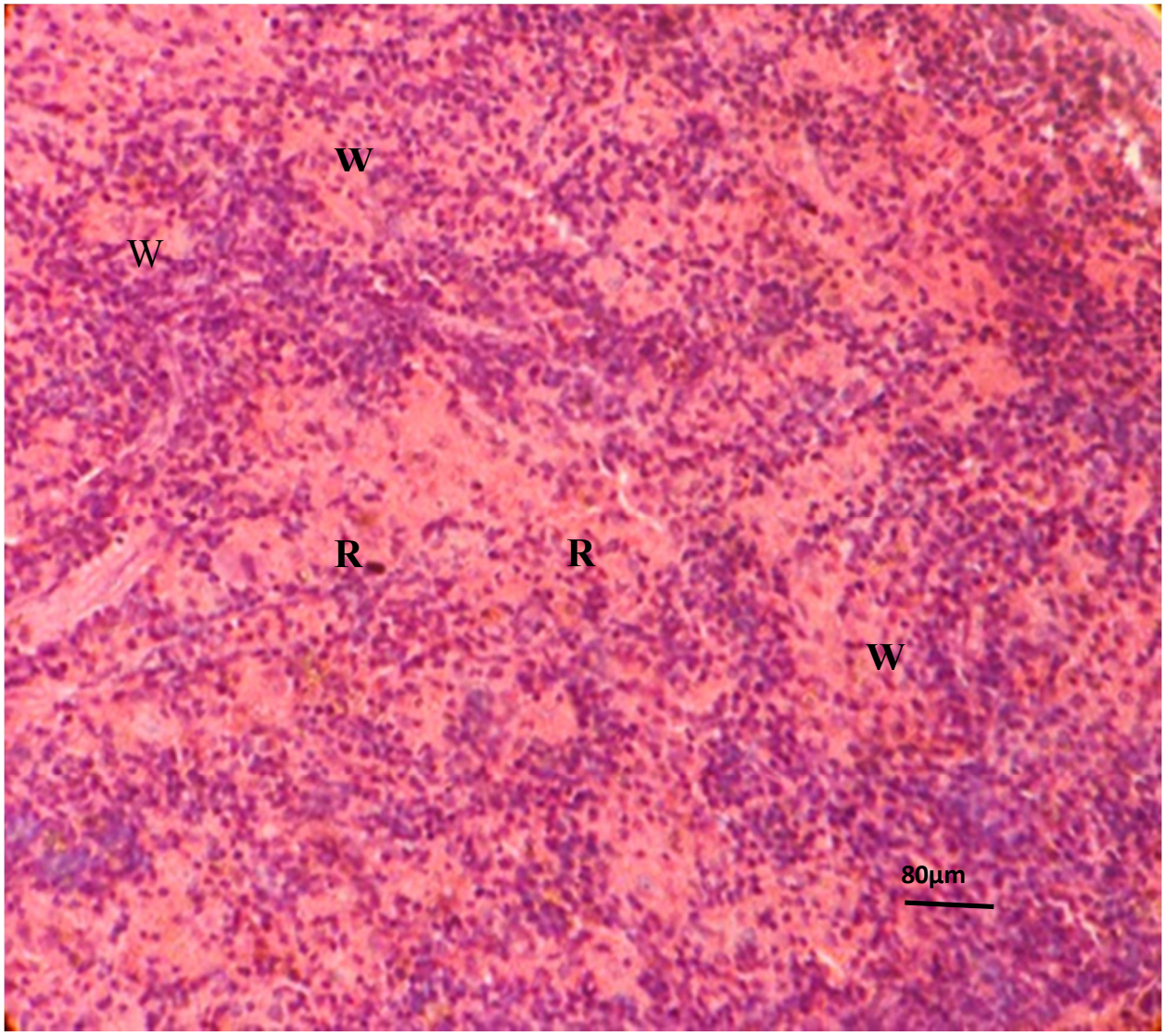


Plate X: Photomicrograph of a section of the spleen of a rat exposed to glyphosate only at 375 mg / kg. Note the depopulated spleenic cells in both the white (W) and the red (R) pulps (H & E Stain).

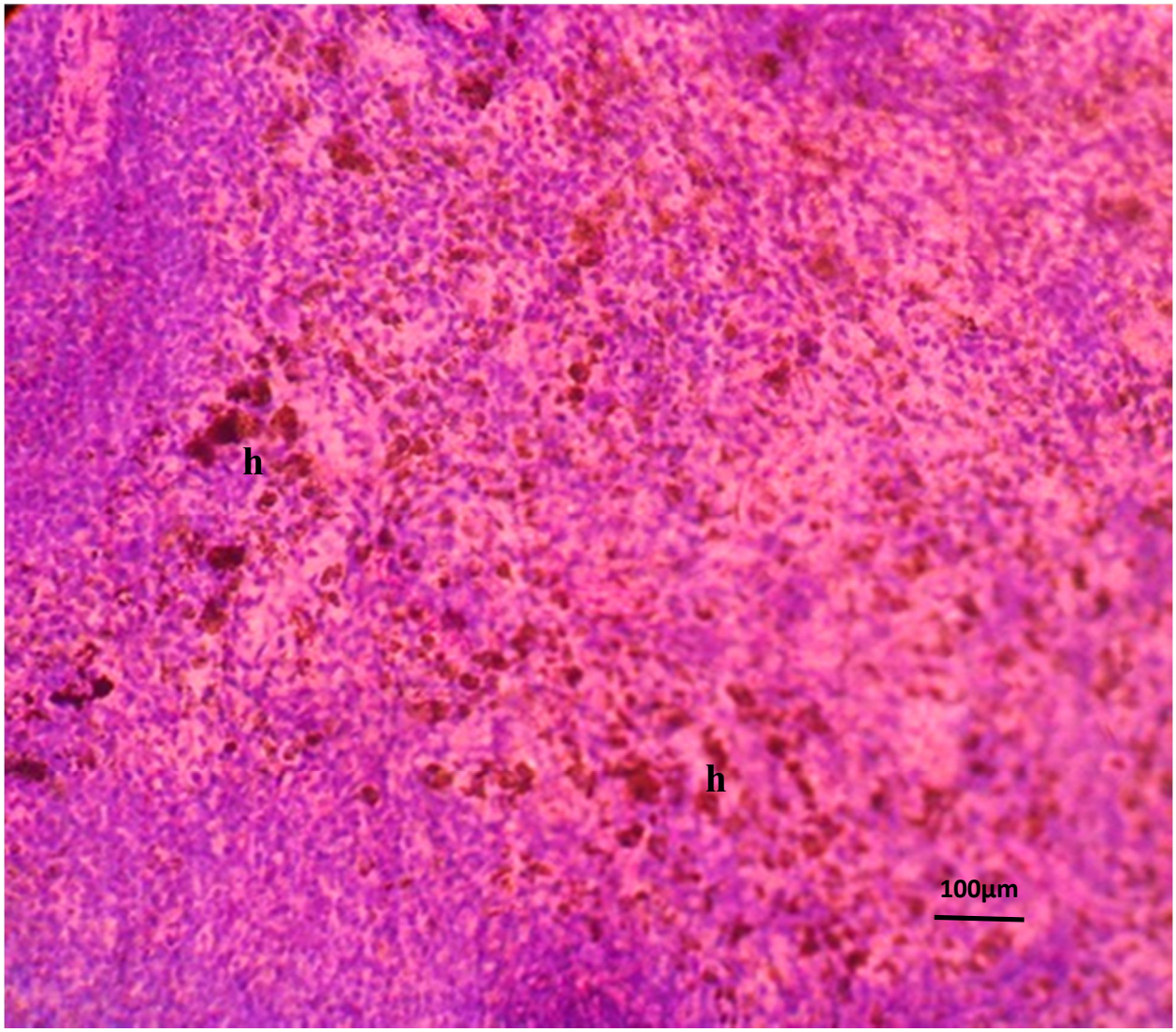


Plate XI: Photomicrograph of a section of the spleen of a rat exposed to zinc and glyphosate at 375 mg / kg. Note the hemosiderosis (h) (H&E Stain).

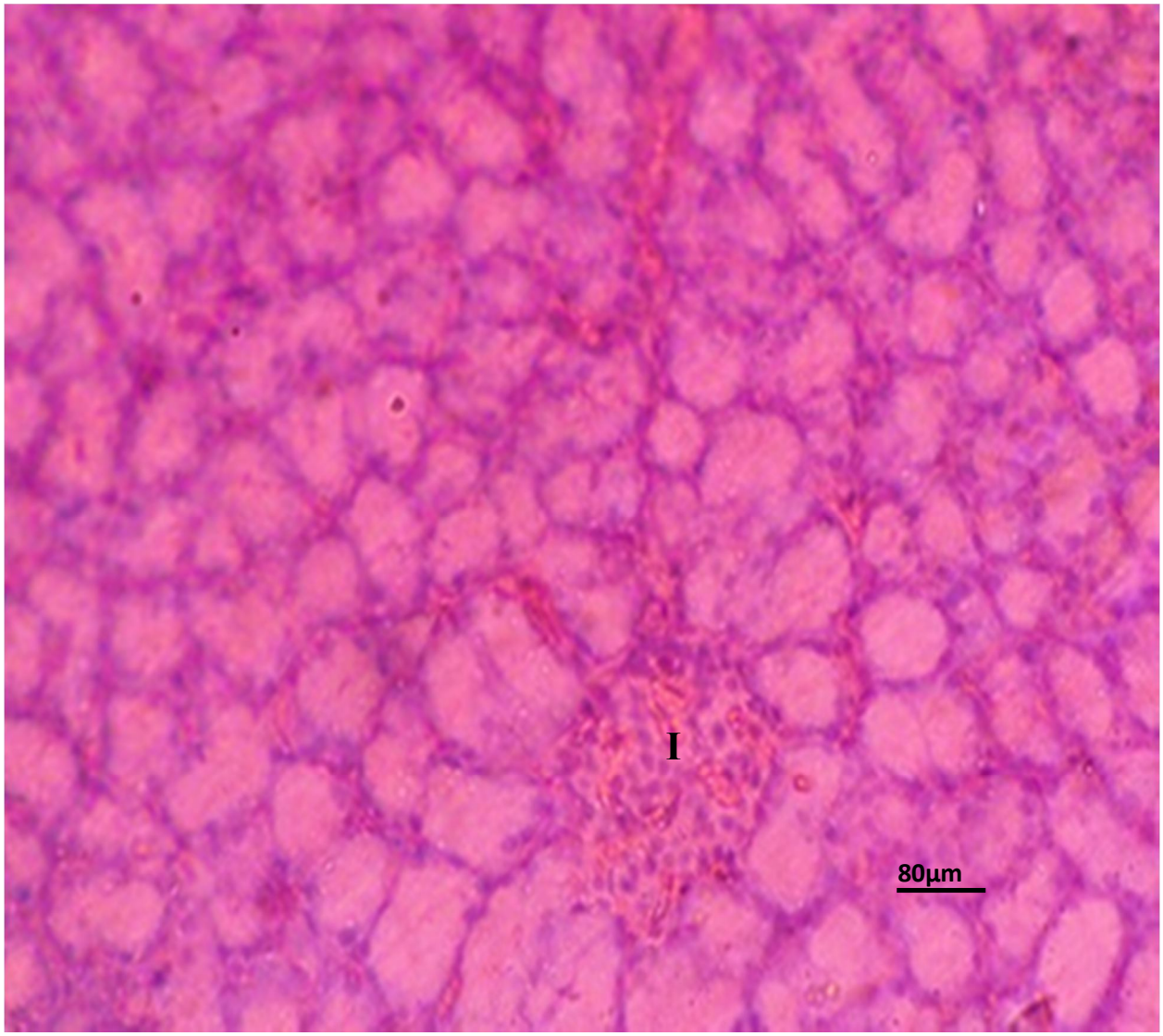


Plate XII: Photomicrograph of a section of the pancreas of a rat exposed to distilled water only. No observable microscopic lesion, note the islet of langerhan's (I) (H and E Stain).

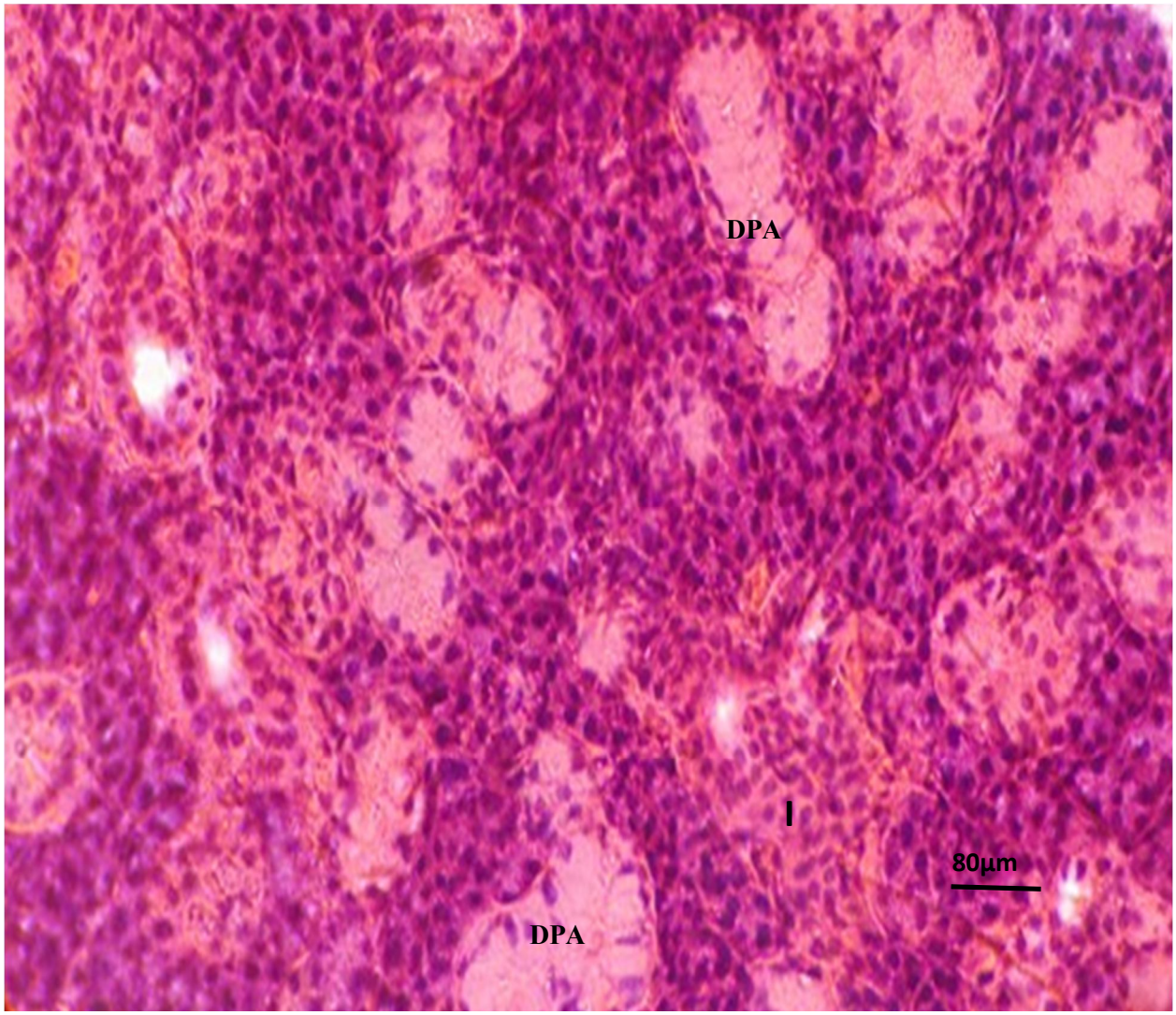


Plate XIII: Photomicrograph of a section of the pancreas of a rat exposed to glyphosate at 375 mg / kg. Note the degenerated glandular pancreatic acinar cells (DPA) and islet of langerhan's (I) (H and E Stain).

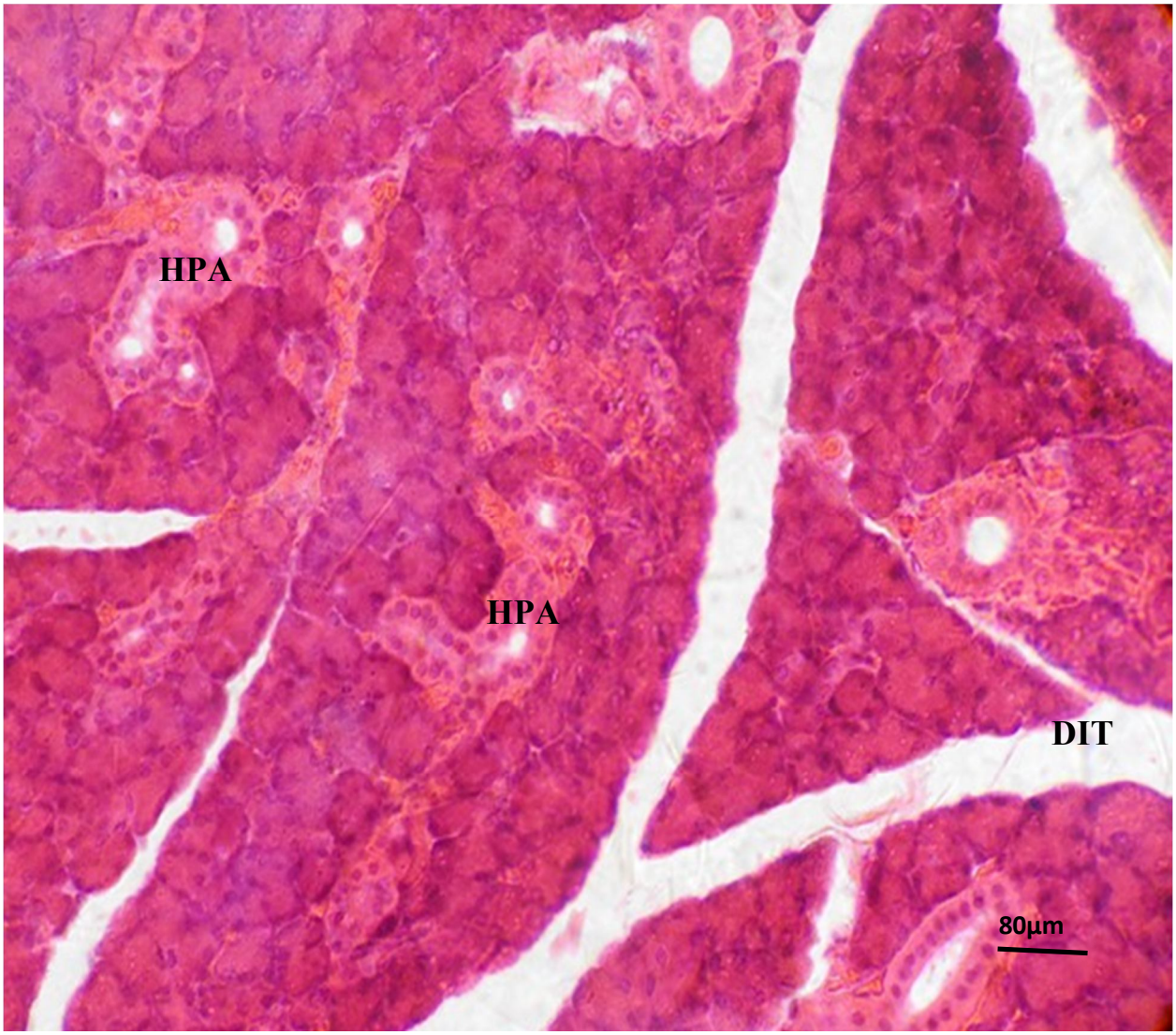


Plate XIV: Photomicrograph of a section of the pancreas of a rat exposed to zinc and glyphosate at 375 mg / kg. Note the hyperplastic glandular pancreatic acinar cells (HPA) and distension of the interlobular septa by transudate (DIT) (H & E Stain).

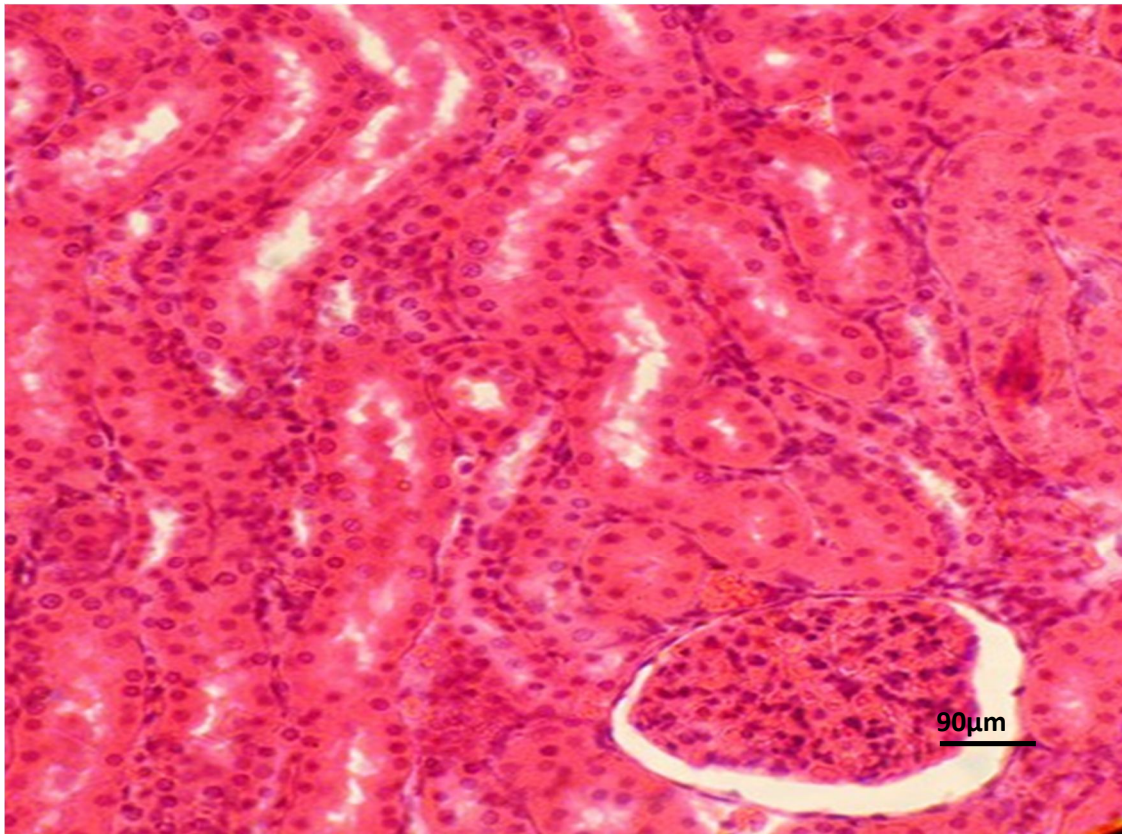


Plate XV: Photomicrograph of a section of the kidney of a rat exposed to distilled water only. No observable microscopic lesion (H and E Stain).

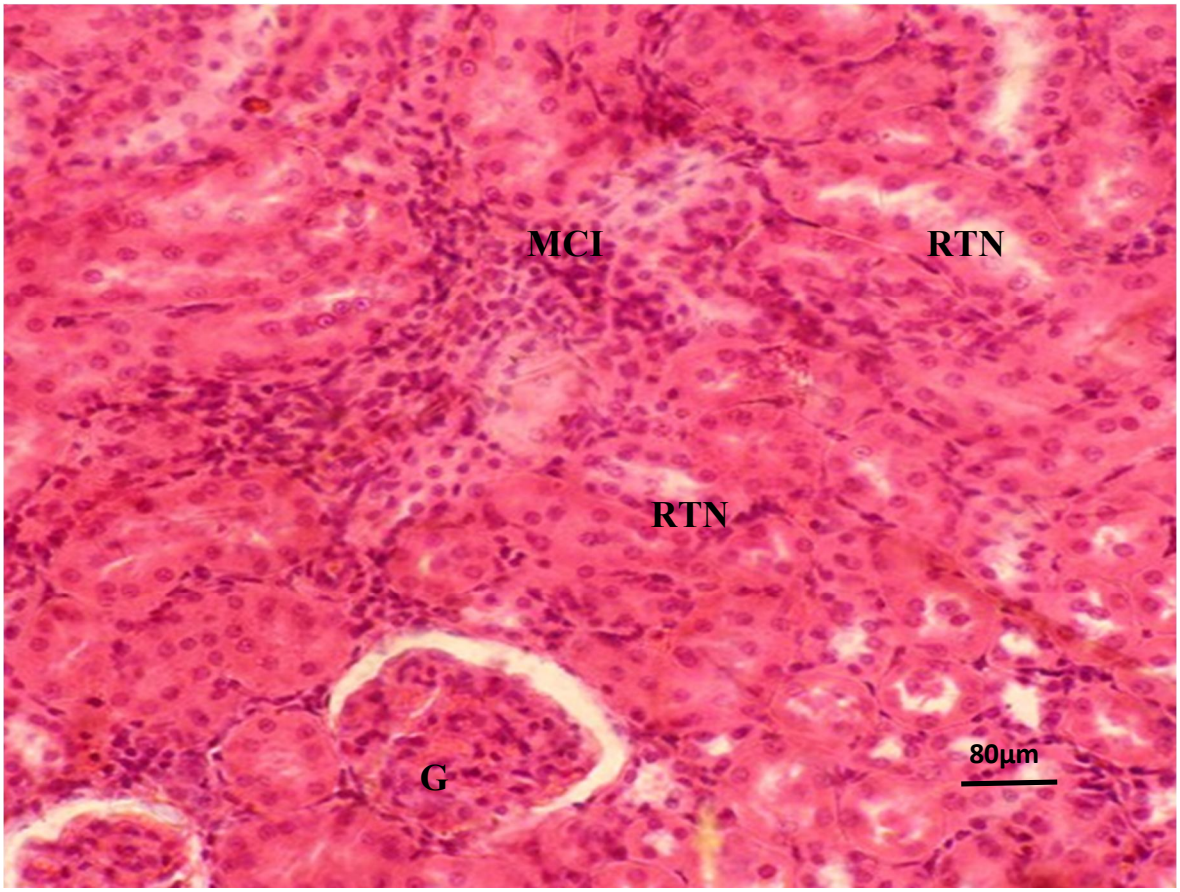


Plate XVI: Photomicrograph of a section of the kidney of a rat exposed to glyphosate at 375 mg / kg. Note the glomerular degeneration (G) and renal tubular necrosis (RTN) with mononuclear cellular infiltration into the interstices (H and E Stain).

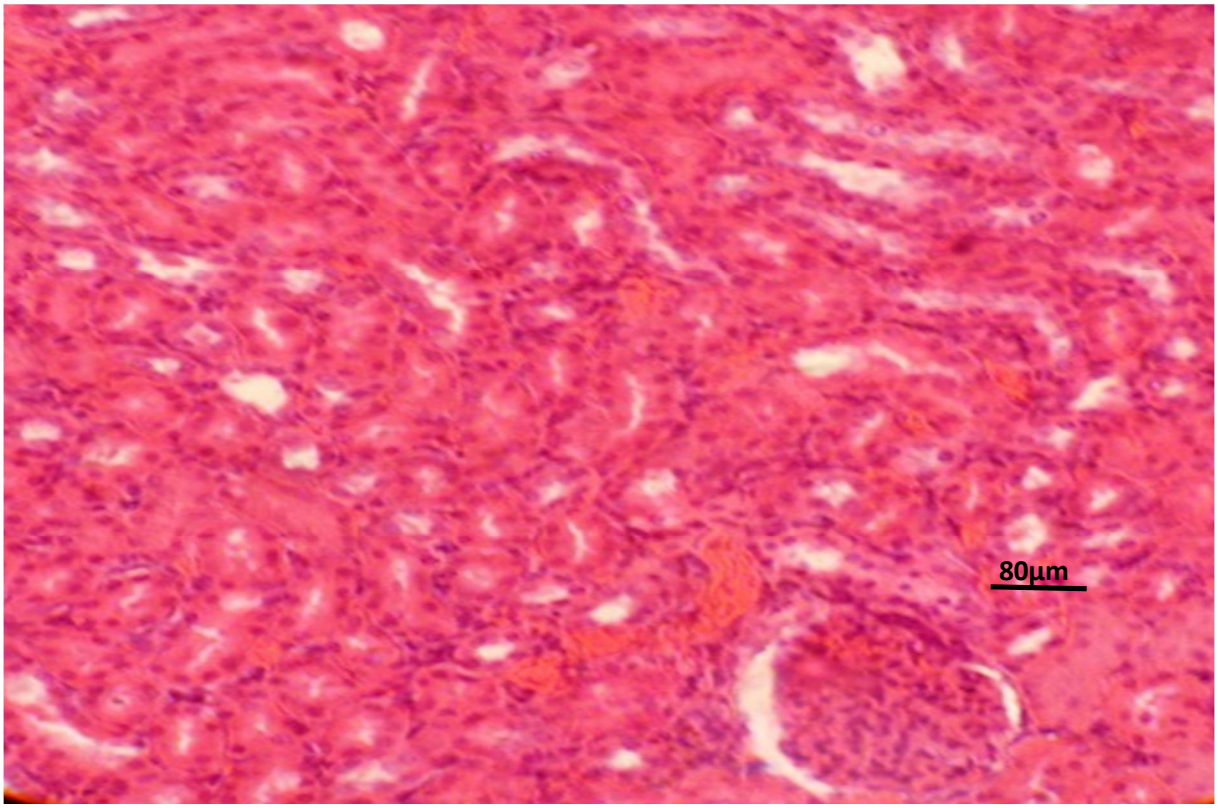


Plate XVII: Photomicrograph of a section of the kidney of a rat exposed to zinc and glyphosate at 375 mg / kg. No observable microscopic lesion (H and Stain).

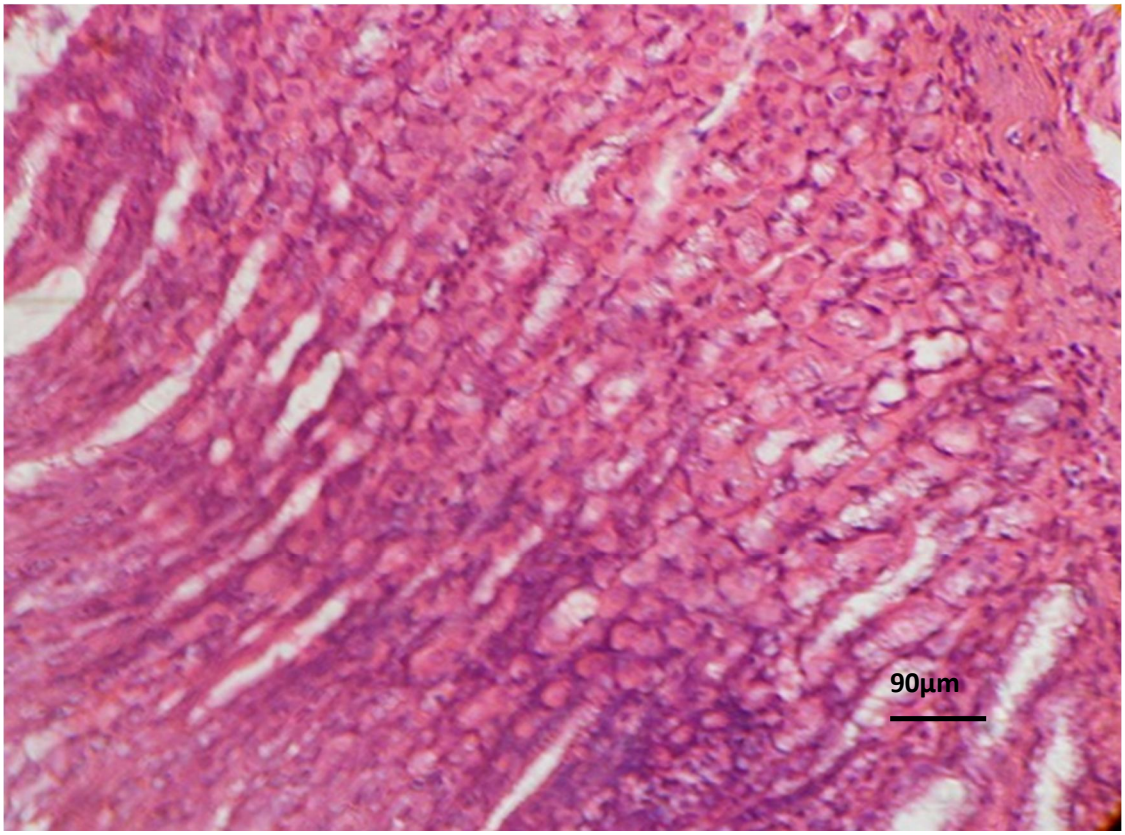


Plate XVIII: Photomicrograph of a section of the stomach of a rat exposed to distilled water only. No observable microscopic lesion (H and E Stain).

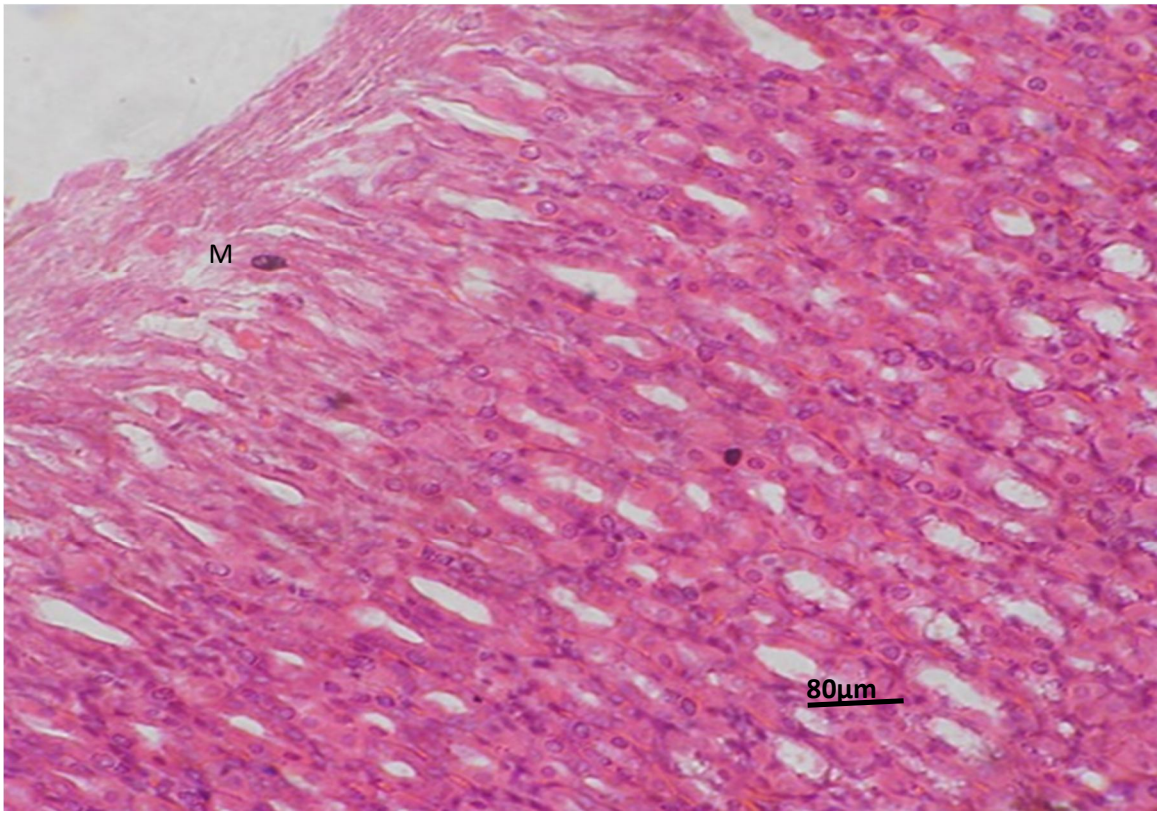


Plate XIX: Photomicrograph of a section of the stomach of a rat exposed to glyphosate only at 375 mg / kg. Note the degenerated mucosal epithelial cells (M) (H and E Stain).

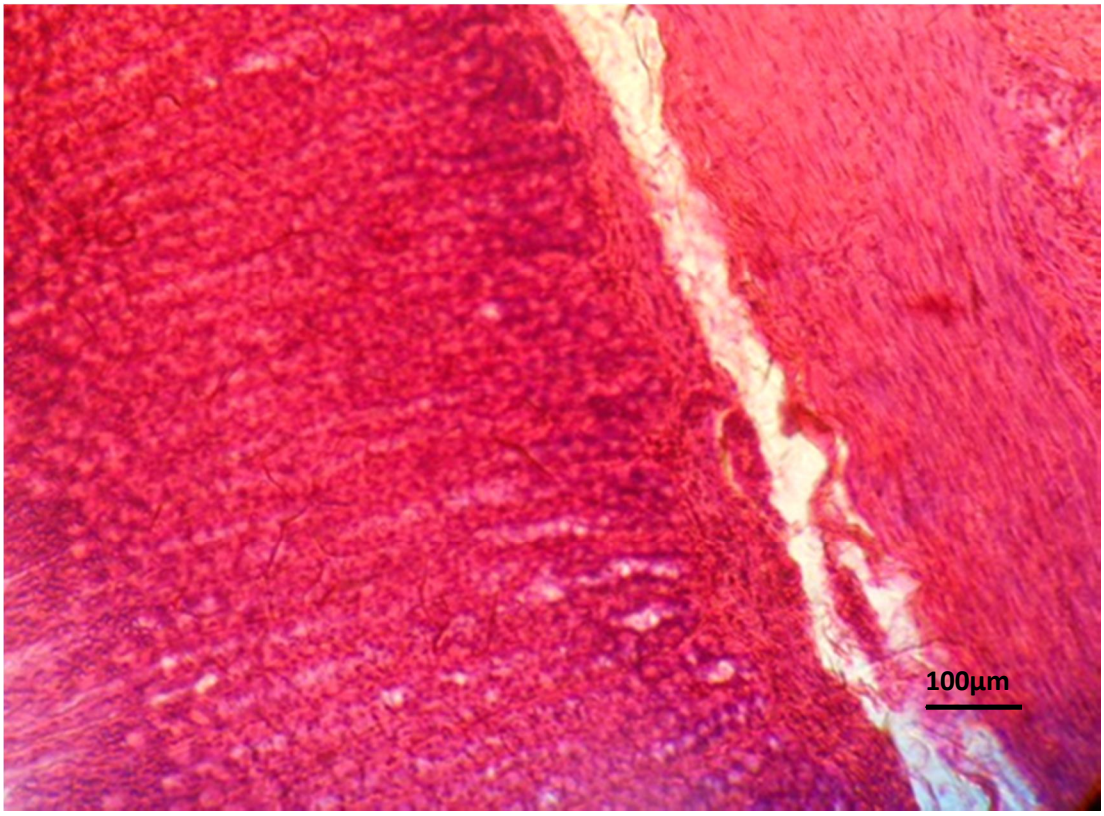


Plate XX: Photomicrograph of a section of the stomach of a rat exposed to zinc and glyphosate at 375 mg / kg. No observable microscopic lesions (H and Stain).

CHAPTER 5

DISCUSSION

The LD_{50} of 3750 mg/kg obtained in this study showed that glyphosate is in the category of chemicals with low toxicity, since chemicals with LD_{50} of 500 – 5000 mg / kg have been categorized as such (NPIC, 2010). This result is slightly different from the earlier report by Rodwell (1980) which placed glyphosate under very low toxicity category, > 5000 mg / kg. The reason for the difference is not clear but might be related to some factors relating to the chemicals, the species of the rats, the method used and the environment. The clinical signs observed during the LD_{50} evaluation included respiratory distress, diarrhoea, rough hair coat and death in one animal. Respiratory signs and diarrhoea have been previously reported following glyphosate poisoning (Olorunsogo *et al* ., 1979; Adam *et al* ., 1997).

The observed toxic signs in the sub – chronic toxicity study in the group exposed to glyphosate alone were respiratory distress, diarrhoea and death. Similarly the respiratory signs were documented by Olorunsogo *et al*.(1979) and were said to be due to uncoupling of oxidative phosphorylation. The diarrhoea observed in this study upholds the observations of Adam *et al*. (1997) who demonstrated glyphosate – induced toxic signs included diarrhoea. The fact that zinc supplemented groups did not manifest clinical signs of toxicity signifies the amelioration of the toxic signs provoked by sub – chronic glyphosate exposure by the antioxidant effect of zinc as reported by previous authors (Mc Clain *et al* ., 1992; Powell, 2000).

In the present study, the PCV and Hb concentration were lowest in the group exposed to glyphosate alone at the dose rate of 375 mg / kg (G) which indicates that sub – chronic

exposure to glyphosate could cause anemia. The anemia observed might have been due to the ability of the herbicide to cause extravascular hemolysis due to the hemosiderosis observed in the spleen in this study or it might be due to its ability to cause oxidative stress (Modesto and Martinez, 2010). The erythrocyte membrane was reported to be highly vulnerable in an oxidative stress condition because it contains high amounts of lipid, iron and is bathed in serum that has low antioxidant properties (Ambali *et al* ., 2010d). The result is similar to that of Glusczak *et al*. (2006) who observed a decrease in PCV and Hb concentration of fish, *Leporinus obtusidens,* exposed to different concentrations of Roundup® (3, 6, 10 and 20 mg l^{-1}) for 96 hours. On the other hand, the rats that were exposed to glyphosate at the dose rate of 14.4 mg / kg (GC), had a relatively high PCV and Hb concentrations when compared to the group administered with distilled water (DW). The relatively high PCV and Hb concentration in this study might be as a result of the release of immature RBC in to the circulation. The increase in PCV and Hb concentrations previously observed were said to be caused by the release of erythrocytes from blood depots and / or from haemopoetic tissues into the blood stream (Svodova *et al*., 1994). The group supplemented with zinc + glyphosate at the dose rate of 50 mg / kg and 375 mg / kg respectively (Z + G) and the group supplemented with zinc + glyphosate at the dose rate of 50 mg / kg and 14.4 mg / kg respectively (Z + GC) had relatively high PCV and Hb concentrations which might be as a result of its role in metabolism serving as a cofactor of many enzymes, coupled with its antioxidant effects (Powell, 2000; Kraus *et al* ., 1997). The relatively low PCV and Hb concentrations in the group administered zinc alone at the dose rate of 50 mg / kg (Z) was probably due to its redox status, since at higher concentration, without a corresponding oxidative challenge, zinc is capable of acting as pro – oxidant,

promoting oxidative stress by eliciting a decline in erythrocyte Cu – Zn superoxide dismutase activity as reported by Abdallah and Samman (1993).

The study revealed a slight increase in WBC count in G and GC group compared to the DW group. This result is similar to the finding of Modesto and Martinez (2010) who reported increase in the total WBC count in oxidative stress condition in fish due to roundup® toxicity. This alteration could be as a result of the activation of the immune system in the presence of contaminants, which in turn may be an adaptive response of the rat resulting in a more effective immune defence (Barreto – Medeiros *et al* ., 2005). The WBC counts in the Z + G and Z + GC groups were relatively higher than what were observed in the other groups, which indicated that supplementation with zinc before glyphosate administration in both cases caused a relatively high WBC count. The protective effect of zinc on the WBC obtained in this study is similar to the earlier study by Powell (2000) who reported that the protective effect of zinc on WBC might be attributed to its antioxidant properties either as a vital component of enzymatic antioxidant Cu – Zn superoxide dismutase (SOD) or due to its ability to antagonize the catalytic properties of redox – reaction of active transition metals namely iron and copper, with respect to production of hydroxyl from hydrogen peroxide and superoxide. Zinc also causes inhibition of both endogenous lipid peroxidation as well as induced lipid peroxidation, thereby resulting in stabilization of biomembranes (Dhawan *et al*., 1992; Srivastava *et al*., 1993). This study revealed a relatively low WBC count in the Z group when compared to the DW group which might be due to its redox status, since at higher concentration without a corresponding oxidative challenge; zinc is capable of acting as pro – oxidant, promoting oxidative stress (Abdallah and Samman, 1993).

In this study, the neutrophil count in the G and the GC groups were relatively higher than that of the DW group. This finding is similar to the normal physiological responses, higher percentage of neutrophils and lower percentage of lymphocytes differential count that were found when fishes were subjected to an array of toxicants (Witneska, 2005). However, this result contradicts the result of Modesto and Martinez (2010) who reported a decrease in the absolute neutrophils count which might be due to differences in species of the rats and the formulations of the glyphosate – based herbicide, Roundup® Transorb and Bush fire®. In the Z + G group, there was relatively low neutrophil count due to the ameliorative effect of zinc, since high neutrophil : lymphocyte ratio was reported to be indicative of stress in rats (Goel *et al* ., 2006; Ambali *et al* ., 2010b). The reason for the relatively high neutrophils count in the Z + GC group is not known, but it might be due to higher neutrophils count in the GC group and might therefore require higher dosage of zinc. The relatively high neutrophils count in the Z group might be due to the pro – oxidant action of zinc on the haemopoietic organs in the rats (Abdallah and Samman, 1993).

The rats in the G and the GC groups in this study showed a relatively higher lymphocyte count when compared to the rats in Z + G and Z + GC groups respectively. Similar to previous reports in studies on oxidative stress (Goel *et al* ., 2006; Ambali *et al* ., 2010a) in which lymphocytic leucopaenia which might be due to either decreased production and / or increased rate of removal due to rapid destruction of lyphocytes. The ameliorative effect of zinc following supplementation with zinc in the Z + G and Z + GC groups are similar to the protective effect of zinc documented by Powell (2000) who reported the protective effect of zinc on WBC count which was attributed to the antioxidant properties either as a vital component of enzymatic antioxidant Cu – Zn (SOD) or due to their ability to antagonize

the catalytic properties of redox – reaction of active transition metals namely iron and copper, with respect to the promotion of hydroxyl radicals formation from hydrogen peroxide and superoxide.

The result of this study showed no significant difference ($P > 0.05$) in total protein concentration in the G and the GC groups compared to the DW group. This is similar to the result obtained by El – Shenawy (2009) who reported that Roundup® and glyphosate did not significantly affect total protein concentration after two weeks of exposure in rats. There were relatively higher total protein concentrations in the Z and the Z + G groups, possibly due to the antioxidant properties of zinc. The total protein concentration in the Z + GC showed a relatively low total protein concentration compared to the DW group. The reason for the relatively low total protein in the Z + GC group is not known especially in the light of the relative increase in the WBC count in the group.

There was a significantly high albumin concentration in the GC group compared to the DW group and a relatively high albumin concentration in the G group compared to the DW group. It was found that roundup® and glyphosate caused a relatively high albumin concentration in rats (El – Shenawy, 2009). The reason for the high albumin level in the blood as observed in the glyphosate (G and GC) treated groups relative to the DW group was not known. However, Vosilenko and Grebenev, (1990) reported that decrease in the serum albumin might be indicative of renal inability that resulted in albuminuria. The relatively high level of albumin in the blood as observed in the present study could be attributed to the observed renal damage. There was a relatively low albumin concentration in Z, Z + G and Z + GC groups. This might be as a result of the ability of zinc to act as an antioxidant (Powell, 2000).

There were relatively low globulin concentrations in the G and the GC groups compared to the DW group. A relatively high globulin concentrations in Roundup® and glyphosate were reported by El – Shenawy (2009). The difference might be due to variation in duration of the experiment, the doses and / or the formulations of the glyphosate, Roundup® and Bush fire® used in this study. On the other hand, zinc alone (Z) and supplementation with zinc was shown to make globulin concentrations relatively high in both the Z + G and the Z + GC groups, indicating the antioxidant effect of zinc to regulate metallothionein, a highly conserved low molecular weight cystein rich metal chelating antioxidant protein, which was demonstrated to share a lot of features with glutathione (Quesada *et al*., 1996; Zhou *et al*., 2007).

The result of this study has shown no significant difference in aspartate aminotransferase (AST) in between the groups; however, there was relatively high AST activity in the G and the GC groups compared to the DW group. The reason for the apparently high level of serum AST might be associated with some level of damage to any or all of the organs producing this enzyme such as liver, cardiac and skeletal muscles (Ambali *et al* ., 2010b). This result agreed with the finding of Benedetti *et al.* (2004). Indeed, it has been reported that sub – chronic exposure to glyphosate caused damage to the liver Benedetti *et al*. (2004). On the other hand, the increase in the serum concentration of AST due to exposure to glyphosate was attenuated due to supplementation with zinc in the Z + G and the Z + GC groups, thus indicating the antioxidant activity of zinc which brought about restoration of the AST activity in rats. The relatively low AST activity in the Z group compared to the DW group might be due to slight pro – oxidant effect of zinc since the rats were not under oxidative stress.

There were no significant changes in the activity of alanine aminotransferase (ALT) in between the groups. However, there was a relatively high ALT activity in the G and the GC groups compared to the DW group. This elevation in the activity of ALT following sub – chronic glyphosate exposure in this study agreed with the result of previous studies (Caglar and Kolankaya, 2008; El – Shenawy, 2009) who reported increased level of ALT in rats which was said to be part of the diagnostic symptoms of liver diseases, and also supported the hypothesis which stated that the increased serum transaminases (ALT and AST) might reflect hepatotoxicity which leads to extensive liberation of enzymes in the blood (Daabees *et al* , 1992). The reason for the apparently high serum ALT activity in the Z group is not known for certain, but it might be due to its pro – oxidant effect since the rats were not under oxidative stress. Supplementation with zinc in Z + GC group attenuated the hepatotoxic effect of the glyphosate on the serum activity of ALT. There was, however, a relatively high ALT activity in Z + G group and the reason for such is not known for certain especially in the light of the attenuated effect observed in Z + GC group and might therefore, require further studies.

The result of this study showed a relatively low serum activity of alkaline phosphatase (ALP) in the G and the GC groups compared to the DW groups. This is at variance with the previous finding (El – Shenawy, 2009) who reported a relatively high ALP activity in rats exposed to Roundup® and glyphosate for two weeks, suggestive of damage to hepatocytes, bone, intestine and kidney which are the sources of this enzyme as reported by Maxam and Gilbert (1980). The reason for the relatively low ALP activity in the glyphosate exposed groups in this study is not known especially in the light of histopathologic lesions observed in the livers of the rats in the G group and this might therefore, suggest that the agent did

not affect the other organs that produce the enzyme since it is not a liver specific enzyme. On the other hand, supplementation with zinc restored the ALP activity to near normal in both the Z and the Z + G groups, thus reflecting the antioxidant effect of zinc. The reason for the significantly high, $p < 0.05$, serum ALP activity in Z + GC compared to GC group is not known especially with the non – observable microscopic lesion in the organs of the rats in the group.

There were no significant changes in the serum sodium ion (Na^+) concentration in between the groups. Oxidative stress was shown not to cause significant alteration of Na^+ in earlier studies (Ambali *et al*., 2007; Ambali, 2009; Ambali *et al.,* 2010a). However, there was a slightly low Na^+ concentration in the G and the GC groups compared to the DW group. These changes might be associated with functional alteration in the proximal tubules due to its nephrotoxic effect. On the other hand, there was a relatively higher decrease in serum Na^+ concentration in zinc treated groups Z, Z + G and Z + GC, probably because the zinc used was zinc chloride.

The result of this study has shown a relatively high serum Potassium (K^+) concentration in the G and the GC groups compared to the DW group. Oxidative stress due to chlorpyrifos (CPF) exposure was shown to cause relatively high serum K^+ concentration (Ambali *et al* ., 2010a). The serum K^+ concentration in zinc (Z) treated group, the Z and the zinc supplemented groups, the Z + G and the Z + GC groups indicated amelioration that resulted in the restoration in the serum K^+ concentration to near normal, probably due to its antioxidant effect.

This study showed relatively low serum Cl^- concentrations in the G and the GC groups compared to the DW group. This marginal variation is apparently associated with functional alteration in the proximal tubules (Krishma and Ramachandra, 2009). Supplementation with zinc caused an improvement in the serum Cl^- concentration in the Z + G and the Z + GC groups. The reason for the relatively lower serum Cl^- concentration in the Z group is not clear, but might be associated with the pro – oxidant effect of zinc since the rats were not under oxidative stress challenge.

The serum bicarbonate (HCO^-_3) concentration in this study showed relatively higher concentrations in G group and without noticeable change in the GC group compared to the DW group. This difference might be due to its nephrotoxic effect (Krishma and Ramachandra, 2009). Supplemention with zinc in the Z + G and the Z + GC groups ameliorated the lower HCO^-_3 concentrations in the glyphosate treated groups. The zinc alone, however, showed a slight difference in serum HCO^-_3 concentration compared to DW group, possibly due to the redox state since supplementation with zinc had ameliorated and resulted in relatively high serum HCO^-_3 concentration.

The result of serum Calcium ion (Ca^{2+}) concentration in this study showed relatively low Ca^+ concentrations in the G and the GC groups compared to the DW group. On the other hand, supplementation with zinc restored the serum Ca^{2+} concentration to relatively higher level in the Z + G group and it was even highly significant ($p < 0.001$) in the Z + GC group compared to the G and GC groups, the effect was highly significant ($p < 0.01$) in the Z + GC group compared to Z + G group and significant ($p < 0.05$) in Z + GC group compared to Z + G group. The high Ca^+ concentration recorded in Z + GC group might be due to intestinal absorption of calcium as reported by Spencer *et al*. (1987) in their study on the

effect of zinc supplementation on the intestinal absorption of calcium who reported that at high concentration of Ca^{2+}, high Zn^{2+} intake did not affect the intestinal absorption of calcium since Yamaguchi (1998) reported that zinc is required for osteoblastic activity by activating the aminoacyl – tRNA synthetase in the osteoblastic cells and Holloway *et al.* (2003) reported that zinc treatment inhibits bone resorption or it might be as a result of dystrophic calcification. In Z group, however, the Ca^{2+} concentration showed no noticeable difference with the DW group, thus underscoring the antioxidant effect of zinc.

The serum Phosphate (PO^{-}_{4}) concentration in this study showed a relatively higher serum PO^{-}_{4} concentration in the G and the GC groups compared to the DW group. On the other hand, supplementation with zinc ameliorated the increase in the serum PO^{-}_{4} concentration in the Z + G and the Z + GC groups. In the Z group, however, there was slightly high PO^{-}_{4} concentration, possibly due to the pro – oxidant effect of zinc.

The present study demonstrated that sub – chronic exposure to glyphosate caused relatively high serum urea concentration in the G and the GC groups compared to the DW group. This finding is in agreement with those reported by Caglar and Kolankaya (2008) who studied the effect of 56 and 560 mg / kg / day Roundup® and glyphosate and reported increased level of urea by 2.1 fold and 1.9 fold compared to the control respectively. The elevation of blood urea is a good indicator for kidney dysfunction. On the other hand, administration of zinc alone resulted into a slightly high serum urea concentration in the Z group and a relatively high in the Z + G group. The reason for the slight elevation of urea in the Z group might be due to the pro – oxidant effect of zinc whereas the reason for the relatively high urea concentration in the Z + G group is not known for certain, but it might be associated with the high exposure and therefore might require high dose of zinc since

supplementation with zinc was able to ameliorate the elevated urea level in the Z + GC group.

The result of this study showed a relatively high serum creatinine concentration in the G and the GC groups compared to the DW group. This result is in agreement with the result of earlier study by (Caglar and Kolankaya, 2008) that reported a relatively high creatinine concentration following exposure to Roundup® in rats and was said to be possible indicator of kidney dysfunction. The relatively high serum creatinine concentrations were ameliorated in the Z + G and Z + GC groups. Administration of zinc alone, however, caused a slightly high serum creatinine concentration when compared to DW group and the reason for that is not clear, but might be associated with pro – oxidant effect of the zinc.

The histopathological examination of the brain samples from rats sub – chronically exposed to high dose of glyphosate in G group in this study revealed neuronal cells degeneration. However, those exposed to low glyphosate concentration in GC group showed no recognizable lesion. This finding is similar to the result of previous study by Ayoola (2008) who reported neuronal degeneration, mononuclear cells infiltration and severe spongiosis in catfish exposed to acute concentrations of glyphosate for 96 hrs. Such neuronal degeneration and mild degeneration in purkinge cells had been similarly observed in oxidative stress condition in chickens (Kammon *et al.*,2011).

There was no recognizable lesion in the brains of rats in the Z, Z + GC and the DW groups. Supplementation with zinc in the Z + G showed ameliorative effects in the brain of the rats since there were relatively preserved neuronal cells in the brains of the rats in the group. Thus, the degeneration of neuronal cells in both the glyphosate – treated group and the

combination of the glyphosate with zinc – treated group indicated that its toxic effects in the brains were not abolished, but ameliorated and that probably, the severity of the neurotoxicity were not reversible or abolished by the antioxidant effect of zinc within the experimental period and condition. Similar oxidative stress condition was shown to be attenuated by the antioxidant effect of zinc following neuronal degenerative changes sub – chronically induced by malathion in rats (Brocardo *et al*., 2007).

Histomorphologically, the portal areas, of the livers had degeneration, focal areas of fatty degeneration or diffusely degenerated hepatocytes in some rats sub – chronically exposed to glyphosate in the G group. The rats exposed to the low dose of glyphosate in the GC group had no observable microscopic lesion. These observations are in tandem with the previous studies (Benedetti *et al* ., 2004; Caglar and Kolankaya, 2008) where increased Kupffer cells were seen in hepatic sinusoids of glyphosate – treated rats exposed to higher doses, 487 mg/kg and 560 mg/kg respectively, unlike in those rats exposed to 4.87, 48.7 or 56 mg/kg. The infiltration of Kupffer cells were followed by large deposition of reticular fibers, mononuclear cell infiltration and congestion of the liver tissues Benedetti *et al*. (2004) reported that these could have contributed to the impairment of regular function of the liver due to xenobiotics modification in detoxification processes.

There were no observable microscopic lesions in the Z, Z + GC and the DW groups. However, supplementation with zinc in Z + G showed an abolishing effect of zinc in the hepato – toxicity induced by the glyphosate in G group, thus demonstrating the antioxidant effect of zinc since there was a positive relationship between the oxidative damage due to glyphosate and the ameliorative effect therein by the zinc. The hepato – protective effect of zinc had been previously observed in an oxidative stress study (Goel and Dhawan, 2001).

Histopathological examination in the present study showed depopulation of splenic cells which involved the lymphoblasts and lymphocytes in both red and white pulps of rats in the G group.The rats had no observable microscopic changes in their spleens in the GC group. Based on the observed degenerative changes of spleenic cells, glyphosate appears to be a potent immuno – suppressive agent under the present experimental condition and period. The probable immuno – suppressive effect of glyphosate was earlier reported by Blackley (1997). The absence of microscopic lesions in the spleens of rats in the Z and the DW groups and the lymphocytes proliferation in both red and white pulps of the spleens from rats with administration of zinc in the Z + G and the Z + GC groups tend to suggest that there was an ameliorative effect, probably due to zinc administration which seemed to have prevented the immuno – suppressive effect that was observed in the G group.

In this study, the pancreas of rats in the G group had focal areas of degenerated pancreatic acinar cells whereas rats in GC group showed no observable microscopic lesion. Kammon *et al*. (2011) similarly recorded degeneration of glandular acini, mild necrosis of the glandular cells and mild degeneration of beta islets of langerhans with distension of interlobular septa of pancreas probably induced by oxidative stress in chickens following chronic chlorpyrifos (CPF) exposure. The rats in the Z, Z + GC and the DW groups had histomorphologically normal pancreas.

In the Z + G zinc supplemented group, there was amelioration of the degenerative changes caused by the glyphosate at 375 mg / kg and the pancreases of the rats were hyperplastic with distension of the interlobular septa by transudate.

Histopathologic lesions seen in the kidney of the rats sub – chronically exposed to glyphosate in the G group were glomerular and renal tubular degeneration with infiltration of mononuclear cells to the interstices of the kidney. In a recent study, mild congestion and haemorrhage, mild focal coagulative necrosis and sloughing of tubular epithelial cells in oxidative stress condition in chickens followed chronic CPF exposure (Kammon *et al*., 2011). Oxidative stress was also said to cause Shrinkage of the glomerular network, necrosis of proximal tubules and ruptured distal collecting tubules (Sastry and Sharma, 1981).

The renal tubules are particularly sensitive to toxic influences because they have high oxygen consumption, vulnerable enzyme systems and complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins during excretion and/or elimination (Tisher and Brener, 1989). The presence of necrosis might be related to the depletion of ATP (Shimizu *et al*., 1996), possibly through the direct toxic effect on the cell function (Kammon *et al*., 2011), which might involve reactive free radicals, oxidative stress or both (Alden and Frith, 1992).

Such nephrotoxic effects induced by glyphosate were absent in the rats in the Z, Z + GC and the DW groups and might have been ameliorated in the Z + G group by the antioxidant effect of zinc.

Histomorphology of stomach specimens exposed sub – chronically to glyphosate in the G group showed degenerated mucosal epithelial cells which involved the glands and the muscularis mucosa. Sastry and Sharma (1981) had reported erosion of the gastric mucosa as the only visible lesion probably caused by oxidative stress following exposure to sub –

lethal concentration of diazinon in fresh water teleost fish, *Ophiocephalus punctatus*. However, the stomach specimens of rats in the GC group in this study showed no observable microscopic lesions.

The stomach specimens of rats in the Z, Z + GC and the DW group also had no recognizable microscopic lesion, however, administration of Zinc in the Z + G group ameliorated the histomorphologic changes in the stomach by acting as an antioxidant as reported by Powell (2000).

The result of this study showed that the testes were histomorphologically normal in the G and the GC groups following sub –chronic exposure to glyphosate. Contrary to the report of Amara *et al* .(2008) that zinc administration reduced elevated levels of malonaldehyde (MDA) in the testes, attenuated DNA oxidation induced by Cadmium in the gonad and restored the testosterone and sperm count to normal levels, the present study did not measure testosterone level and sperm counts of the rats. Similarly, there were no microscopic changes in the uterus in any of the rats in all the groups, suggesting that glyphosate might not induce histological lesion in the gonads as evident within this experimental period and condition.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The present study has shown that subchronic exposure of adult Wistar rats to glyphosate caused decreased packed cell volume (PCV) and hemoglobin (Hb) concentration, increased white blood cell (WBC) count, increased neutrophil count, increased lymphocyte count, increased total protein, significantly increased albumin concentration, decreased globulin concentration, increased aspartate aminotransferase (AST) activity, increased alanine aminotransferase (ALT) activity, decreased alkaline phosphatase (ALP) activity, decreased Na^+ concentration, increased K^+ concentration, decreased Cl^- concentration, increased HCO^-_3 concentration, decreased Ca^{2+} concentration, increased PO^-_4 concentration, increased urea concentration, decreased uric acid concentration and increased creatinine concentration. The study also showed that subchronic exposure to glyphosate in adult Wistar rats caused histopathological changes in the brains characterized by neuronal cells degeneration; livers, degeneration in hepatic cells especially at the portal areas of the liver; spleens, depopulated splenic cells (lymphoblast and lymphocytes) in both red and white pulps; pancreas, degenerated pancreatic acinar cells; kidney, glomerular and renal tubular degeneration with few mononuclear cells infiltration into the interstices of the kidney and in stomach, there were degenerated mucosal epithelial cells that involved the muscularis mucosa and the glands whereas there were no observable microscopic lesions in the gonad. In addition, the study also revealed that pretreatment with zinc ameliorated and also

abolished the haematological, serum – biochemical and histopathological changes induced by subchronic glyphosate exposure in adult Wistar rats.

6.2 CONCLUSION

In conclusion, the study has shown that subchronic exposure to glyphosate in Wistar rats caused:

- Alterations in the haematological parameters
- Alterations in serum – biochemical parameters
- Histopathological lesions in the brain, liver, spleen, pancreas, heart, kidney and stomach
- Pretreatment with zinc differentially ameliorated glyphosate – induced haematological, serum – biochemical and the histopathological lesions in the brains as well as ameliorated the histopathological lesions in the liver, spleen, pancreas, kidney and stomach.

6.3 RECOMMENDATIONS

i. Further research should be conducted on the chronic toxicity of glyphosate exposure in Wistar rats.

ii. Further investigation should be made on the curative potential of zinc in glyphosate – induced toxicity by administering zinc after some period of exposure to glyphosate based on the pharmacokinetics of glyphosate in Wistar rats.

iii. Additional study should be carried out to compare the combined effect of zinc and another antioxidant vis – a – vis their individual effects on glyphosate induced toxicity in

Wistar rats because of the inability of the zinc to completely abolish the microscopic lesions observed in the brain and the pancreas of the rats exposed to glyphosate at 375 mg / kg body weight.

iv. Further research should be carried out on the chronic effect of glyphosate and zinc and glyphosate combination on the reproductive systems and reproduction in rats.

v. Farmers should prevent their animals from indiscriminate grazing on pastures that have been sprayed with glyphosate because of the likely attendant risks thereof.

vi. Animals on extensive management systems should be administered with zinc on regular basis especially at the onset of the rainy season when there is indiscriminate use of this herbicide, Bushfire®, or any other glyphosate based herbicide.

REFERENCES

Abdallah S.M and Samman S. (1993). The Effect of Increasing Dietary Zinc on the Activity of Superoxide dismutase and Zinc Concentration in Erythrocytes of Healthy Female Subjects. *European Journal of Clinical Nutrition*. 47: 327 – 332.

Abdel – Rahman, A., Dechkovskaia, A.M., Goldstein, L.B., Bullman, S.H., Khan, W., El – Masry, E.M. and Abou – Donia, M.B. (2004). Neurological Deficits Induced by Malathion, DEET and Permethrin, Alone or in Combinnation in Adult Rats. *Journal of Toxicology and Environmental Health*, 67: 331 – 356.

Abou Donia, M. B. and Graham, D. G. (1978). Delayed Neurotoxicity from Long – term Low level Administration of Leptophos to the Comb of Hens. *Toxicology and Pharmacology*, 46: 199 – 213.

Adachi, M. and Ishii, H. (2002). Role of Mitochondria in Alcoholic Liver Injury. *Free Radical Biology and Medicine*, 32: 487 – 491.

Adam, A., Marzuki, A. and Abdul, A. (1997). The oral and intratrachael toxicities of Roundup® and its components to rats. *Veterinary and Human Toxicology*, 39: 147 – 151.

Adedeji, O. B., Adedeji, O. A., Adeyemo, O. K. and Agbede S. A. (2009). Acute Effects of Diazinon on Blood Parameters in the African Catfish (*Clarias gariepinus*). *The Internet Journal of Hematology*, 5(2): 32 – 49.

Agius, C. and Coushman, W. (1986). Introduction of Enhanced Alkaline Phosphatase Activity in the Melano – Macrophage Centres of *Oreochromis aureus* (Steindachner) Through Starvation and Vaccination. *Journal of Fish Biology*, 28: 87 – 92.

Alden, C. L. and Frith, C. H. (1992). Urinary System. In: Hashek, W.M. and Rousseaux, C.G (ed) 1992. *Handbook of Toxicologic Pathology*, 316 – 379.

Amara, S., Abdelmelek, H., Garrel, C., Guiraud, P., Douki, T., Ravanat, J., Favier, A., Sakly, M. and Ben R.K. (2008). Preventive Effect of Zinc Against Cadmium – Induced Oxidative Stress in the Rat Testis. *Journal of Reproduction and Development*, 54(2): 129 – 134.

Ambali, S. F (2009). Ameliorative Effect of Vitamin C and E on Neurotoxicological, Haematological and Biochemical Changes Induced by Erythrocte Osmotic Fragility in Wistar Rats. PhD. Thesis, Ahmadu Bello University, Zaria, 356.

Ambali, S. F., Abubakar A. T., Shittu M.. Yaqub .S L., Anafi S.B. and Abdullahi A. (2010a). Chlorpyrifos-induced Alteration of Haematological parameters in Wistar Rats : Ameliorative Effects of Zinc. *Research Journal of Environmental Toxicology* .pp, 1-12.

Ambali, S. F., Onukak, C., Idris, S. B., Yaqub, S. L., Shittu, M., Aliyu, H. and Kawu, M. U. (2010b). Vitamin C Attenuates Short – term Hematological and Biochemical Alterations Induced by Acute Chlorpyrifos Exposure in Wistar Rats. *Journal of Medicine and Medical Sciences*, 1(10): 465 – 477.

Ambali, S. F., Akambi, D. O., Shittu, M., Giwa, A. and Oladipo, O. O (2010c). Chlorpyrifos – Induced Clinical, Haematological and Biochemical Changes in Swiss Albino Mice – Mitigating Effect by Co – administration of Vitamins C and E. *Life Science Journal*, 7(3): 37 – 44.

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*, 4(3): 25 – 36.

Ambali, S. F., Akanbi D. O., Igbokwe N., Shittu M., Kawu M. and Ayo J. (2007). Evaluation of Subchronic Chlorpyrifos Poisoning on Haematological Changes in Mice and Protective Effect of Vitamin C. *Journal of Toxicological Science*, 32: 111 – 120.

Ambali, S. F., Ayo J.O., Ojo S.A. and Esievo K. A. N. (2009). Ameliorative Effect of Vitamin C on Chronic Chlorpyrifos – Induced Erythrocyte Osmotic Fragility in Wistar Rats. *Human and Experimental Toxicology*, 21: 44 – 52.

Ansari, B. A. and Kumar, K. (1988). Diazinon Toxicity: Effect on Protein and Nucleic Acid Metabolism in Liver of Zebrafish, Brachydani rerio (Cyprinidae). *Journal of Environmental Science*, 76: 63 – 68.

Attai, A. M. and Nasr, H. M. (2009). Dimethoate – Induced Change in Biochemical Parameters of Experimental Rats – Serum. *Slovenian Journal of Animal Science*, 42(2): 87 – 94.

Ayoola, S. O.(2008). Histopathological Effects of glyphosate on Juvenile African Catfish (*Clarias gariepinus).American- Eurosian Journal of Agriculture and Environmental Science*, 4(3): 362-367.

Bailey, S. M. and Cunningham, C. C. (2002). Contribution of Mitochondria to Oxidative Stress Association with Alcoholic Liver Disease. *Free Radical Biology and Medicine*, 32: 11 – 16.

Baker, J., Silverton, R. E. and Pillister, C. J. (2000). Dehydration,Impregnation,Embedding Technique and Section Preparation. *Introduction to Medical Laboratory Technology,7th edition,* 199-242

Baker, H. W. G., Brindl, J. and Irvine, D. S. (1996). Protective Effects of Antioxidants on the Impairment of Sperm Motility by Activated Polymorphonuclear Leucocytes. *Fertility and Sterility*, 65: 411 – 419.

Barna – Lloyd, T., Szabo J. R and Davis N. L. (1991). Chlorpyrifos – Methyl (Reldan R) Rat Chronic Dietary – Toxicity/Oncogenicity Study. *WHO, Indianapolis, USA*.

Barna – Lloyd, T., Szabo, J. R. and Davis, N. L. (1990). Chlorpyrifos – Methyl (Reldan R) Rat Subchronic Dietary Toxicity and Recovery Study. Report, TXT: K-046193-026 From Dow Chemical, Texas, USA. Submitted to WHO by Dow Elonco, Indianapolis, USA.

Barreto – Medeiros, J. M., Feitoza, E. G., Megalhaes, K., Da Silva, R. R., Manhaes – de – Castro, F. M., Manhaes – de – Castro, R., De – Castro, C. M. M. B. (2005). The Expression of an Intraspecific Aggressive Reaction in the Face of a Stressor Agent Alters the Immune Response in Rats. *Brazilian Journal of Biology*, 65: 203 – 209.

Baselt, R. C. (1982). Disposition of Toxic Drugs and Chemicals in Man. *Biomedical Publications, Davis, California*, 1795.

Bebarji, A. and Rajendranth, H. (1990). Haematological Changes Induced by an Organophosphorous Insecticide in Fresh Water Fish, *Clarias batrachus* (Linnaeus). *Tropical Fresh Water Biology*, 2: 197 – 202.

Bebarji, J. and Rajendranth, R. (1990). Haematological Changes Induced by an Organophosphorous Insecticide in a Fresh Water Fish, *Clarias batrachus* (Linnaeus).*Tropical and Fresh Water Biology*, 2: 197 – 202.

Begum, G. (2004). Carbofuran Insecticide Induced Biochemical Alterations in Liver and Muscle Tissues of the Fish, *Clarias batrachus* (Linn) And Recovery Response. *Aquatic Toxicology*, 66: 83 – 92.

Begum, G. and Vijayaraghavan, S. (1995). *In vivo* Toxicity of Dimethoate on Protein and Transaminases in the Liver Tissues of Fresh Water Fish, *Clarias batrachus*. *Bulletin of Enviromental Contamination and Toxicology*, 54(3): 370 – 375.

Belongia, E. A., Berg, R. and Liu K. A. (2001).A Randomized Trial of Zinc Nasal Spray for Adults. *American Journal of Medicine*, 111 (2):103-108.

Benedetti, A. L., Vituri, C., Trentin, A. G., Domingues, M. A. C and Alvarez – Silva, M. (2004). The Effects of Sub – chronic Exposure of Wistar Rats to the Herbicide Glyphosate – Biocarb. *Toxicology Letters*, 153(2004): 227 – 232.

Beuret, C. J., Fanny, Z. and Maria S. G. (2004). Effects of the Herbicide Glyphosate on Liver Lipoperoxidation in Pregnant Rats and Their Fetuses. *Reproductive Toxicology,* 19(4): 501-504.

Bhattacharya, S., Ghosh, R. K., Mondal, T. K., Chakrabarty, A. K. and Basak, D. K. (1993). Some Histhopathological Changes in Chronic Endosulfan Toxicity in Poultry. *Indian Journal of Animal Health*, 32: 9 – 11.

Blair, D., Hoadley, E. C. and Hutson, D. H. (1975). The Distribution of Dichlorvos in the Tissues of Mammals after its Inhalation or Intravenous Administration. *Toxicology and Applied Pharmacology*, 31: 243 – 353.

Blakley, B. R. (1997). Effect of round up and tordon 202C herbicides on antibody production in mice. *Veterinary and Human Toxicology*, 39: 204 – 206.

Bondy, S. C. (1992). Ethanol Toxicity and Oxidative Stress. *Toxicology Letters*, 63: 231 – 242.

Brocardo, P. S., Assin, F., Franco, J. L., Pandolfo, P., Muller, Y. M. R., Takahashi, R. N., Dafre, A. L. and Rodrigues, A. L. S. (2007). Zinc Attenuates Malathion – Induced Depressant – Like Behaviour and Confers Neuroprotection in the Rat Brain. *Toxicological Sciences*, 97(1): 140 – 148.

Caglar, S. and Kolankaya, D. (2008). The effect of sub – acute and sub – chronic exposure of rats to the glyphosate based herbicide Roundup. *Environmental Toxicology and Pharmacology*, 25(2008): 57 – 62.

Campbell, J. D., Cole, M., Bunditrutavorn, B. and Vell, A. T. (1999). Ascorbic Acid is a Potent Inhibitor of Various Forms of T – Cell Apoptosis. *Cell Immunology*, 194: 1 – 5.

Caudray, C., Richard, M.J., Laporte, F., Faure P., Roussel, A. M. and Favier, A. E. (1992). Superoxide Dismutase and Zinc Status: A study in Man and Animals. *Journal of Nutrition and Medicine*, 3: 13 – 26.

Chance, B., Sies, H. and Boveris, A. (1979). Hydroperoxide Metabolism in Mammalian Organisms. *Physiological Reviews*. 59: 527 – 605.

Chatterjea, M. N. and Shinde, R. (2005). *Text Book of Medical Biochemistry, 6th Edition*. Jaypee Brothers. New – Delhi . 644.

Chaudhary, N., Sharma, M., Verma, P. and Joshi, S. C. (2003). Hepato and Nephrotoxicity in Rat Exposed to Endosulfan. *Journal of Environmental Biology*, 24(3): 305 – 308.

Chaudiere, J. and Ferrari – Illiou, R. (1999). Intracellular Antioxidants: from Chemical to Biochemical Mechanisms. *Food and Chemical Toxicology*, 37(9 – 10): 949 – 962.

Corcoran, G. B., Fix, L., Jones, D. P., Mosten, M. T., Nicotera, P., Oberhammer, F.A. and Buttyan, R. (1994). Apoptosis: Molecular Control Point in Toxicity. *Toxicology and Applied Pharmacology*, 128: 169 – 181.

Cornelius, C. E. (1989). Serum Enzyme Activities and other Markers for Detecting Hepatic Necrosis, Cholestasis or Co Carcinoma. In: Kaneko J.J., Harvey J.W. and Bruss, L.M. (ed) 1989. *Clinical Biochemistry of Domestic Animals*, 381 – 386.

Cox, C. (1995). Herbicide factsheet: Glyphosate, Part 1: *Toxicology Journal of Pesticide*s *Reform,* 15:3.

Daabees, A.Y., El – Damiaty, N. A., Soliman, S. A. and El – Toweissy, M. Y. (1992). Comparative Action of Three Synthetic Pesticides on Serum, Liver, Brain of the Fresh Water Fish, *Clarias lazera*. *Journal of Egyptian German Society for Zoology. 9(A). Comparative Physiology*, 105 – 109.

Davies, K. (1995). Oxidative Stress: The Paradox of Aerobic Life. *Biochemical Society Symposium,* 61: 1 – 31.

De Groot, H. (1994). Reactive Oxygen Species in Tissue Injury. *Hepato – Gastro – Enterology*, 41: 328 – 332.

Decoursey, T. E. and Ligeti, E. (2005). Regulation and Termination of NADPH Oxidase Activity. *Cellular and Molecular Life Sciences*, 62(19 – 20): 2173 – 2193.

Dhawan, D., Goel, A. and Gautam, C. S. (1992). Effects of Zinc Intake in Liver Enzymes in Carbontetrachloride Induced Liver Injury. *Medical Science Research*, 20: 55 – 56.

Driscoll, E. R. and Bettger, W. J. (1992). Zinc Deficiency in the Rat Alters the Lipid Composition of the Erythrocyte Membrane Triton Shell. *Lipids*, 27: 972 – 977.

Durham, W. F., Wolfe, H. R. and Elliot, J. W. (1972). Absorption and Excretion of Parathion by Spraymen. *Archiv Za Higijenu Rada Toksikologiju*, 27: 381 – 387.

Ecobichon, D. J., Ozere, R. L., Reid, E. and Crocker, J. F. S. (1977). Acute Fenitrothion Poisoning. *Canadian Medical Association Journal*, 116: 377 – 379.

Edson, E. F., Jones, K. H. and Watson, W. A. (1967). Safety of Dimethoate Isecticide. *British Medicine Journal*, 4: 554 – 555.

El – Boushy, M. A. (1994): The Effect of Molluscicides Pollution on the Blood Picture and Serum Biochemical Parameters in *Clarias lazera*. M.V.Sc Thesis, Faculty of Veterinary Medicine, Snez – Canal University.

El – Shenawy, N. S. (2009). Oxidative Stress Responses of Rats Exposed to Roundup® and it's Active Ingredient Glyphosate. *Environmental Toxicology and Pharmacology*. 28 (2009), 379 – 385.

Elias, M. A. S. (2010). Toxic Effect of Dimethoate and Diazinon on the Biochemical and Hematological Parameters in Male Rabbits. *Jordan Journal of Biological Sciences*, 3(2): 77 – 82.

Environmental Protection Agency U. S (2007). Pesticide. September 15th, 2007.

Environmental Protection Agency, United States (2010).

Food and Agriculture Organization of the United Nations (2002). International Code of Conduct on the Distribution and use of Pesticides. October 25th, 2007.

Franz, J. E., Mao, M. K. and Sikorski J. A. (1997). Glyphosate : A unique Global Herbicide , Acs Monograph 189. *American Chemical Society, Washington D.C. U.S.*

Fredrikson (1961). Percutaneous Absorption of Parathion and Paraoxon. *Archives of Environmental Health*, 3: 67 – 70.

Gaafar, A. Y., El- Manakhly, E. M., Soliman, M. K., Soufy, H., Mona, S. Z., Safinaz, G. M. and Shahenaz, M. H. (2010). Some Pathological, Biochemical and Hematological Investigations on Nile Tilapia (*Oreochromis niloticus*) Following

Chronic Exposure to Edifenfos Pesticide. *Journal of American Science*, 6(10): 542 – 551.

Gaithin, D. M. III. And Wilson, R. P. (1986). Dietary Copper Requirement of Fingerling Channel Catfish. *Aquaculture*, 54: 277 – 285.

Gallo, M. A. and Lawryk, K. N. J. (1991) Organic Phosphorus Pesticides. In: Hayes, W.J. and Laws Edition, Pesticides Studied in Man. Williams and Wilkins, B. 917 – 1123.

Glusczak, L., Miron, D. S., Crestani, M., Fonseca, M. B., Pedron, F. A., Duarte, M. F., Vieira, V. I. P. (2006). Effect of Glyphosate Herbicide on Acetylcholinesterase Activity and Metabolic and Hematological Parameters in Piava (*Leporinus obtusidens*). *Ecotoxicology and Environmental Safety*, 65: 237 – 241.

Goel, A. and Dhawan, D. K. (2001). Zinc Supplementation Prevents Liver Injury in Chlorpyrifos Treated Rats. *Biology and Trace Elements Research*, 82 (1-3): 185 – 200.

Goel, A., Danni, V. and Dhawan, D. K. (2006). Role of Zinc in Mitigating Toxic Effects of Chlorpyrifos on Haematological Alterations and Electron Microscopic Observations in Rat Blood. *BioMetals*, 19(15): 483 – 492.

Goel, A. Danni, V. and Dhawan, D. K. (2005). Protective Effects of Zinc on Lipid Peroxidation, Antioxidant Enzymes and Hepatic Histoarchitecture in Chlorpyrifos – Induced Toxicity. *Chemical and Biological Interactions*, 156: 131 – 140.

Gokalp, O., Gulle, K., Sulak, O., Cicek, E. and Attuntas, I. (2003). The Effects of Methidathion on Liver: Role of Vitamins E and C. *Toxicology and Industrial Health*, 19: 63 – 67.

Gomes, J., Dawodu, A. H., Lloyd, O., Revitt, D. M. and Anilal, S.V. (1999). Hepatic Injury and Distributed Amino Acid Metabolism in Mice Following Prolonged Exposure to Organophosphorous Pesticides. *Human and Experimental Toxicology*, 18: 33 – 37.

Gupta, M., Bagchi, G., Bandyopadhyay, S., Sasmal, D., Chatterjee, T. and Dey, S. N. (1982). Hematological Changes Produced in Mice by Nuvacron or Furadan. *Toxicology*, 25: 255 – 260.

Gupta, P. K and Chandra, S.V. (1977). Toxicity of Endosulfan after Repeated Oral Administration to Rats. *Bulletin of Environmental Contamination and Toxicology*, 18: 378 – 384.

Haley, P. J. and Weiser, M. G. (1985). Erythrocyte Volume Distribution in Rainbow Trout. *American Journal of Veterinary Research*, 46: 2210 – 2212.

Halliwell, B. (1999). Antioxidant Defence Mechanisms: From:Beginning to End. *Free Radical Research*, 31: 261 – 272.

Holloway W. R., Collier F. M., Herbst R. E., Hodg, J. M. and Nicholson, G. C (2003). Osteoblast – mediated Effects of Zinc on Isolated Rat Osteoclast : Inhibition of

Bone resorption and Enhancement of Osteoclast Number. *ScienceDirect.*19(2): 137 – 142.

Howe, C. M., Berrill, M., Pauli, D. B., Helbing, C. C., Werr, K., Veldhoen, N. (2004). Toxicity of Glyphosate – based Pesticides to four North American Frog Species. *Environmental Toxicology and Chemistry*, 23: 1928 – 1938.

Imlay, J. (2003). Pathways of Oxidative Damage. *Annual Review Microbiology*, 57: 395 – 418.

Iqbal, K., Khan, A. and Khattak, M.A.K. (2004). Biological Significance of Ascorbic Acid in Human Health – A Review. *Pakistanian Journal of Nutrition*, 3(1): 5 – 13.

Jirachayabhas,V.,Visuthismajarn, P., Hore, P. and Robson, M.G. (2004).Organophosphate Pesticide Exposure of Traditional and Integrated Pest Management Farmers from Working air Conditions:A case study of Thailand. *International Journal of Occupational and Environmental Health*, 10:289-295.

Josephine, D. L (2011). Pesticide Classification. *Archives of Insect Experts*, 1 – 5.

Joshi, S. C., Mathur, R. and Gulati, N. (2007). Testicular Toxicity of Chlorpyrifos (An Organophosphate Pesticide) in Albino Rats. *Toxicology and Industrial Health*, 23(7): 435 – 439.

Kammon, A. M., Brar, R. S., Banga, H. S. and Sodhis S. (2010). Patho – Biochemical Studies on Hepatotoxicity and Nephrotoxicity on Exposure to Chlorpyrifos and Imidacbprid in Layer Chickens. *Veterinary Archives*, 80(5): 663 – 672.

Kammon, A. M., Brar, R. S., Sodhi, S., Banga, H. S., Singh, J. and Nagra, N. S. (2011). Chlorpyrifos Chronic Toxicity in Broilers and Effect of Vitamin C. *Open Veterinary Journal*, 1: 21 – 27.

Kossmann, S., Magner – Krezel, Z., Sobjeraj, R. and Szwed, Z. (1997). The Assessment of Nephrotoxic Effect Based on the Determination of the Activity of Some Selected Enzymes in Urine. *Jordan Journal of Biological Sciences*, 54(10): 707 – 711.

Kraus, A., Roth, H. and Kirchgessner, M. (1997). Supplementation with Vitamin C, Vitamin E and β – Carotene Influences Osmotic Fragility and Oxidative Damage of Erythrocytes of Zinc Deficient Rats. *Journal of Nutrition*, 127: 1290 – 1296.

Krishma, 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.

Kumar, A., Malhotra, A., Nair, P., Garg, M. and Dhawan, D. K. (2010). Protective Role of Zinc in Ameliorating Arsenic – Induced Oxidative Stress and Histopathological Changes in Rat Liver. *Journal of Environmental Pathology, Toxicology and Oncology*, 29(2): 91 – 100.

Liska, D. and Kolesar, D. (1982). Toxicological Classification of Pesticides. *Czech Medical*, 5(3): 137 – 145.

Lorke, D.(1983).A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, 54: 250-287.

Lyon, J., Taylor, H. and Ackerman, B. (1987). A Case Report of Intravenous Malathion Injection with Determination of Serum Half – Life. *Clinical Toxicology*, 25(3): 243 – 364.

Majumder, S., Chakraborty, A. K., Mandal, T. K., Bhattacharya, A. and Basak, D. K. (1994). Subacute Toxicity of Fenavalerate in Broiler Chicks. Concentration, Cytotoxicity and Biochemical Profiles. Indian *Journal of Experimental Biology*, 32(10): 752 – 756.

Malik, J., Barry G. and Kishore G. (1989). The herbicide glyphosate. *Biofactor,* 2: 17-25.

Mana, A. H. Y., Sabah, G. and Aly, B.O. (2007). Diazinon Toxicity Affects Histophysiological and Biochemical Parameters in Rabbits. *Experimental and Toxicologic Pathology*, 59(2007): 215 – 225.

Matin, M., Hussain, K. and Khan, S. (1990). Modification of Diazinon – Induced Change in Carbohydrates Metabolism by Adrenalectomy in Rats. *Biochemistry and Pharmacology*, 39: 1781 – 1786.

Maxam, A. M and Gilbert, W (1980). Sequencing end – labeled DNA with Base – Specific Chemical Cleaves. *Methods in Enzymology*. 65 (1) : 499 – 560.

McClain C. J., Staurt M. and Vivian B. (1992). Zinc status before and after zinc supplementation of eating disorders in patients. *Journal of American College of Nutrition,*11: 694-700.

Mccord, J. M., Gao, B. and Leff, J. (1994). Neutrophil Generated Free Radicals: Possible Mechanisms of Injury in Adult Respiratory Distress Syndrome. *Environmental Health Perspective*, 102(10): 57 – 60.

Meister, A. (1994). Glutathione – Ascorbic Acid Antioxidant System in Animals. *Journal of Biology and Chemistry*, 265(26): 15361 – 15364.

Meydani, M. (2001). Vitamin E and Artherosclerosis: Beyond Prevention of Low Density Lipids (LDL) Oxidation. *Journal of Nutrition Supplement*, 366 – 368.

Miller, R. A. and Britigan, B. E. (1997). Role of Oxidants in Microbial Pathophysiology. *Clinical Microbiology*, 10(1): 1 – 18.

Minton, N. A. and Murray, V. S. G. (1988). A Review of Organophosphate Poisoning. *Medical Toxicology*, 3: 350 – 375.

Modesto, K. A. and Martinez C. B. R. (2010). Effects of Roundup® Transcorp on Fish: Hematology, Antioxidant Defences and Acetylcholinesterase Activity. *Chemosphere*, 81 (2010): 781 – 787.

Mohiuddin, S. M. and Ahmed, M. N. (1986). Effect of Feeding Ekalux (Quinolphos) Pesticide in Poultry. *Indian Veterinary Journal*, 63: 796 – 798.

Monsanto advertising supplements, farmers weekly, 5th June, 1992.

Nabb, D. P., Stein, W. J. and Hayes, W. J. Jr. (1966). Rate of Skin Absorption of Parathion and Paraoxon. *Archives of Environmental Health*, 12: 501 – 505.

Naidu, K. A. (2003). Vitamin C in Human Health and Disease is Still a Mystery? An Over View. *Nutrition Journal*, 2: 7 – 16.

Nakazawa, J., Genka, C. and Fujishima, M. (1996). Pathological Aspects of Reactive Oxygen/Free Radicals. *Japanese Journal of Physiology*, 46: 15 – 32.

Nath, R. and Banerjee, V. (1996). Effect of Pesticides Methylparathion and Cypermethrin on the Air – Breathing Fish Heropneuster fossilis. *Environmental Ecology*, 14: 163 – 165.

National Pesticide Information Centre (2010). Toxicity Category Signal Word (9), 1 – 800 – 858 – PEST (7378).

Neskovic, N. K., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M. (1996). Biochemical and Histopathological Effects of Glyphosate on Carp, *Cyprinus Carpio*. *Bulletin of Environmental Contamination and Toxicology*. 56: 295 – 302.

O'Dell, B. L. (2000). Role of Zinc in Plasma Membrane Function. *Journal of Nutrition*, 130: 1432S – 1436S.

Olurin, K. B., Olojo., E. A. A., Mbaka, G. O. and Akindele, A. T. (2006). Histopathological Responses of the Gill and Liver Tissues of Clarias gariepinus Fingerlings to the Herbicide, Glyphosate. *African Journal of Biotechnology*, 5(24): 2480 – 2487.

Olurunsogo O., Bababunmi E. and Bassir, O. (1979). Effect of glyphosate on rat liver mitochondria in vivo. *Bulletin of Environmental Toxicology,* 2: 257-264.

Packer, L. (1992). Free Radical Scavengers and Antioxidants in Prophylaxy and Treatment of Brain Diseases. Free Radicals in the Brain Aging, Neurological and Mental Disorders. *Springer – Verlag Berlin*, 1 – 20 .

Parrat, M. O., Richard, M. J., Beani, J. C. and Favier, A. (1997). Involvement of Zinc in Intracellular Oxidant /Antioxidant Balance. *Biology of Trace Element Research*, 60: 187 – 204.

Pesticide Action Network Asia and the Pacific (2009). Glyphosate, 1 – 3.

Powell, S. R. (2000). The Antioxidant Properties of Zinc. *Journal of Nutrition*, 130: 1447S – 1454S.

Prasad, A. S. and Kucuk, O. (2002). Zinc in Cancer Prevention. *Cancer Metastasis Review*, 21: 291 – 295.

Quesada, A. R., Byrnes, R. W., Krezoski, S. O. and Pettering, D. H. (1996). Direct Reaction of H_2O_2 with Sulfhydryl Groups in HL – 60 Cells: Zinc – Mettalothionein and other Sites. *Archives of Biochemistry and Biophysics*, 334: 241 – 250.

Rabideau, C. L. (2001). Pesticide Mixtures Induce Immunotoxicity : Potentiation of Apoptosis and Oxidative Stress. M.Sc Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 170.

Ray, D. E. (1992). Pollution.*Pollution and Health, Wiley Eastern Limited, New Delhi*.

Rebecca L.T., Guang-Yang Y., Wei-Jin T., Hsiao-min, C. and Jou-Fang, D. (1991). Survey of Glyphosate-surfactant Herbicide Ingestion. *Clinical Toxicology*, 29 (1) : 91-109.

Rehulka, J (2002). *Aeromonas* Causes Severe Skin Lesions in Rainbow Trout (*Oncorhynchus mykiss*): Clinical Pathology, Haematology and Biochemistry. *Acta Vterinaria Brno*, 71 – 351 – 360.

Rehulka, J. (2000). Influence of Astaxanthin on Growth Rate Condition and Some Blood Indices of Rainbow Trout, *Onchorhynchus mykiss*. *Aquaculture*, 190: 27 – 47.

Rehulka, J. (2002). Effect of Polychlorinated Biphenyls (PCBs) Delor 103 on Some Haematological and Biochemical Indices of the Blood Plasma of the Rainbow Trout, *Oncorhynchus mykiss*. In: The Second PCBs Workshop. Recent Advances in the Environmental Toxicology and Health Effects of PCBs. *Brno Czech Republic*.

Rhee, S. G. (2006). Cell Signalling. H_2O_2 a necessary Evil for Cell Signalling. *Science*, 312 (5782): 1882 1883.

Robert, R. J. (2001). *Fish Pathology*, 3rd Edition, Bailliere Tindall, London, Philadelphia, Sydney, Tokyo, Toronto.

Rodwell, D. E. (1980). Teratology Study in Rats With Technical Glyphosate. International Research and Development Corporation, Mattawan, MI, USA. No_ 79/016.

Rostan, E. F., DeBubys, H. V., Madey D. L. and Pinnell S. R. (2002). Evidence Supporting Zinc as an Important Antioxidant for Skin. *International Journal of Dermatology*, 4 (9):606-611.

Saeed, R. M. A. (1983). Biochemical and Physiological Studies on the Effect of Some Pesticides on the Nile Fish *Clarias lazera*. PhD. Thesis, Faculty of Girls Ain Shams University.

Sahin, K. and Kucuk, O. (2003). Zinc Supplementation Alleviates Heat Stress in Laying Japanese Quail. *Journal of Nutrition*, 133: 3808 – 2811.

Santillo, D. J., Leslie, D. M. and Brown, P. W. (1989).Responses of Small Mammals and Habitat to Glyphosate Application on Clearcuts. *Journal of Wildlife Management,* 53(1): 164-172.

Santos, J. B., Ferreira, E. A., Kasuya, M. C. M., Silva, A. A. and Procopio, S. O. (2005). Tolerance of Bradyrhizobium Strains to Glyphosate Formulations. *Crop Protection*, 24: 543 – 547.

Sastry, K. V. and Sharma, K. (1981). Diazinon – Induced Histopathological and Hematological Alterations in a Fresh Water Teleost, *Ophiocephalus punctatus*. *Ecotoxicology and Environmental Safety*, 5(3): 329 – 340.

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.

Seiverd, C. E. (1972). *Hematology for Technologists*, 4th Edition Philadelphia Ilea and Fabiger, 300 – 360.

Shalby, A. A. (1998). Toxicological Studies on Experimental Rats. M.Sc Thesis, Faculty of Agriculture, University of Mansoura.

Shimizu, S., Eguchi, Y., Kamiike, W., Waguri, S., Uchiyama, Y., Matsuda, H. and Tsujimoto, Y. (1996). Retardation of Chemical Hypoxia – Induced Necrotic Cell Death by Bcl – 2 and ICE Inhibitors: Possible Involment of Common Mediators in Apoptotic and Necrotic Signal Transductions. *Oncogene*. 12: 2045 – 2050.

Sidhu, P., Garg, M. L. and Dhawan, D. K. (2004). Protective Effects of Zinc on Oxidative Stress Enzymes in Liver of Protein Deficient Rats. *Nutricion Hospitalaria*, XIX (6) : 341 – 347.

Sies, H. (1993). Strategies of Antioxidant Defense. *European Journal of Biochemistry*, 215(2): 213 – 219.

Sies, H. (1997). Oxidative Stress: Oxidants and Antioxidants. *Experimental Physiology*, 82(2) 291 – 295.

Singh, A., Naidu, P.S., Gupta, S. and Kulkarni, S. K. (2002). Effect of Natural and Synthetic Antioxidants in a Mouse Model of Chronic Fatique Syndrome. *Journal of Medicinal Food,* 5(4): 211 – 220.

Singh, R. K. and Sharma, B. (1998). Carbofuran – Induced Biochemical Changes in *Clarias batrachus*. *Pesticide Science*, 53(4): 285 – 290.

Snieszko, S. F. (1961). Microhaematocrit Values in Rainbow Trout and Brook Trout. *The Progressive Fish – Culturist*, 23: 114 – 119.

Snow, D. H. (1973). The Acute Toxicity of Dichlorvos in the Dog. *II Pathology. Australian Veterinary Journal*, 49: 120 – 125.

Spencer H., Rubio N., Kramer L., Norris C. and Osis D. (1987). Effect of Zinc Supplements on the Intestinal Absorption of Calcium. *Journal of American College of Nutrition*. 6(1): 47 – 51.

Srivastavan, R. C., Hassan, S. K., Gupta, J. and Gupta, S. (1993). Protective Role of Mettalothionein in Nickel Induced Oxidative Damage. *Biochemistry and Molecular Biology International*, 30: 261 – 370.

Stohs, S. and Bagchi, D. (1995). Oxidative Mechanisms in the Toxicity of Metal Ions. *Free Radical Biology and Medicine*, 18(2): 321 – 336.

Svobodova, M. V., Luskova, J., Drastihova, V. and Zlabek, V. (2001). The Effect of Diazinon on Haematological Indicies of Common Carp (Cyprinus carpio). *Acta Veterinaria Brno*, 70: 457 – 465.

Svobodova, Z., Machova, J., KolaA – Ova, J., Vykusora, B. and Piaaka, V. (1996). The Effect of Selected Negetive Factors on Haematological Parameters of Common Carp, *Cyprinus carpio l*. and *Tench, Tinca tinca l*. *Proceedings of Science Papers to 75th Anniversary of Foundation of the RIFCH VodAany*, 95 – 105.

Svodova, Z., Vykusova, B., Machova, J. (1994). The Effects of Pollutants on Selected Hematological and Biochemical Parameters in Fish. In: Muller, R., Llyd, R. (Eds), Sub – lethal and chronic Effects of Pollutants on Fresh Water Fish. *FAO Fishing News Books, Great Britain,* 39 – 52.

Szabo, J. R., Young, J. T. and Granjean, M. (1988). Chlorpyrifos: 13 – Weeks Dietary Toxicity Study in Fisher 344 Rats. *Jackson Research Centre, Health and Environmental Sciences. Texas Laboratory Study No: TXT: K-044793-071. Report.*

Teitelman, U., Adler, M. M., Levy, L. and Dikstein, S. (1975). Treatment of Massive Poisoning by the Organophosphate Pesticide Methidathion. *Clinical Toxicology*, 8: 277 – 282.

Temple, W. A. and Smith, N. A. (1992). Glyphosate herbicide poisoning experience in NewZealand. *NewZealand. Medical. Journal*, 105:173-174.

Thaker, A. M. (1988). Toxicological and Immunological Studies on Long Term Exposure to Malathion and Endosulfan in White Leghorn Chicks. PhD. Thesis Submitted to Haryana Agriculture University, Hisar.

Tisher, C. C. and Brenner, B. M. (1989). Renal Pathology with Clinical and Functional Correlation. *Volume (1) J.B. Lippincott Company. Philadelphia*

Toykuni, S. (1999). Reactive Oxygen Species – Induced Molecular Damage and its Application in Pathology. *Pathology International*, 49: 91 – 102.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M., Mazur, M. and Telser, J. (2007). Free Radical and Antioxidants in Normal Physiological Functions and Human Disease. *International Journal of Biochemistry, Cell Biology*, 39(1) 44 – 84.

Varshneya, C., Bahga, H. S. and Sharma, L. D. (1986). Toxicological Evaluation of Dietary Lindane in Cockerels. *Indian Journal of Poultry Science*, 21: 312 – 315.

Verhaegh, G., Parat, M. O., Richard, M. J. and Hainut, P. (1998). Modulation of P53 Protein Conformation and DNA Binding Activity by Intracellular Chelation of Zinc. *Molecular Carcinogene*, 21: 205 – 214.

Verstraeten, S. V., M. P. Zago, G. G. Mackenzie, C. L. Keen and P. I. Oteiza (2004). Influence of Zinc Deficiency on Cell – Membrane Fluidity in Jurka 3T3 and IMR – 32 Cells. *Biochemistry Journal*, 378: 579 – 587.

Vertuani, S., Angustin, A. and Manfredini, S. (2004). The Antioxidants Network: An Overview. *Journal of Currerent Pharmaceutical Design*, 10(14): 1677 – 1694.

Vivian, D. C. L. and Claudia, B. R. M. (2007).Toxicity and effects of a glyphosate –based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* 147(2):222-231.

Vosilinko, V. and Grebenev, A. (1990). Internal Diseases. *Jordan Journal of Biological Sciences*, 406.

Vural, S., Cetin, E. T. and Tuzlaci, U. (1986). Klink Teshiste Laboraturvar. Nutrettin Uycan Ciltve, Basim Sanayii, Istanbul. http://www.nadirkitap.com/klinik-teshiste-laboraturvar1986-kitap635537.html.

Wannee, J., Upatham, E. S., Maleeya, K., Somphong, S., Suksiri, V. and Prayad, P. (2002). Histopathological Effects of Roundup®, a Glyphosate Herbicide, on Nile Tilapia (*Oreochromis niloticus*). *Science Asia*, 28 : 121 – 127.

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

Witeska, M. (2005). Stress in Fish – Hematological and Immunological Effects of Heavy Metals. *Electronic Journal of Ichthyology*, 1: 35 – 41.

Wolfe, H. R., Staiff, D. C. and Armstrong, J. F. (1978). Exposure of Pesticide Formulating Plant Workers to Parathion. *Bulletin of Environmental Contamination and Toxicology*, 20: 340 – 343.

World Health Organization (1986). Organophosphorous Insecticides : A general Introduction. *Environmental Health Criteria* 63. WHO, Geneva, 181.

World Health Organization (1994a).Glyphosate Environmental Health Critaria No_ 159, Geneva, SwitZerland, 132.

World Health Organization (1994b).Glyphosate Environmental Health Critaria No_ 159. UNEP/ILO/WHO, Geneva, 177.

Yamaguchi, M. (1998). Role of Zinc in Bone Formation and Bone Resorption. *Journal of Trace Elements and Experimental Medicine*. 11: 119 – 135.

Yoshida, A., Kosaka, T., Miyaoka, T., Maita, K., Goto, S. and Shirasu, Y. (1985). Chlorpyrifos – Methyl: 28 – Day Oral Toxicity Study in Mice. Report No: G.HF-R 80, From the Institute of Environmental Toxicology Tokyo, Japan. Submitted to Dow Elanco. Indianapolis, USA.

Zhou, Z., Kang, X., Jiang, Y., Song, Z., Feng, W., Mcclain, C. J. and Kang, Y. J. (2007). Preservation of Hepatocyte Nuclear Factor – 4a is Associated with Zinc Protection Against TNF – α Hepatotoxicity in Mice. *Experimental Biologys and Medicine*, 232: 622 – 628.

APPENDIX

BRIEF HISTORY OF THE STUDY

A total of sixty Wistar rats made up of 30 males and 30 females that weighed between 150g and 350g were used for this study. Twelve were used for LD_{50} determination as described by Lorke (1983) and this was evaluated to be 3750 mg/kg body weight whereas forty eight were divided in to six groups of eight rats each. The rats were grouped based on their weight and each group was made up of four males and four females.

The treatment regiment for the six groups was administered *per os* by gavage once daily for eight weeks as outlined below:

Group I (DW): The rats in this group were administered with distilled water at 2 ml/kg body weight.

Group II (Z): Zinc chloride at the dose rate of 50 mg/kg body weight was administered to the rats in this group.

Group III (G): The rats were administered with glyphosate at the dose rate of 375 mg/kg body weight (10% of the LD_{50}).

Group IV (Z + G): Zinc chloride followed by glyphosate after 30 minutes were administered to the rats in this group at the dose rate of 50 mg/kg and 375mg/kg body weight respectively.

Group V (GC): The rats in this group were administered with glyphosate at the dose rate of 14.4 mg/kg body weight (Recommended concentration by the manufacturers for spraying weeds).

Group VI (Z + GC): Zinc chloride followed by glyphosate after 30 minutes were administered to the rats in this group at the dose rate of 50 mg/kg and 14.4 mg/kg body weight respectively.

The rats in groups II, IV and VI were administered with zinc chloride at the dose rate of 50 mg/kg body weight *per os* by gavage once, two days before the commencement of the research.

Group	Organs	Microscopic Lesion
I (DW)	Brain	No observable lesion
a	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,
	Brain	No observable lesion
	Liver	,, ,, ,,
b	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,

C	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,
d	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,
	Intestine	,, ,, ,,
e	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,

Group	Organs	Microscopic Lesions
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
f	Kidney	No observable lesion
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
g	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,

	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
H	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
II (Z)	Brain	No observable lesion
a	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,

Group	Organs	Microscopic lesions
b	Brain	No observable lesion
	Liver	,, ,, ,,
d	Spleen	No observable lesion
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,
	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Testis	,, ,, ,,

e	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
f	Brain	No observable lesion
	Liver	,, ,, ,,
	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,
	Brain	No observable lesion
	Liver	,, ,, ,,

G	Spleen	,, ,, ,,
	Pancreas	,, ,, ,,
	Heart	,, ,, ,,
	Kidney	,, ,, ,,
	Stomach	,, ,, ,,
	Uterus	,, ,, ,,