AMELIORATIVE EFFECT OF *BORRERIA VERTICILLATA* AQUEOUS EXTRACT ON PATHOLOGY INDUCED BY *ADENIUM OBESUM* AQUEOUS EXTRACT IN JUVENILE *CLARIAS GARIEPINUS* (BURCHELL 1822)

BY

BENEDICT OLUROTIMI MUYIWA

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

OCTOBER, 2018

AMELIORATIVE EFFECT OF *BORRERIA VERTICILLATA* AQUEOUS EXTRACT ON PATHOLOGY INDUCED BY *ADENIUM OBESUM* AQUEOUS EXTRACT IN JUVENILE *CLARIAS GARIEPINUS* (BURCHELL 1822)

By

Benedict Olurotimi MUYIWA, DVM (UNIMAID, 1997)

(P15VTPT8001)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PATHOLOGY

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

OCTOBER, 2018

DECLARATION

I declare that this study entitled "AMELIORATIVE EFFECT OF *BORRERIA VERTICILLATA* AQUEOUS EXTRACT ON PATHOLOGY INDUCED BY *ADENIUM OBESUM* AQUEOUS EXTRACT IN JUVENILE *CLARIAS GARIEPINUS* (BURCHEL L 1822)" has been carried out by me in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of reference provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Benedict Olurotimi MUYIWA ……. ……
Signature Date

CERTIFICATION

This dissertation entitled "AMELIORATIVE EFFECT OF *BORRERIA VERTICILLATA* AQUEOUS EXTRACT ON PATHOLOGY INDUCED BY *ADENIUM OBESUM* AQUEOUS EXTRACT IN JUVENILE *CLARIAS GARIEPINUS* (BURCHELL 1822)" by Benedict Olurotimi MUYIWA meet the regulation governing the award of the degree of masters in Veterinary Pathology of Ahmadu Bello University, and is approve for its contribution to knowledge and literary presentation.

Dr. S.J. SAMBO (DVM, MSc, PhD) ……. ……
Department of Veterinary Pathology, Signature Date
Chairman, Supervisory Committee.

Prof. S.J. Oniye (BSc, MSc, Ph.D) ……. ……
Department of Zoology, Signature Date
Member, supervisory Committee.

Prof. S.B. Oladele (DVM, MSc, PhD, FCVSN) ……. ……
Head of Department, Signature Date
Department of Veterinary Pathology.

Prof. S.Z. Abubakar (B.Sc, MSc, PhD) ……. ……
Dean, School of Postgraduate Studies. Signature Date

ACKNOWLEDGEMENTS

I give the Almighty God the glory for enabling me to initiate and complete this study.

My appreciation goes to my supervisory team-Dr. S.J. Sambo and Professor S.O. Oniye for their fatherly care, mentorship and contribution towards the success of this research work.

Special gratitude goes to the Head of Department of Veterinary Pathology, Professor S.B. Oladele,for the encouragement and fatherly role he played throughout the period of this study.My profound gratitude goes to my Departmental postgraduate lecturers,especially, Professors C.O.Njoku,K.A.N.Esievo,S.O.Akpavie,N.D.G.Ibrahim,S.Adamu, and M.Bissa llah.

My colleagues, Drs.Ochoku Orepoghene,Isaac Akefe, and Austin Ochowechi, contributed to the success of this research.

I am indebted to Mallam Kabir Ibrahim of the Department of Pharmacognosy and Therapeutics, A.B.U., Zaria, for the extraction of the plants and the phytochemical screening, and to Mr. Bitrus Bako for the preparation of the histopathology slides.

My parents, Mr G.K.O. Ogunmuyiwa and Late (Mrs) Deborah Ajayi Ogunmuyiwa, and siblings, Late Mr Victor Olushola Muyiwa, Ms Qristee Muyiwa, Mr David Muyiwa, Mrs Gloria Waba were responsible for whom I have become today.

I also want to thank members of my family, especially my immediate family members for their sacrifices and love throughout this period. My special thanks go to the love of my life my wife, Chinwe (Nkem), for her unfailing support throughout this study. My little angels Mercy (Boluwatinfe), Victoria (Boluwayo) and Jason (Boluwajoba) bearing with my absence during the course of the research.

ABSTRACT

The study was conducted to determine the effect of aqueous extract of *Borreria verticillata* aerial part (BVE) on *Clarias gariepinus* juveniles exposed to *Adenium obesum* stem bark (AOE).The study consisted of ten (10) groups each of fifteen (15) fish per group. Group one,G1(CONTROL),had no extract treatment, G2 (25mg/L, BVE), G3 (50mg/L, BVE), G4 (100mg/L, BVE), G5 (200mg/L, BVE), G6 (0.838 mg/L AOE), G7 (25mg/L BVE + 0.838mg/L AOE), G8 (50mg/L BVE+0.838mg/L AOE), G9 (100mg/L BVE+0.838mg/L) and G10 (200mg/L BVE + 0.838mg/L). The haematological parameters, oxidative stress markers and tissue changes were determined for the ten groups. The phytochemical screening of both aqueous extracts of *Borreria verticillata* (BVE) and *Adenium obesum* (AOE) revealed the presence of glycoside, saponin, steroids, triterpenes, tannins, flavonoid and alkaloids in varying quantities, while cardiac glycoside was found in *Adenium obesum* only. The LC_{50} of aqueous extract *Adenium obesum* stem bark was established as 8.38mg/l in this study. The red blood cell (RBC) counts and erythrocytic indices as well as the serum total protein in G6 decreased significantly ($P<0.05$) compared to groups1-5. The total white blood cell count, differential white blood cell count, serum amino transaminases, serum malondialdehyde, serum reduced glutathione and serum super oxide dismutase increased significantly ($P<0.05$) in G6 with increase in days of exposure to 0.838mg/L of AOE. These values decreased significantly ($P<0.05$) in groups 7-10 during the period compared to group 6 in a concentration and time dependent manner. Histology of gill, liver, intestine and kidney showed no changes in Groups 1 – 5. In Group 6, there were complete fusion of secondary lamella, club-shaped primary lamella, congested branchial blood vessels complete fusion of secondary lamella, club-shaped primary lamella, congested brachial blood vessels and vacuolated primary lamella. The liver showed, congested centrmulti-focal vacuolation, mononuclear

cell infiltration, and presence of melanomacrophage centres. The intestines had hyperplasia of goblet cells. Aggregates of mononuclear cells, tubular degeneration, hypertrophied glomerulus, congestion and perivascular fibrosis were seen in kidneys. Alterations noticed in groups 7-10 on days 14 and 28 include one-sided fusion of the secondary lamella in the gills, some of which were atrophied or hypertrophied. The liver showed congested blood vessels and vacuolation, while the intestine had hyperplasia of goblet cells, hypertrophy of lamina propria and the muscularis mucosa. There were dilated tubules and hypertrophied glomerulus. There was no clear cut distinction between the intestinal lesions seen in G6 compared to G7-10 on days 14-28. Based on the haemato-biochemical and histopathological findings in this study, it was concluded that the aqueous extract of *Borreria verticillata* has an ameliorative effect on the pathology induced by the aqueous extract of *Adenium obesum* stem bark extract in *Clarias gariepinus*, (Burchell, 1822).

TABLE OF CONTENTS

COVER PAGE.....i

FLY PAGE.....ii

TITLE PAGE.....iii

DECLARATION.....iv

CERTIFICATION.....v

ACKNOWLEDGEMENTS.....vi

ABSTRACT.....vii

LIST OF TABLES.....xiv

LIST OF PLATES.....xvii

ABBREVIATIONS.....xx

CHAPTER ONE.....1

1.0 INTRODUCTION.....1

1.1 Background of Study.....1

1.2 Statement of Research Problem.....2

1.3 Justification of the Research.....3

1.4 Research Hypothesis.....5

1.5 Aim and Objectives of the Study.....5

1.5.1 Aim of the study.....5

1.5.2 Objectives of the study.....5

CHAPTER TWO.....7

2.0 LITERATURE REVIEW.....7

2.1 Aquaculture.....7

2.1.1 Aquaculture as source of protein and nutrition.....7

2.1.2 Aquaculture as source of livelihood.....8

2.1.3 Aquaculture and farm efficiency and sustainability in agricultural systems 9
2.2 Fish Species in Modern Aquaculture 10
2.2.1 Tilapia (Oreochromis niloticus) 10
2.2.2 Common carp (*Cyprinus carpio L*) 10
2.2.3 Clariids 11
2.2.3.1 Clarias gariepinus 11
2.2.3.2 Geographical distribution 11
2.2.3.3 Biological description 12
2.2.3.4 Natural food and feeding habit 13
2.2.3.5 Economic importance of Clarias gariepinus 14
2.3 Biomarkers in Fish Health 14
2.3.1 Haematological alteration 15
2.3.2 Biochemical alteration 16
2.3.2.1 Serum total protein 16
2.3.2.2 Serum malondialdehyde 16
2.3.2.3 Aminotransaminases 17
2.3.2.4 Alkaline phosphatase 19
2.3.3 Antioxidants 19
2.3.3.1 Super oxide dismutase 21
2.3.3.2 Reduced glutathione 21
2.4 Selected Target Organs 23
2.4.1 Gills 24
2.4.2 Liver 25
2.4.3 Intestine 27
2.5 Potential of plant extracts in aquaculture 30

2.5.4 The Phytochemical constituents of Plant extracts of importance in aquaculture 33
2.6 The Plant – Borreria verticillata.....35
2.7 The Plant: *Adenium obesum*.....37
CHAPTER THREE 40
3.0 MATERIALS AND METHODS.....40
3.1 Plant Collection, Authentication and Processing.....40
3.2 Extraction Process.....40
3.3 Determination of the Phytochemical Constituents of the Aqueous Extracts.....41
3.3.1 Qualitative Phytochemical Screening.....41
3.4 Study Design.....45
3.4.1 Experimental Animal.....45
3.4.2 Toxicity Bioassay.....46
3.4.3 Experimental design.....46
3.5 Physicochemical Analyses.....50
3.6 Haematological Studies 50
3.6.1 Packed Cell Volume Determination 50
3.6.2 Red Blood Cells and Total White Blood Cell count.....51
3.6.3 Heamoglobin concentration.....52
3.6.4 Differential white blood cell count 53
3.6.5 Haematological indices.....53
3.7 Serum Biochemical Studies.....53
3.7.1 Serum Aminotransferases and Alkaline Phosphatase.....54
3.7.1.1 Serum Aspartate Aminotransferase (AST) Determination 54
3.7.2 Antioxidants.....55
3.7.2.1 Estimation of serum reduced glutathione (GSH) level.....55

3.7.2.2 Determination of serum superoxide dismutase activity.....55

3.7.3 Determination serum total protein 56

3.7.4 Determination of Serum Malondialdehyde (MDA).....57

3.8 Histopathological Studies 57

3.8.1 Tissue processing.....58

3.8.2 Haematoxyline and eosine staining technique.....58

3.8.3 Microscopic evaluations 58

3.9 Data Analyses 59

CHAPTER FOUR.....60

4.0 RESULTS 60

4.1 The Extractive Yield of Aqueous Extracts of *Adenium obesum* Stem Bark and *Borreria verticillata* Aerial Part.....60

4.2 Phytochemical constituents of aqueous extracts of *Adenium obesum* stem bark and *Borreria verticillata* aerial part 60

4.3 Clinical signs in *Clarias gariepinus* exposed to aqueous extract of A*denium obesum* stem bark 63

4.4 Median lethal concentration of aqueous extracts of *Adenium obesum* stem bark and *Borreria verticillata* in *Clarias gariepinus* juveniles.....63

4.4.1 The median lethal concentration of aqueous extract *Adenium obesum* stem bark ...63

4.4.2 The median lethal concentration of aqueous extract *Borreria verticillata* aerial part.....63

4.5.1 Mean Red Blood Cell Count Sub-Lethal Treatment.....65

4.5.2 Mean Hemoglobin Concentration Sub-Lethal Treatment.....65

4.5.3 Mean Packed Cell Volume (PVC) Sub-lethal treatment 65

4.5.4 Mean Erythrocytic Corpuscular Values Sub-Lethal Treatment.....66

4.5.5 Mean Total White Blood Cell Count Sub-lethal Treatment 73

4.5.6 Mean Serum Total Protein Sub-lethal Treatment 73

4.6 Mean Serum Levels of Aminotransferases and Alkaline phosphatase of *Clarias gariepinus* Juveniles Sub-Lethal Treatment.....80

4.6.1 Mean Serum Aspartate Aminotransferase levels Sub-Lethal Treatment.....80

4.6.2 Mean Serum Alanine Aminotransferase Levels Sub-Lethal Treatment.....80

4.6.3 Mean Serum Alkaline Phosphatase Levels Sub-Lethal Treatment.....81

4.7 Effect of aqueous extract *Borreria verticillata* aerial part on serum oxidative stress markers in *Clarias Gariepinus* Juveniles.....85

4.7.1 Mean Serum levels of Malondialdehyde.....85

4.7.2 Mean Serum levels of reduced glutathione.....85

4.7.3 Mean Serum levels of Superoxide Dismutase.....85

4.8 Histopathlogical Studies.....90

4.8.1 Histopathological changes in fish gills during Sub-Lethal Treatment.....90

4.8.2 Histopathological changes in fish Liver during Sub-Lethal Treatment.....90

4.8.3 Histopathological changes in fish Intestine during Sub-lethal treatment.....90

4.8.4 Histopathological changes in fish kidney with Sub-Lethal Treatment.....91

CHAPTER FIVE.....120

5.0 DISCUSSION.....120

CHAPTER SIX.....139

6.0 CONCLUSIONS AND RECOMMENDATIONS.....139

6.1 Conclusions.....139

REFERENCES.....142

APPENDICES.....184

LIST OF TABLES

Table 4.1: Phytochemical constituents of aqueous extract of *Adenium obesum* stem bark and aqueous extract of *Borreria verticillata* aerial part.....61

Table 4.2: Percentage of aqueous extract of *Adenium obesum* stem bark and aqueous extract of *Borreria verticillata* aerial part.....62

Table 4.3: Behavioral Display of *Clarias gariepinus* juveniles *Borreria verticillata* in Range Finding Test.....64

Table 4.4: Mean Red Blood Cell Count (RBC) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part.....67

Table 4.5: Mean Hemoglobin Concentration (Hb) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part.....68

Table 4.6: Mean Packed Cell Volume (PCV) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part.....69

Table 4.7: Mean Corpuscular Volume (MCV) of *Clarias gariepinus* juveniles exposed aqueous extract *Adenium obesum stem* bark treated with *Borreria verticillata* aerial part.....70

Table 4.8: Mean Corpuscular Hemoglobin (MCH) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....71

Table 4.9: Mean Corpuscular Hemoglobin Concentration (MCHC) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....72

Table 4.10: Mean Total White Blood Cell Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....74

Table 4.11: Mean Heterophil Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....75

Table 4.12: Mean Eosinophil Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....76

Table 4.13: Mean Monocyte Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part.....77

Table 4.14: Mean Lymphocyte Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium Obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 78

Table 4.15: Mean Serum Total Protein (TP) level of *Clarias gariepinus* juveniles exposed to sub lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 79

Table 4.16: Mean Serum Aspartate Aminotransferase (AST) Level of *Clarias gariepinus* juveniles exposed to Sub-Lethal Treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 82

Table 4.17: Mean Serum Alanine Aminotransferase (ALT) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 83

Table 4.18: Mean Serum Alanine Phosphatase (ALP) Levels of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 84

Table 4.19: Mean Serum Malondialdehyde (MDA) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 87

Table 4.20: Mean Serum Reduced Glutathione (GSH) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 88

Table 4.21: Mean Serum Superoxide Dismutase (SOD) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part 89

Table 4.22: The pH profile acute bioassay aqueous extract *Adenium obesum* stem bark 96-hr exposure 192

Table 4.23: Dissolved oxygen profile Acute Bioassay Aqueous Extract *Adenium besum* Stem Bark 96-hr exposure 192

Table 4.24: Total dissolved solid profile bioassay aqueous extract *Adenium obesum* stem bark 96-hr exposure 193

Table 4.25:Electrical conductivity profile acute bioassay aqueous extract *Adenium Obesum* stem bark 96-hr exposure 193

Table 4.26: Temperature profile acute bioassay aqueous extract *Adenium obesum* stem bark 96-hr exposure 194

Table 4.27: Probit analysis aqueous extract of *Adenium Obesum* stem bark 195

Table 4.28: Clinical sign exhibiteb in acute bioassay of *Adenium Obesum* stem bark (24Hr-48Hr) 196

Table 4.29: Clinical signs exhibited in the contact phase (72-hr to 96-hr) of the toxicity of aqueous extract of *Adenium Obes*um stem bark 197

Table 4.30: Acute bioassay of aqeuous extract of adenium obesum stem bark mortality profile 96-hour 198

LIST OF PLATES

Plate I : Photomicrograph of the gill in G1 (CONTROL) on D14, showing a normal primary lamella (Black arrow) and a normal secondary lamellae (blue arrow)(H & E, X200). 92

Plate II: Photomicrograph of the gill in G5 on day 14, showing secondary lamellae (black arrow), atrophied secondary lamellae (blue arrow) (H & E, X200). 93

Plate III: Photomicrograph of the gill in G6 on day 14, showing vacuolated primary lamella (yellow arrow), hypertrophy secondary lamella (red arrow).(H & E, X400) 94

Plate IV: Photomicrograph of the gill in G10 on day 14,showing a one-sided fusion of the secondary lamella(red arrow),mis-shapened secondary lamella (black arrow), Telangiectasia within the primary lamella (yellow arrow) (H&E, X100). 95

PlateV: Photomicrograph of the gill G5 on day 28, showing,denuded ,fused,and atrophied secondary lamellae (blue arrow). (H&E, X400). 96

Plate VI: Photomicrograph of the gill in G6 on day 28, showing fusion of secondary lamella (Yellow arrow),clubbed-shaped primary lamella with complete fusion of the secondary lamella (Black arrow) and congested blood vessel (Red arrow) (H&E, X200). 97

Plate VII: Photomicrograh of the gill in G10 on day 28; showing atrophied secondary lamelae(black arrow). (H & E, X100). 98

Plate VIII: photomicrograph of the liver G1; showing a normal parenchyma of the liver tissue, bile duct (Black arrow),central vein (yellow arrow),melanomacrophage centre (blue arrow). (H & E, X200). 99

Plate IX: Photomicrograph of the liver in G5 on day 14, hepatopancrease (yellow arrow), melanomacrophage (blue arrow), diffuse areas of vacuolar degeneration of hepatocytes (black arrow). (H & E,X400). 100

Plate X: Photomicrograph of the liver in G6 on day 14, showing severe vacuolation (blue arrow) (H & E, X2400). 101

Plate XI: Photomicrograph of the liver in G10 on day 14, showing;centrilobular mononuclear cell aggregations(black arrow), hepatocyte vacuolation (yellow arrow).(H & E, X1360). 102

Plate XII: Photomicrograph of the liver in G5 on day 28; showing normal liver parenchyma. (H & E, X40). 103

Plate XIII: Photomicrograph of the liver in G6 on day 28; showing congested central vein (black arrow),severe widespread hepatocellular vacuolation (yellow arrow). (H & E, X2400). 104

Plate XIV: Photomicrograph of the liver G10 on day 28; atrophied hepatopancrease (yellow arrow),blood vessel (blue arrow)(H & E, X200). 105

Plate XV: Photomicrograph of the posterior intestine G1(Normal); showing,intestinal lumen (L), intestinal villi (red arrow), lamina propria (blue arrow), muscularis mucosa (double arrow), serosa (yellow arrow) (H & E, X1440). 106

Plate XVI: Photomicrograph of the posterior intestine G5 on day 14; showing intestinal lumen (L),atrophy of villi (green arrow), muscularis mucosa (double arrow), serosa (yellow arrow). (H & E, X1440). 107

Plate XVII: Photomicrograph of the proximal intestine in G6 of day 14; showing hyperplasia of goblet cells (black arrow), lumen (L). (H & E, X400). 108

Plate XVIII:Photomicrograph of the posterior intestine G10 on day 14; showing,intestinal lumen (L), goblet cell hyperplasia (yellow arrow), muscularis mucusa (double arrow). (H & E, X1280). 109

Plate XIX: Photomicrograph of the sagittal section of the posterior intestine G5 day 28, showing,
normalsecondaryvillus (black arrow), submucosa (blue arrow), muscularis mucosa (doub le black arrow), (H & E, X1280). 110

Plate XX: Photomicrograph of the sagittal section of the posterior intestine in G6 on day 28; showing, intestinal lumen (L), hyperplasia of the goblet cells (yellow arrow), lamina propria (blue arrow),muscularis mucosa (double arrow). (H & E, X1280). 111

Plate XXI: Photomicrograph of the sagittal section of the posterior intestine in G10 on day 28; showing, intestinal lumen (L), villi (green arrow), goblet cell (yello arrow), sub mucosa (blue arrow), muscularis mucosa(double arrow),serosa (black arrow),(H & E, X1280). 112

Plate XXII: Photomicrograph of the transverse section of the kidney G1(Normal), showing; distal convoluted tubule (yellow arrow), proximal convoluted tubule (blue arrow), hematopoietic tissue (H) , glomerulus (black arrow), (H & E, X1280). 113

Plate XXIII: Photomicrograph of the transverse section of the kidney in G5 day 14; showing, heamatopoietic tissue (H), proximal convuluted tubule (red arrow).(H & E, X1680). .. 114

Plate XXIV: Photomicrograph of the transverse of the kidney in G6 on day 14; showing, depleted hematopoietic tissue (H), tubular degeneration (black arrow), distal convoluted tubules (blue arrow). (H & E, X1680). 115

Plate XXV: Photomicrograph of the transverse section of the kidney in G10 on day 14 showing,proximal convuluted tubule (yellow arrow), hematopoietic tissue (H), distal convoluted tubule (black arrow). (H & E, X1120). 116

Plate XXVI: Photomicrograph of the transverse section of the kidney in G5 on day 28; showing, hematopoietic tissue (H),glomerulus (yellow arrow), proximal convuluted tissue (black arrow), distal convuluted tubule (blue arrow). (H & E,X1680). 117

Plate XXVII: Photomicrograph of the transverse section of the kidney in G6 on day28; showing, generalized tubular degeneration and necrosis (H) (H & E, X1680). .. 118

Plate XXVIII: Photomicrograph of the transverse section of the kidney in G10 on day 28; showing, dilated proximal convuluted tubule (blue arrow),distal convuluted tubule (yellow arrow), hematopoietic tissue (H), hypercellularity of the glomerulus (black arrow). (H & E, X1120). 119

Plate XXIX: *Adenium obesum* plant 184

Plate XXX: Borreria verticillata plant 185

Plate XXXI: Clarias gariepinus (Burchell, 1822) 186

Plate XXXII: Concrete Tank for Acclimatization of Fish 187
Plate XXXIII: Acute Toxicity Bioassay AOE 188
Plate XXXIV: Glass Aquirium Tank Sub Lethal Treatment Set-Up before Introduction of Extracts and Stocking 189
Plate XXXV: Fish in sublethal treatment tanks after stocking before the introduction of extracts 190
Plate XXXVI: Aspirating blood from the caudal vein lateral approach using a wet towel to calm the fish 191

ABBREVIATIONS

%	Percentage
ppm	Part per million
% w/w	Percent weight by weight
Kg	Kilogram
g	Gram
mg	Milligram
ml	Millilitre
mg/l	Milligram per litre
$mgKg^{-1}$	Milligram per kilogram
L	Litre
spp	species
Conc.	Concentration
PCV	Packed cell volume
Hb	Haemoglobin
RBC	Red blood cells
TRBC	Total red blood cells
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
WBC	White blood Cell
TWBC	Total white count
Lym	Lymphocyte
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
TP	Total protein
MDA	Malondialdehyde

ALP	Alkaline phosphatase
SOD	Superoxide dismutase
GSH	Reduced glutathione
BE	Aqueous extract *Borrena veitiallala* aerial parts
AOE	Aqueous extract *Adenium obesum* Stem bark
LC_{50}	Median lethal concentration
GESAMP	Group of Experts on the Scientific Aspect of Marine Environmental Protection

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Aquaculture is the fastest growing food sector in the world, accounting for an estimated 43 per cent of all fish consumed by humans globally (Allsopp *et al.,* 2008). Growth in the global supply of fish for human consumption has outpaced population growth in the past five decades, increasing at an average annual rate of 3.2 per cent in the period 1961–2013, twice that of human population growth. This has resulted in an increased average per capita availability and world per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and 19.7 kg in 2013, with preliminary estimates for 2014 and 2015, pointing towards further growth beyond 20 kg (FAO, 2016).

The recent global surge in intensive aquaculture practices has prompted high interest in understanding the various fish diseases, for prevention and treatment purposes. It has been widely proven that the occurrence of diseases in fish farm is due to various factors such as, the rearing methods, environmental conditions and infectious causes. Consequently, farmed fish can become predispoded not only to pathogenic but also to opportunistic bacteria infection (Woo and Bruno,1998).

The use of antibiotics and chemotherapeutics in intensive aquaculture for treatment and prophylaxis has been largely criticized for its negative outcome, and researches on interactions between growth, immunity and development of eco-friendly alternatives to antibiotics to keep fish healthy such as probiotics and plant based immunostimulants has increased, with indigenous developed technological knowledge for treating fish diseases and fish health management is enjoying attention (Sahu,2007).

The global demand for safe food has prompted the search for natural alternative growth promoters for usage in the aquaculture industry. There has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds such as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been assessed for possible development for use in the aquaculture industry (Denev,2008).

Adenium obesum extract has been reported to illicit various histopathological responses in exposed *Clarias gariepinus* (Abalaka *et al.,* 2015), and as a piscicide globally (Badwen-Davis,2010).

Studies have established that extracts from Borreria and Spermacoce species as well as their isolated compounds possessed diversed biological activities,including analgesic,anti-inflammmatory,antitumor,antimicrobial,larvicidal,antioxidant,gastrointestinal,antiulcer,and hepatoprotective in man and rat,with alkaloids and iridoids as the major active principles (Balde *et al*.,1991; Shajiselvin *et al*.,2010 ; Conserva and Ferreira, 2012; Abdullahi-Gero *et al*.,2014,Murtala *et al*.,2015).

1.2 Statement of Research Problem

Aquaculture production is generally vulnerable to disease outbreaks associated with culture intensification, resulting in partial or total loss of production (Bondad-Reantaso *et al.,* 2005). Factors such as overcrowding, periodic handling, high or sudden changes in temperature, poor water quality and nutritional status contribute to physiological changes in fish ,leading to stress or immunosuppression and thus, heighten susceptibility to infection. Moreover, high concentration in a handling tank as is in aquaculture facilitate the spread of pathogens, thereby producing high mortality levels (Cabello, 2006; Naylor *et al.,* 2000; Quesada *et al.,* 2013), leading to economic losses.

In order to avoid economic losses several veterinary drugs are commonly used in aquaculture to prevent or treat disease outbreaks. Antimicrobials and other veterinary drugs are administered regularly as additives in fish feed or sometimes in baths and injections and are used as prophylactics or growth promoters (Rico *et al.,* 2013).The use of veterinary drugs for these purposes present numerous side-effects for the environment and health safety. For example, massive use of antibiotics have resulted in the development of resistant bacteria strains (Mirand and Zemelman, 2002; Seyfried *et al.,* 2010).Therefore the observation of residual antibiotics in the muscle of fish has potential consequences on human health (Cabello, 2006; Romero Ormazábal *et al.,* 2012) Indeed, resistance genes have been found located on transferable plasmids and integrons in pathogenic bacteria such as *Aeromonas* spp., *Edwardsiella* spp. and *Vibrio* spp. (Defoirdt *et al.,* 2011). It has been reported that drug like trichlorfon or praziquantel in bath treatments for ectoparasites in fish also have numerous disadvantages like development of resistance (Umeda *et al*., 2006), hazardous for animal health and detrimental to the environmental (Forwood *et al*., 2013; Kiemer and Black, 1997). Vaccination has also been regarded as a potential treatment against disease outbreaks in aquaculture but commercial vaccines are too expensive for wide spread use by fish producers and apart from that a single vaccine is effective against only one type of pathogens (Harikrishnan *et al*., 2011a; Pasnik *et al*., 2005; Sakai, 1999).

1.3 Justification of the Research

Aquaculture had become a viable industry, with fewer, but much larger farms, infectious diseases are always a hazard and may cause significant stock losses and problems with animal welfare. Intensive aquaculture (shrimp and fish farming) has led to growing problems with bacterial diseases, the treatment of which now requires the intensive use of

antimicrobials (Defoirdt *et al.*, 2007; 2011). Although various authors have emphasized the putative negative effects of using antimicrobial agents in fish farms (Alderman and Hastings, 1998; Cabello, 2006), few studies on antimicrobial resistance in the aquaculture industry have been performed *in situ* (Fernández-Alarcón, 2010; Miranda and Zemelman, 2002).

Considering the potential harm of veterinary drug treatments on the environment and human health and in some cases their limited efficacy, disease management should focus on harmless, preventive and lasting methods. Moreover, disease outbreaks are frequently associated with fish fitness and health, most pathogens being opportunistic and taking advantage of immunocompromised or stressed fish, thus alternate solutions should maximize fish immunity and fitness to avoid and reduce pathogen infections (Ruane *et al*., 1999; Davis *et al*., 2002; Iguchi *et al*., 2003; Ashley, 2007). Some of the proposed solutions are the use of natural products (plant extracts) or probiotics (beneficial microbial strains) in the culture of fish and shrimp (Makkar *et al.*, 2007; Panigrahi and Azad, 2007; Lee *et al*., 2009; Citarasu, 2010; Mohapatra *et al.*, 2013). Interestingly, channel catfish (*Ictalurus punctatus*) fed with the natural oregano essential oil extracted from *Origanum heracleoticum* had the lowest mortality following an *Aeromonas hydrophila* infection compared with fish fed a combination of carvacrol and thymol, which are the principal active components of oregano essential oil (Zheng *et al*., 2009). The utilization of nonconventional feedstuffs of plant origin had been limited as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, momosine, cyanoglycosides, linamarin to mention a few despite their nutrient values and low cost implications (Sogbesan, 2006).

In view of the above given reasons, it is imperative to conduct investigations to find new and possible plant extracts to address antibiotic resistance and synthetic drug that will be useful in the aquaculture industry. There appears to be paucity of literature on the toxicopathology of *Borreria verticillata* on fish species.

1.4 Research Hypothesis

Null Hypothesis (H_o): Aqueous extract of *Borreria verticillata* aerial part has no ameliorative effect on the pathology induced by aqueous extract of *Adenium obesum* on juvenile *Clarias gariepinus.*

1.5 Aim and Objectives of the Study

1.5.1 Aim of the study

To evaluate the effect of *Borreria verticillata* aqueous extract on histopathological, haematological and serum biochemical changes induced by *Adenium obesum* aqueous extract in juvenile *Clarias gariepinus*.

1.5.2 Objectives of the study

The objectives of the study were:

i. To determine the effect of *Borreria verticillata* aqueous extract on alterations in some haematological and serum biochemical parameters of juvenile *Clarias gariepinus* exposed to *Adenium obesum* aqueous extract.
ii. To determine the effect of *Borreria verticillata* aqueous extract on oxidative stress markers in juvenile *Clarias gariepinus* exposed to *Adenium obesum* aqueous extract.

iii. To determine the effect of *Borreria verticillata* aqueous extract on tissue changes induced by *Adenium obesum* aqueous extract in the gill, liver, intestine and kidney of juvenile *Clarias gariepinus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aquaculture

The farming or culturing of aquatic animals and plants in a controlled environment is referred to as aquaculture. Asia, particularly China was believed to be the origin of fish farming as far back as 1100 BC, when common carp were raised in freshwater pond for food (Bondad-Reantaso *et al.,* 2005). Aquaculture is internationally acknowledged as the fastest growing sector of the food producing industry (Bondad-Reantaso *et al.,* 2005). Currently, aquaculture accounts for nearly half (45.6%) of the world's food fish consumption, compared with 33.8% in 2000 (FAO, 2011). Estimates show that this growth will continue as demand increases with the growth in the human population (SOFIA, 2006). World fish-food supply has grown significantly at an average growth rate of 3.2% per year in the period 1961-2009, outpacing the increase of 1.7% per year in the world's population. World per capita food fish supply has also increased from an average of 9.9 kg in the 1960s to 18.4 kg in 2009 (FAO, 2011). Global production of fish from aquaculture has grown substantially in the past decade and hit a landmark in 2009, supplying half of the total fish and shellfish demand for human consumption (Brinker and Reiter, 2011). In 2010, aquaculture production reached 63.6 million tonnes, compared with 32.4 million tonnes in 2000 (FAO, 2012).

2.1.1 Aquaculture as source of protein and nutrition

A unique combination of high-quality protein and vital nutrients make fish an invaluable food. Fish is not only a source of animal protein – 150 g of fish provides about 50 to 60% of an adult's daily protein requirements – but also of fatty acids, vitamins and other

essential elements such as iodine and selenium, which do not occur in such quantity and diversity in cereals, other crops or meat (Beveridge *et al.,* 2013; Kawarazuka and Béné 2011; WRI, 2013). In a global comparison, Africa and Latin America consume relatively little fish (around 10 kg per person per year), whereas per capita consumption in Asia, North America Europe is above the global average (20 kg) at around 22 kg per year. Dietary tradition relates to the fact that countries with a strong fishing tradition due to a long coastline, many fish-rich rivers or islands tend to still consume more fish (FAO, 2016). For 3.1 billion people, fish accounts for 20% of their animal protein; for 4.3 billion people, this share is 15% (FAO, 2016). Some small island states such as Kiribati, Micronesia and the Maldives depend almost exclusively on fish as a protein source (FAO, 2016). The average daily dietary contribution of fish in terms of calories is about 34 calories per capita. In countries where there is a lack of alternative protein food and where there is a traditional preference for fish (e.g. Senegal), as well as in several small island states such as the ones named above, the daily fish calorie intake reaches 130 calories per capita or more (FAO, 2016). Poly-unsaturated fatty acids (PUFAs) in fish lower blood pressure, reduce the risk of heart disease (Wang *et al*., 2006), and possibly benefit infant growth and cognitive development (Koletzko *et al*., 2007). The daily requirement of protein for women is 30-56 g, depending on body weight and age. During pregnancy and lactation, an additional 6 g/day and 17.5 g/day of protein are required respectively, and children's requirement is about 1.5-2.5 times higher by body weight than that of adults, as protein is essential for growth (WHO, 2007).

2.1.2 Aquaculture as source of livelihood

Poverty is a complex phenomenon, which cannot be understood in purely sectoral terms. A series of consultations on small-scale rural aquaculture concluded that aquaculture

should not be viewed as an isolated technology but be considered as one aspect of rural development and form part of a holistic approach to development (APFIC, 2000). A recent FAO/World Bank Farming Systems study noted the importance of five major household strategies for escaping poverty for 70 farming systems across the world: intensification, diversification, increased asset base, increased off farm income, and exit from agriculture. Diversification, which includes aquaculture, was judged to be the single most promising source of farm poverty reduction in the coming years (Dixon *et al.*, 2001). In coastal areas, the farming of mudcrabs, oysters, mussels, cockles, shrimps, fish and seaweeds provides employment for the rural poor, mainly for direct labour inputs, as well as seed and feed collection (Tacon, 2001). Aquaculture can also benefit the landless from utilization of common resources, such as finfish cage culture, culture of molluscs and seaweeds, and fisheries enhancement in communal water bodies (IIRR *et al.* 2001, Tacon 2001).

2.1.3 Aquaculture and farm efficiency and sustainability in agricultural systems

An important, though often overlooked, benefit which is particularly relevant for integrated agriculture-aquaculture systems, is their contribution to increased farm efficiency and sustainability (FAO, 2011). Agricultural by-products, such as manure from livestock and crop residues, can serve as fertilizer and feed inputs for small-scale and commercial aquaculture. Fish farming in rice fields not only contributes to integrated pest management, but also management of vectors of human medical importance (Halwart, 2001). Furthermore, ponds become important as on-farm water reservoirs for irrigation and livestock in areas where there are seasonal water shortages (Lovshin, 2000).

2.2 Fish Species in Modern Aquaculture

2.2.1 Tilapia (Oreochromis niloticus)

Tilapia refers to several freshwater species that belong to the family Cichlidae, and comprises of about 80 species of fish. Tilapia originates from Africa, and because of its easy adaptability to various environmental conditions, has been introduced to many countries. The low cost of production combined with the fact that tilapia is widely accepted by consumers as a food fish, promotes its culture worldwide (Rad *et al.*, 2006; Shalloof and Salama, 2008; El-Kashief *et al*., 2013). World tilapia production for the year 2012 exceeded 4.51 million metric tonnes (FAO, 2012).

2.2.2 Common carp (*Cyprinus carpio L*)

The common carp is a member of the family *Cyprinidae*. Carp are extensively farmed in Europe, Asia, and the Middle East, and are a very popular angling fish in Europe, but in North America, Canada and Australia they are considered a pest (Fisheries Section of NSW Department of Primary Industries, 2006). Most common Carp have broad deep bodies and, compared with the more frequently-fished cultivated varieties of carp, true wild carp are more barbell-like in shape, with the long and lean-body of a hard fighting, fast moving fish. They lack the distinctive hump behind the head of the cultivated carp and rarely reach 35 kg in weight (Fisheries Section of NSW Department of Primary Industries, 2006).

2.2.3 Clariids

Clariids are of great economic importance as food fish, aquaculture candidate and are cultured mostly in Europe, Asia and Africa especially Nigeria (Nwafili and Gao, 2007). In Nigeria, *Clarias* and *Heterobranchus* species are commonly cultured due to their good taste, good flavor, acceptability among fish farmers and consumers (Ayinla, 2007; Olagunju *et al.*, 2007).

2.2.3.1 Clarias gariepinus

Clarias gariepinus, which belongs to the family Claridae, has the synonyms: *Clarias lazera Valenciennes*, 1840; *Clarias mossambicus Peters*, 1864; *Clarias longiceps Boulenger*, 1899 and *Clarias muelleri* Pietsmann, 1939 (FAO, 2011a). *Clarias gariepinus* is named after its locality, the Gariep River, which is a Hottentos name for Orange River in South Africa (Teugels, 1986).

Clarias gariepinus is internationally called "African-Sharp-Tooth catfish" or "North African-Sharp-Tooth catfish" or "Sharptoothed catfish" in English; "Poisson-chat nord-africain" in French; "pez gato africano" in Spanish;" Afrikaanse meerval' Dutch and "Yuzhnoafrikanskaya zubatka" in Russian (FAO, 2011a). However in Nigeria, the fish is locally called "Tarwada" in Hausa; "Arira" in Igbo; "Aro" in Yoruba, "Ejengi" in Nupe and "Emono" in Ijaw language (Yaro, 1967; FAO, 2011a). It is also known as "ailo" amongst the Igala ethnic group of Kogi State in North Central Nigeria.

2.2.3.2 Geographical distribution

Clarias gariepinus has a pan-African distribution, but are naturally absent from the magreb, upper and lower guinea and Cape provinces (FAO, 2011). However, the fish do occur in the Asia Minor of Israel, Syria, Southern Turkey and India (Yildirim and Turan,

2010). In Africa, the fish is widely distributed where it inhabits tropical swamps, lakes, rivers and flood plains that are usually subjected to seasonal drying (FAO, 2011). Although *C. gariepinus* is indigenous to Africa, it is one of the most cultured fish species globally (Lal *et al.,* 2003;Achionye-Nzeh and Jimoh, 2010; Yildirim and Turan, 2010).

2.2.3.3 Biological description

Catfish are generally eel or anguilliform shaped, having an elongated cylindrical body with a dorsal and anal fins, which nearly reaches the caudal fins (Rahman *et al.,* 1992; De Graaf and Jassen, 1996). The head is large, depressed and highly ossified with small eyes (De Graaf and Jassen, 1996).The body is darkly pigmented on the dorsal and lateral sides and through body color is uniformly marbled, it may become mosaic-like pattern of dark and light spots or change from greyish to live to blackish under stress or according to the substrate in addition to the fish turning lighter upon exposure to light (Rahman *et al*., 1992; De Graaf and Janssen, 1996). Clarias gariepinus has light terminal mouth with four barbells (FAO, 2011a), which are used to detect preys (De Graaf and Jassen, 1996).

There are accessory breathing organs (ABOs) within pear-shaped air chambers on both sides of the head that are supported by the second and fourth gill arches with vascular supply from the first and fourth afferent branchial arteries, which are used to extract oxygen directly from the atmosphere (Moussa, 1956). The fish therefore, tolerate low oxygen concentration by utilizing both atmospheric and dissolved oxygen contents of the fish culture water (Okechi, 2004) with help of these accessory respiratory structures, especially in fish above 12 – 14 days old with functionally developed ABOs (Peteri *et al*., 1992). The paired pectoral fins have strongly developed spines that are for both locomotive and protective purposes as the fish can move over long distance as l0ng as 180 m between water bodies (Prinsloo and schoonbee, 1987; Rahman *et al*., 1992).

Clarias gariepinus has a typically pointed cleithrum that is narrow with longitudinal ridges that are sharp, which is used to distinguish it from its closest relative, *C. anguillaris* (Benech *et al.,* 1993). However, the males of *C. gariepinus* have a distinct sexual papilla that is absent in their female counterparts (FAO, 2011a).

2.2.3.4 Natural food and feeding habit

Clarias gariepinus is a slow moving omnivorous predatory fish, which feeds on a variety of food items ranging from zooplanktons to fish that is half of its length or 10% of its own body weight (Bruton, 1979c; De Graaf and Janssen, 1996). The food items of the fish includes insects, crabs, planktons, crustaceans, snails and small fish, fruits, rotten flesh and plants (FAO, 2011). However, the feed composition of *C.gariepinus* change with size (Munro, 1967), becoming zooplankton based in large fish (De Graaf and Jassen, 1996) because of their comparative[y increased gape and increased number of gill rakers (Jubb, 1961; Groenewald, 1964). *Clarias gariepinus* have some adaptations for feeding under low visibility and these include the wide mouth; broad band of jaw and pharyngeal teeth; abundant network of sensory on the body, head, lips and barbels; long gill rakers and a short with dilatable oesophagus and wide rounded caudal fins that is typical of fish which ambush their prey amongst others (Bruton, 1996c). *Clarias gariepinus* is able to switch between feeding on one type of prey to another depending upon availability and the fish's ability to catch its prey as it feeds mainly on invertebrate during the daylight, which are abundant and relatively easy to catch but switches to the more vulnerable fish prey at night (Bruton, 1979b). This is because the fish is physiologically equipped to efficiently utilize infrequent and irregular meals due to the rapid secretory response of the enzymes activity and the absence of a rhythmic cycle of digestive activity (Uys and Hecht, 1987; Uys *et al.,* 1987). Approximately 70% of feeding activity by *C.gariepinus* takes place at

night (FAO, 2011). *Clarias gariepinus* are known to either feed by grasping from the surface or forage or shovel feed while feeding in circles as individuals or as a group (Bruton, 1976). However, the fish is known to cannibalize its own under certain condition but this aberration tend to practically stop after the fish reaches a size of between 50 – 100g in properly fed fish pond (Rahman *et al*., 1992).

2.2.3.5 Economic importance of Clarias gariepinus

Clarias gariepinus is used as "police fish" to control over-breeding in mixed-sex tilapia Culture in earthen ponds (FAO, 2011) thereby enhancing pond productivity for greater aquaculture economic returns. *Clarias gariepinus* have high consumer preference (Huisman and Ritcher, 1987; Fafioye *et al.*, 2004; Ayotunde *et al.*, 2010) and therefore high market price due to its high quality and oily tasty flesh (Sogbesan and ugwumba, 2008; Kori-Siakpere and Ubogu, 2008; Ayotunde *et al.*, 2010).This is in addition to the fact that the fish has high growth rate, it is very resistance to stress and highly appreciated in a number of African countries (de Graaf *et al.*, 1995). These may be why *C. gariepinus* has remained the most cultured fish species worldwide (FAO, 1977) with its attendant economic benefits in terms of employment generation and economic empowerment, especially in developing countries.

2.3 Biomarkers in Fish Health

According to meyer *et al.*, (1992) the term biomarker refer to the use of physiological, biochemical and histological changes as indicators of exposure and/or effects of xenobiotics at organismal. The National Research Council defined biomamker as "a xenobiotically-induced variation in cellular or biochemical components or process,structures or functions that is measurable in a biological system or sample" (NRC,1989).

2.3.1 Haematological alteration

It has been illustrated that the blood variables are indicators of stress and give information on the physiological response of fish to a changing external environment (Hrubec *et al.,* 2001). *Oreochromis niloticus* exposed to cadmium showed significant reduction in RBC, Hb and PCV (Kaoud *et al*., 2011).Blood parameters are considered patho–physiological indicators of the whole body (Adhikari and Sarkar, 2004) and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants. Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition (Venkatesan *et al.,* 2012). Blood of living organisms are very sensitive to changes and are widely used in Ichthyology research, aquaculture research as well as toxicology and biological monitoring (Svoboda *et al*., 2001; Adedeji *et al.,* 2007; Adeyemo, 2008). Because blood is the medium of intercellular and intracellular transport, that is, in direct contact with various organ and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood. Thus, blood provides an ideal medium for toxicity studies. The blood parameters have been considered as diagnostic indices of pathological condition, findings are important for the assessment of systemic functions and overall health of animals. Furthermore, the findings also helps in diagnosing the structural and functional status of animals exposed to the toxicant (Atamanalp and Yanik, 2003; Talas and Gulhan, 2009; Suvetha *et al.,* 2010). It is important in toxicological research because a haematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. A thin epithelial membrane separates fish blood from the water and any unfavourable changes in the water body is reflected in the blood (Shahi and Singh, 2011; Kori-Siakpere and Ubogu, 2008). Haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful conditions such

as handling, pollutants, metals, hypoxia, anaestehtics and acclimation (Jhosi and Bose, 2003; Hori *et al.,* 2008; Alwan *et al.,* 2009).

2.3.2 Biochemical alteration

Biochemical and enzyme activities tend to be more sensitive, less variable, highly conserved between species and often easier to measure as stress indices (Agrahari *et al*., 2007).Diagnosis of metabolic disturbances and disease processes can be detected through the bioassay of serum constituents (Campbell, 2012).

2.3.2.1 Serum total protein

The quantification of serum total protein does not directly indicate the presence of a disease condition but it is indicative of a disease process and it assist in confirmatory diagnosis (Walencik and Witeska,2007). The total protein concentrations are especially valuable in determining a fish's state of hydration. In serum, total protein, albumin, globulin and albumin-globulin ratios are measured.A decrease in protein content is known as hypoproteinemia and it has been reported in cases of starvation, toxications affecting the kidneys (Asztalos, 1990); the liver (Wagner and Congleton, 2004) and in parasitic disease (Roberts, 2012). An increase in total serum protein termed hyperproteinemia has been reported in chemical toxicosis affecting the circulatory system (Adeyemo, 2003;Roberts, 2012).

2.3.2.2 Serum malondialdehyde

When fish are subjected to oxidative stress conditions, fatty acid peroxidation can occur. Indeed, increased ROS production and subsequent oxidative damage has been associated with pollutant-mediated mechanisms of toxicity in fish liver (Livingstone *et al.,* 1993). Malondialdehyde (MDA) production is a well-known oxidation product of

polyunsaturated fatty acids, influencing cell membrane fluidity as well as the integrity of biomembranes (Ercal *et al.,* 2001; Almroth *et al.,* 2005), and can be used as an indicator of lipid peroxidation.

Lipid peroxidation secondary product such as thiobarbituric acid (TBARS) formed by reaction of singlet oxygen with unsaturated fatty acids and is considered as useful and reliable indicator of oxidative damage in cells and tissues due to susceptibility of membranes to attack by reactive oxygen species (Draper *et al.,* 1993; Hodges *et al.,* 1999). Aquatic organisms detoxify organic xenobiotics by phase I metabolism, however, during the detoxification process, highly reactive oxygen species (ROS) such as superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are produced as by-products.Excessive ROS production in response to xenobiotic detoxification can overwhelm natural defense mechanisms leading to cumulative damage to biomolecules namely nucleic acid, proteins and lipids (Kelly *et al.,* 1998). A radical attack on lipids leads to the formation of lipid peroxides, which can decompose to yield alkanes, ketones and aldehydes. The aldehydes most extensively studied are 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal and malondialdehyde (MDA) (Zielinski and Portner, 2000). The quantification of the diverse products of peroxidation especially level of MDA is now being exploited as biomarker of oxidative stress. This quantification has enabled early detection of stress in exposed organisms before lethal or pathological effects are observed (Otitoju and Olagoke, 2011).

2.3.2.3 Aminotransaminases

The activity of AST and ALT enzymes in blood may be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James *et al*., 1991; Svoboda, 2001). Alanine

aminotransaminase (ALT) and aspartate aminotransaminase (AST) are blood serum enzymes which function as a link between carbohydrate and protein metabolism by catalysing the inter conversion of strategic organic compounds such as α–ketoglutarate to pyruvic acid and alanine to glutamic acid (Tiwari and Singh, 2004).

Aspartate transaminase (AST) and alanine transaminase (ALT) are enzymes frequently used in the diagnosis of damage caused by pollutants in various tissues such as the liver, muscle and gills (de la Torre *et al.*, 2005). Of these two enzymes, ALT is specific for liver damage because it predominates in organs with intensive glycogenesis (Ulrich, 1994; Torre *et al.*, 2000). AST is present in myocardium, skeletal muscle, kidney and brain and increased plasma activity may be indicative of degenerative changes in these organs. Increased plasma ALT and AST activities are a measurable biochemical symptom of cytolysis as aminotransaminases play vital roles in carbohydrate-protein metabolism in fish (Eze, 1983). They occupy a central position in amino acid metabolism in that they help in retaining amino groups during degradation of amino acid and are involved in the biochemical regulation of intracellular amino acid group. They also provide necessary intermediates for gluconeogenesis as such elevated levels in plasma may have an adverse effect on amino acid metabolism (Murray *et al*., 1996). An increased activity of these aminotransaminases is an indication of amplified transamination process (Kori-Siakpere *et al*., 2005). Also alterations in the metabolism of proteins and carbohydrates are used for a similar purpose. The treatment of *Clarias batrachus* with carbaryl led to a marked increase in the activities of liver transaminases (Sharma, 1999). Cells contain enzymes that are necessary to their function. When the integrity of a cell is disrupted, enzymes escape into plasma/serum, where their activity can be measured as a useful index of cell integrity (Coppo *et al*., 2002). Changes in the biochemical blood profile indicates changes in metabolism and biochemical processes of the organism, resulting from the effect of

various pollutants and they make it possible to study the mechanisms of the effects of various pollutants (Luskova *et al*., 2002).

2.3.2.4 Alkaline phosphatase

Chronic hepatic disorders and excessive steroids results in increase plasma alkaline phosphatase (ALP) in most animal. During normal bone growth in young animals, a large amount of ALP is found in plasma; also ostheopathies result in increase of plasma ALP (Coppo *et al*., 2002). According to Sevigler *et al*. (2004), alkaline phosphatase an enzyme involved in bone growth and liver function, may be affected by low level fluoride intake and fluoride switches off the enzyme cytochrome c oxidase, an oxygen-carrying respiratory enzyme by oxidation.

2.3.3 Antioxidants

The oxidative stress defenses are provided by the markers which includes enzymes and scavengers. Enzymatic defenses include glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) (Jakoby and Habig, 1980; George, 1994; Yang *et al.,* 2000; Sanchez *et al.,* 2008; Haluzova *et al.,* 2011). Reduced glutathione (GSH) and ascorbic acid are the most important antioxidant found in the extracellular fluid (Sies and Stahl, 1995). They are water soluble and scavenge a number of different reactive oxygen species (ROS) including thiol radicals generated during xenobiotic reduction (Palozza and Krinsky, 1992; Kelly *et al.,* 1998; Palace *et al.,* 1999). The scavenging and enzymatic antioxidants are linked in many ways, often if one is deficient; others increase to compensate (Puangkaew *et al.,* 2005).

Fish are particularly sensitive to water contamination and pollutants may impair many physiological and biochemical processes when assimilated by fish tissue (Durmaz *et al.,*

2006). When abnormal or xenobiotic-induced ROS production exceeds the endogenous protection, damage to cellular components can often be observed, this process is known as oxidative stress (Oakes and Van der Kraak, 2003). The antioxidant defense system includes enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GST) and other low molecular weight scavengers such as reduced glutathione (GSH) (Storey, 1996; Droge, 2002; Monteiro *et al.,* 2009). Oxidative damage is counteracted by antioxidant defense systems and repair mechanisms. The antioxidant defense systems comprise of a number of enzymes which act as scavengers of the highly reactive intermediates produced in cells during hydrocarbon metabolism to maintain cell homeostasis (Olushola *et al*., 2014). Notable antioxidant enzymes include (a) superoxide dismutase (SOD), which converts superoxides (O^{2-}) generated in peroxisomes and mitochondria to hydrogen peroxide; (b) catalase (CAT) which removes the hydrogen peroxide by converting it to water and oxygen; (c) glutathione *S*-transferase (GST); (d) glutathione peroxidase and (e) glutathione reductase all of which are involved in the removal of hydrogen peroxide from the system in conjunction with reduced glutathione (GSH) (Olushola *et al*., 2014).

Antioxidant enzymes of fishes, that play a crucial role in maintaining cell homeostasis, have received much attention in ecotoxicology since oxidative damage was considered a mechanism of toxicity in aquatic organisms exposed to environmental contaminants in general (Santos *et al*., 2004). Aerobic organisms generate reactive oxygen species (ROS), such as superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), because of oxidative metabolism. The hydroxyl radicals can initiate lipid peroxidation (LPO) in tissues. To attenuate the negative effects of ROS, fish possess an antioxidant defense system like other vertebrates that utilizes enzymatic and non-enzymatic mechanisms. The most important antioxidant enzymes are superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST). The non-enzymatic defense system includes Vitamins E, C, and A, glutathione, carotenes and ubiquino (Filho, 1996).

Antioxidants protect an organism against oxyradical damage, such as DNA strand breaks, protein oxidation and the induction of lipid peroxidation (Winzer *et al.,* 2000). The increase in the activated form of molecular oxygen species due to overproduction and/or to the inability to destroy them may lead to damage in the DNA structure and thus may cause mutations, chromosomal aberrations, and carcinogenesis. A shift to a more oxidative state or any imbalance between production and degradation of reactive oxygen species (ROS) in animal tissues may cause lipid peroxidation, plasma membrane alterations, and inactivation of enzymes (Anand *et al.,* 2000). The use of the biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish. The use of oxidative stress biomarkers for stress evaluation have rapidly increased in the field of ecotoxicology, therefore, it has been suggested that they could be used in environmental monitoring systems (Pandey *et al.,* 2003).

2.3.3.1 Super oxide dismutase

Super oxide dismutase (SOD) is an antioxidant enzyme important in inhibiting oxyradical formation and is used as a biomarker to indicate oxidative stress. SOD catalyzes the transformation of superoxide radicals to H_2O_2 and O_2, and is the first enzyme to deal with oxy-radicals (Zhang *et al.,* 2007).

2.3.3.2 Reduced glutathione

Reduced glutathione (GSH) is a powerful antioxidant as it binds to many different toxicants, inactivating them (Kelly *et al.,* 1998). GSH protect the membrane-

polyunsaturated fatty acid hydrogen of sulfuhydryl group instead of methylene hydrogen .of unsaturated lipid (Arima and Shiba, 1992). Moreover, GSH serves as a cofactor for glutathione transferase, which facilitates the removal of certain chemicals and other reactive molecules from the cells. It can also interact directly with certain ROS (e.g., hydroxyl radical) for their detoxification as well as perform other critical activities in the cell (Stara *et al.*, 2012**).**

2.3.4 Histopathological observation

Microscopic observations, either through light microscopy or electron microscopy, is an inevitable tool in the interpretation of the parameters used to measure toxicity, and contributes to the establishment of measures that aim to prevent the environmental contamination, a risk not only to ecosystems, but also to human health. In this context, cellular biomarkers including histopathological and ultrastructural effects represent an intermediate level of biological organization between lower-level biochemical effects and higher-level population effects (Adams *et al.*, 1989, 2001) which ultimately provide a better evaluation of organism health than a single biochemical response (Triebskorn *et al.*, 1997; Hued *et al.*, 2012). Abalaka *et al.* (2015) reported, lamellar necrosis, mononuclear infiltration, fusion of secondary lamellar, lamellar edema, lamellar hemorrhage and epithelial detachment in the gill of *Clarias gariepinus* exposed to ethanol extract of *Parkia biglobosa.* Skin histopathology such as proliferated and hypertrophied mucous cells; eroded epithelia surface, elaborated mucous cells, and eroded epithelial surface was also reported by Abalaka *et al.* (2015) in *Clarias gariepinus* exposed to *Parkia biglobosa.* Tasneem *et al*. (2014) reported dilation of secondary lamellar, atrophy, mononuclear cell infiltration in the primary lamellar, vacuolation of the secondary lamellar in the gill, and

vacuolation in the villi, inflammatory cell infiltration in the epithelium of the intestine of *Oreochromis mossambicus* exposed to *Carica papaya* and *Nerium oleander*.

2.4 Selected Target Organs

Pathology, as a standard part of environmental monitoring programmes on effects of pollution, was approved by Moore (1980); Balouet and Poder (1981) and Couch (1985). Histopathology is the study of lesions or abnormalities on a cellular level in fishes. Histopathological examination is widely recognized as a reliable method for disease diagnosis and for assessing acute and chronic effects of exposure to toxicants at the cellular level in both marine and fresh water species (Gary-Ostrander, 1996).As an indicator of exposure to contaminants, histology constituents a useful tool for assessing the degree of pollution, particularly for sub lethal and chronic effects (Bernet *et al.,* 1999). Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. Histopathological lesions are related to the biochemical changes that occur in the organism. As well as from chemical insult, histopathological lesions may arise from infectious diseases and parasites, provoking necrotic and degenerative alterations to which the organismresponds with an inflammatory, defensive reaction (Velkova-Jordanoska, 2002; Roganovic-Zafirova *et al.,* 2003). Gills (Poleksic and Mitrovic-Tutundzic, 1994), Kidney (Bucher and Hofer, 1993), and liver (ICES, 1997) are suitable organs for histological examination in order to determine the effect of pollution. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Thophon *et al.,* 2003) and field studies (Schwaiger *et al.,* 1997; The *et al.,* 1997). Histopathological biomarkers in environmental monitoring is that this category of

biomarkers allows examining specific target organs, including gills, kidney and liver that are responsible for vital functions in the fish (Gernhofer *et al.,* 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta *et al.*, 2003) and serve as warning signs of damage to animal health (Hinton and Lauren, 1990). Histopathological changes were observed in the muscle of fish exposed to different toxicants (Das and Mukherjee, 2000; Elnemaki and Abuzinadah, 2003; Abbas and Ali, 2007).

2.4.1 Gills

The gill arches and filaments are supported by a branching system of cartilaginous rods. Two sets of striated muscles (abductor and adductor) are present in the gill arch. Embedded in the superficial tissue of the gill arches are numerous taste buds, supplementing those scattered abundantly throughout the buccal cavity (Yasutake and wales, 1983). The filament (also known as the primary lamellae) consist of blood vessels, cartilaginous gill rays, melanocytes, lymphocytes, macrophage, coarse eosinophillic whites, undifferentiated cells in the central part of the primary lamellar epithelium are thought to be the stem cells of all the other types of cells (Dang, 2000). The lamellae (secondary lamellae) consist of mucus cells, rodlet cells, chloride cells, pavement cells, pillar cells with lacunae and blood vessels. The importance of the gills in respiration mechanism and ionic regulation of fish had encouraged many investigations of the effects caused to this organ by changes in environmental factors (De La Torre, 2005; Nigro *et al.*, 2006). The gills, participates in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water and are considered the primary

target of the contaminants (Poleksic and Mitrovic-Tutundzic, 1994; Mazon *et al*., 2002; Fernandes and Mazon, 2003).

2.4.1.1 Gill as biomarker of exposure to toxicant

The function of the gills necessitates the exposure of a system of capillaries with sufficient surface area to the water to facilitate the required gas exchange. Gills of fish constitute up to 90% of the total body surface area and are in intimate contact with ambient water (Yasutake and Wales, 1983;Mayer-Gostan *et al.,* 1987; Perry and Laurent, 1993). Fish gills are the primary uptake site of toxicologically effective concentrations water metals (Pelgrom *et al.,* 1995; Sprague, 1987) and are considered the crucial organ when it comes to the induction of compensatory responses, whether adaptive or pathological to the organism (Pelgram *et al.,* 1995; Tao *et al.,* 2001; Taylor *et al.,* 2002).

2.4.1.2 Histopathology of the gill

Gill histopathological changes are, in general, responsive but nonspecific to pollutant exposure. Epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, edema with epithelial lifiing and epithelial desquamation are typical histopathological lesions of gills in response to a wide range contaminants, including organochlorins, petroleum compounds, organophosphates, carbamates, herbicides and heavy metals (Baker, I969; Gardner and Yevich, 1970; Van der Putte and Paert, 1982; Hemalatha and Banerjee, 1997; Global Tox, 1997).

2.4.2 Liver

Fish liver is a dense organ located ventrally in the cranial region of the body cavity. The size, shape and volume of the liver are adapted to the space available between other visceral organs (Brusle and Gonzalez, 1996; Vincetini *et al.,* 2005). Fish liver is generally

reddish-brown because of its rich supply of blood. The liver is nourished by a dual blood supply via two afferent blood vessels (hepatic artery and portal vein) and a single efferent vessel (hepatic vein) located at the helium (Munshi and Dutta, 1996).

2.4.2.1 Liver as biomarker of exposure to toxicant

Constant exposure to xenobiotic substances, example toxicants, may overwhelm the liver's detoxification capability and cause some degree of structural damage within the liver parenchyma. The liver may thus be expected to be the primary target of toxic substances, providing an excellent biomarker of aquatic pollution (Braunbeck and Volki, 1993). The liver is the major site for the enzymatic detoxification of xenobiotics, nutrient derived from gut absorption are stored in the hepatocytes and released for further catabolism by other tissues; bile synthesized within hepatic cells aids in the carrying of conjugated metabolites of pollutants to the intestine for excretion; and vitellogenin, a major yolk protein is exclusively synthesized within the liver (Lauren, 1990).

2.4.2.2 Histopathology of the liver

Mathur (1965) reported marked degeneration of liver in *Ophiocephalus punctatus, Barbus stigma, Trichogaster fascitus* and *Heteropneustes fossilis* due to dieldrin toxicity. Welling and McCain (1976) and Maline (1980) reported high frequency of hepatic hypertrophy, hepatic tumours and other liver diseases of bottom dwelling fishes due to polynuclear hydrocarbon contamination in sediments of aquatic system. Amminikutty and Rege (1977) reported rapid degeneration and vacuolation in liver tissues of fish due to chronic exposure of Thiodon E.C and Agallol 3. According to Dubale and Shah (1979), necrosis and cytoplasmic disintegration occurred in hepatopancreas of *Channa punctatus*, due to Malathion exposure. According to Mandal and Kulashrestha (1980) toxicity of DDT could produce empty blood vessels, necrosis, vacuolation, break down of cell

boundaries and formation of multinucleated giant cells in the liver of *Clarias batrachus*. Altemirano-Reynoso (1984) observed lindane induced hydropic degeneration and epithelial necrosis in the liver of *Tilapia hornorum*. Ray and Bhattacharya (1984) in their histopathological study on liver of *Anabas testudineus* found that precipitation of cytoplasm, vacuolation of cells, nuclear degeneration, hepatic chord disarray *etc.*, occurred due to chronic exposure of cythion. Urdaneta (1989) also observed hepatic necrosis in some species of cultivable fishes due to exposure of 12 different pesticides. John *et al.* (1993) explained the impact of endosulfun on liver of *Cyprinus carpio* and observed some important histopathological alterations characterized by indistinct cell boundaries, loss of polygonal shape of hepatocytes. Risbourg and Bastide (1995), reported that vacuolization of hepatocytes, focal necrosis, hydropic degeneration, cloudy swelling occurred in fish liver exposed to atrizine herbicide. Neskovic *et al.* (1996) recorded early sign of fibrosis, congestion of sinusoids in liver of *Cyprinus carpio* exposed to glyphosate for sub-acute toxicity test.

2.4.3 Intestine

Histological structure of this part of the digestive system is: mucosa, which consists of the lamina epitelialis (simple columnar and glandular epithelium) and lamina propria (connective tissue); submucosae, consisting of two layers (stratum compactum and stratum granulosum); muscular layer and serosa (Poleksić *et al.,* 2006). This histological structure is not altered even in experiments in which fish were fed food containing heavy metals (Andreozzi *et al.,* 1994; Kruatrachue *et al.,* 2003). The histology of the intestine in many teleosts has also been studied (Ezeasor and Stokoe, 1981; Park and Kim, 2001; Cinar and Senol, 2006; Diaz *et al.,* 2008). The structure and function of the stratum compactum seen in the sub mucosa have been reported in teleost intestine. The cells of

the gut associated lymphoid tissues which include eosinophilic granular cells, intraepithelial lymphocytes and wandering leukocytes have been documented as part of intestinal local defense mechanism (Ezeasor and Stokoe, 1981; Dorin *et al.,* 1993; Powell *et al.,* 1993; Delashoud *et al.,* 2010). The nature and form of teleost mucosal folds have also been reported (Reifel and Travill, 1979), even their role in nutrient absorption (Ezeasor and Stokoe, 1981).

2.4.3.1 Intestine as biomarker of exposure to toxicant

Different authors like Llyod (1960), McCarty *et al.,* (1978), Datta and Sinha (1989), Ghosh and Chakrabarty (1990) in different occasions studied the effects of different xenobiotics on fish alimentary canal. Braunbeck *et al.,* (1992) found that the fish gut was less sensitive to the impact of environmental pollutants

2.4.3.1 Histopathology of the intestine

In fish intestine enteritis represents a set of changes visible at the light microscopy level, described and named by Baeverfjord and Krogdahl (1996) as "non-infectious subacute enteritis": shortening of intestinal villi, loss of supranuclear vacuolization of the enterocytes, widening of lamina propria of villi, and infiltration of inflammatory cells in the lamina propria.

Kumari and Kumar (1997) reported several alterations in intestine of *Channa punctatus* like, desquamation, degeneration and proliferation in villi, hyperactivity of goblet cells, focal necrosis, dialated blood vessels and accumulation of mucous in lumen due to aquatic pollution. Braunbeck and Appelbaum (1999) described distension of the intercellular space, proliferation of lysosomal compartment and stimulation of lysosomes within the cells in the intestine of *Cyprinus carpio*, exposed to endosulfun.

Histopathological effects of insecticides on intestine of fish have been studied by several authors (Tilak *et al.,* 2003, 2005; Prashanth, 2003; Tilak and Yacobu, 2002; Thorat, 2001). Triebskorn *et al.* (2004) observed enlargement and distortion in mucous cells, heavily dialated epithelial cells in the intercellular spaces between epithelial cells, in the intestine of rainbow trout *(Onchorhynchus mykiss*) exposed to anti-inflammatory drug diclofenac at a dose of 1 to 500 µg/l. Soufy *et al.* (2007) revealed epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosal oedema in the intestine of tilapia fish exposed to carbofuran. Ramírez-Duarte *et al.* (2008) described there was no morphological alteration occurred in the intestine of fish cachama blanca (*Piaractus brachypomus*) due to Roundup exposure. They also observed the mild hyperplasia of mucous cells in stomach of *Piaractus brachypomus* after the exposure of Roundup at 7.5 to 120 mg/l dose. Mohamed (2009) observed necrosis, oedema in mucosa and submucosa layer; dilation of blood vessels of serosa and atrophy in muscularis and submucosa in intestine of *Tilapia zillii* and *Solea vulgaris* due to environmental pollutants in aquatic body. Ravanaiah and Narasimhamurthy (2010) observed vacuolization, pycnotic nuclei, distorted villi and serosa layer, necrosis in mucous epithelium, enlarged goblet cells in intestine of *Tilapia mossambica* due to irritants.

2.4.4 Kidney

2.4.4.1 Kidney as biomarker of exposure to toxicant

The teleostean kidney is one of the organs to be affected by contaminants in the water (Thophon *et al.,* 2003). The kidney is a highly dynamic organ in most of the vertebrates; it receives about 20% of the cardiac output, chemicals substances in the systemic circulation are delivered in relatively high amounts to the kidney. The kidney of the fish

receives largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz *et al.,* 2003).

2.4.4.2 Histopathology of the kidney

Most common alterations found in the kidney of fishes exposed to water contamination are tubular degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman's space (Takashima and Hibiya, 1995). Exposure to metals frequently causes alterations in the tubules and glomerulus, such as described by Thophon *et al.* (2003) for the perch (Lates calcarzfer) exposed to cadmium, Handy and Penrice (1993) found swollen Bowman's capsule cells and melanomacrophages in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to mercuric chloride. Similar alterations were found in fishes exposed to organic contaminants (Veiga *et al*., 2002) and mixed environmental; contaminants (Schwaiger *et al.*, 1997; Pacheco and Santos, 2002). These reports suggest that the histopathological changes in the kidney, like in gills, could not be considered specific to the stressors. Granules may be formed inside the cells or by the re absorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton and Lauren, 1990). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995).

2.5 Potential of Plant Extracts in Aquaculture

Plant extracts have been reported to favor various activities like antistress, growth promotion, appetite stimulation, enhancement of tonicity and immunostimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish and shrimp aquaculture due to active principles such as alkaloids, terpenoids , tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils (Chakraborty and Hancz,

2011; Citarasu, 2010). Besides, their use could reduce costs of treatment and be more environmentally friendly as they tend to be more biodegradable than synthetic molecules and they are less likely to produce drug resistance in parasites due to the high diversity of plant extract molecules (Blumenthal *et al.,* 2000; Logambal *et al.,* 2000; Olusola *et al.,* 2013).

2.5.1 Plant extracts as appetite stimulators and growth promoters

Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured fish (Pavaraj *et al.,* 2011; Takaoka *et al.,* 2011; Harikrishnan *et al.,* 2012a). Shalaby *et al.* (2006) showed that food intake, specific growth rate and final weight of Nile tilapia (*Oreochromis niloticus*) increased when garlic was incorporated in the diet. In another study, grouper Ephinephelus tauvina fed with a diet supplemented with a mixture of methanolic herb extracts of Bermuda grass (*Cynodon dactylon*), Long pepper (*Piper longum*), stonebreaker (*Phyllanthus niruri*), coat buttons (*Tridax procumbens*) and ginger (*Zingiber officinalis*) displayed 41% higher weight than fish fed with the control (Punitha *et al.,* 2008). Ji *et al.* (2007a) showed that olive flounder (*Paralichthys olivaceus*) fed with a herbal mixture of medicated leaven (*Massamedicata fermentata*), hawthorne (*Crataegi fructus*), virgate wormwood (*Artemisia capillaris*) and *Cnidium officiale* (2:2:1:1) had higher weight gain than the control fish and showed higher total carcass unsaturated fatty acid content and lower carcass saturated fatty acid content, indicating that feeding with the herbal mixture improves fatty acid utilization. The authors suggested that this could be caused by a lower plasma triglyceride and high plasma HDL-CHO (high-density lipoprotein cholesterol) levels in the herbal mixture diets. Besides, plant extracts have been shown to improve digestibility and availability of nutrients resulting in an increase in feed conversion and leading to a higher protein

synthesis (Nya and Austin, 2009; Citarasu, 2010; Talpur and Ikhwanuddin, 2013).Putra *et al.* (2013) showed that supplemented diet with 1% of ethanolic katuk extract (*Sauropus androgynous*) stimulated appetite, growth and improved food utilization (lower feed conversion ratio) in grouper *Ephinephelus coioides.*

2.5.2 Plant extracts as immunostimulants

The immune system is classified into innate (non-specific) and adaptive (specific) immune systems. The innate immune system is the first line of defense against invading pathogens and their major components are macrophages, monocytes, granulocytes, and humoral elements, including lysozymes or complement systems (Harikrishnan *et al.,* 2009b). An immunostimulant is a substance that enhances the defense mechanisms or immune response (both specific and nonspecific), thus rendering the animal more resistant to diseases and external aggressions (Anderson, 1992). An increasing interest in the use of plant extracts as fish immunostimulants has arisen in the last decade (Galina *et al.,* 2009; Vaseeharan and Thaya, 2013). Several studies have monitored the immunological parameters after intraperitoneal injection or orally administered plant extracts on distinct fish species and they found that treated fish showed increased lysozyme activity, phagocytic activity, complement activity, increased respiratory burst activity and increased plasma protein (globulin and albumin) (Dugenci *et al.,* 2003; Wu *et al.,* 2010; Yuan *et al.,* 2007).

Other studies analyzed the haematological parameters which provides a clue of the fish health status and they found that erythrocytes, lymphocytes, monocytes, hemoglobin and hemocrit levels significantly increased in fish treated with plant extracts compared to control fish (Shalaby *et al.,* 2006; Innocent *et al.,* 2011; Harikrishnan *et al.,* 2012a).

Lysozyme activity and burst respiratory activity of olive flounder increased more when treated with plant extract (Harikrishnan *et al.,* 2011b).

2.5.3 Plant extracts as fish anti-pathogenic

Bioactivities displayed by natural products from plants, fungus and algae, have revealed to be of great interest in the prevention or treatment of pathogens (Tagboto and Townson, 2001; Zahir *et al.*, 2009; Zheng *et al.*, 2007).

2.5.4 The Phytochemical constituents of Plant extracts of importance in aquaculture

Essential oils (EOs) are volatile liquid fractions that contain the substances responsible for the aromas of plants; they are obtained from different organs, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Bakkali *et al*., 2008). There are several methods for extracting essential oils, including expression, fermentation, enfleurage, extraction, and the use of liquid carbon dioxide or microwaves, but the steam distillation method is most commonly used for the commercial production of EOs (Burt, 2004). The chemical composition of Eos depends on the extraction method and can also vary according to conditions such as climate, soil composition, origin, season, plant organ, age and vegetative cycle stage (Angioni *et al.,* 2006; Abu-Darwish *et al.,* 2011; Ben Marzoug *et al.,* 2011; Chung *et al.,* 2011; Ennajar *et al.,* 2011; Karakaya, 2011 Masotti *et al.,* 2003).The composition of EOs is very complex and can include more than sixty components; however, a few major components constitute up to 85% of the EOs and generally determine their biological properties (Bakkali *et al.,* 2008), whereas other components are present only as traces (Rali *et al.,* 2007; Bader *et al.,* 2010). The components include two groups of distinct biosynthetic origin that are synthesized in secondary metabolism (Pichersky *et al.,* 2006; Nagegowda, 2010). The main group is

composed of terpenes, terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weights (Bakkali *et al.,* 2008).

The primary roles of essential oils in plants are believed to be as pollinator attractors and defenses against pathogens and pests due to their antibacterial, antiviral and antifungal.

2.5.5 The potency and mode of action of plant extracts

The effect of plant products on fish is dose-dependent and there is a potential for overdosing, so determining the suitable extract concentration is of great importance (Kajita *et al.,* 1990; Harikrishnan *et al.,* 2011a). The molecular basis of the antibacterial action of essential oil is poorly understood. It has been suggested that they can disrupt the permeability of the bacterial cell membrane (Turina *et al.,* 2006; Pascua *et al.,* 2007; Bouhdid *et al.,* 2009), leading to the disruption of the proton motive force, electron flow and active transport (Helander *et al.,* 1998; Cox *et al.,* 2000; Lambert, 2001;Ultee *et al.,* 2002; Fisher *et al.,* 2009). Other proposed mechanisms are related to the coagulation of cell contents (Lambert, 2001, Becerril *et al.,* 2007), and recently, evidence has been provided for the inhibition of quorum sensing (Brackman *et al.,* 2008; Kahn *et al.,* 2009), the induction of heat shock proteins and the prevention of flagella development (Burt, 2004). Interestingly, cinnamaldehyde was recently found to interfere with autoinducer-2 (AI-2), which is involved in quorum sensing in *Vibrio* spp., by decreasing the DNA-binding ability of LuxR, resulting in several marked phenotypic changes, including reduced virulence.Because inhibitors of AI-2-based quorum sensing are rare, and given the role of AI-2 in several processes, these compounds may provide useful leads for antipathogenic drugs (Brackman *et al.,* 2008).

There is need for quantifying and characterizing chemical plant extracts in order to identify active molecules responsible for the observed activity and thus facilitate the

establishment of a standardized protocol including different methods of extraction depending on the bioactive molecules observed, adequate extract concentration and regularized administration procedure (Reverter *et al.,* 2014).

2.6 The Plant – Borreria verticillata

Borreria verticillata is a perennial shrub belonging to the family *Rubiaceae*. It is commonly called shrubby false button weed or shrubby false button wood (Burkill, 2000). It is distributed in tropical and subtropical America, Africa, Asia, and Europe (Dessein *et al.,* 2006). It is originated from South and Central America (Burger and Taylor, 1993; Chiquieri *et al.,* 2004). *Borreria verticillata* species is used medicinally in various manners and are reputed in traditional medicine of Latin America, Asia, Africa, and West Indies. In Brazil, the infusion of the flowers is used as antipyretic and analgesic (Vieira *et al.,* 1999; Moreira *et al.,* 2010). The roots extract is emetic, the leaves extracts as antidiarrheal, and the remedy against erysipelas and hemorrhoids (Lorenzi and Matos, 2002). It is also commonly used effectively to cure eczema (*Tinea versicolor),* ring worm (*Tinea capitis),* scabies and other skin lesions (e.g. infectious dermatitis), toothache, headache, and dyspepsia (Chopra *et al.,* 1956). The juice obtained from the aerial part is applied topically for the treatment of skin diseases. A lotion is prepared to relieve skin itches (Liogier, 1990). In West India, the decoction of this plant is used for diabetes and dysmenorrhea, and when prepared with *Cuscuta* and *Zebrina schnizlein* is used for amenorrhea (Ayensu, 1978).While in Senegal it is used to treat bacterial skin infections and leprosy (Maynart *et al.,* 1980).

In Nigeria, it is common to all vegetation particularly in Sudan savanna, normally found in wastelands, in non-cultivated fields or within the vicinity of ponds, mainly in the rainy season. It is known as *Fasa- kaba* by the Hausa people of northern Nigeria, *Irawo-ile* by

the Yoruba people and *Abia-ikana* by the Ibibio people of southern Nigeria. The leaves are used in some parts of Nigeria, as in many West African countries for curative purposes as one of the mainstream traditional medicines. Studies have confirmed that extracts from *Borreria* and *Spermacoce* species as well as their isolated compounds possessed diverse biological activities, including analgesic, anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer, and hepatoprotective, with alkaloids and iridoids as the major active principles (Shajiselvin *et al.,* 2010; Conserva and Ferreira, 2012; Abdullahi *et al.,* 2014). The roots are used to treat malaria, the leaves as ophthalmic, inflammation of eye and gums, blindness, fever, spleen complaints, sore, hemorrhage, dysentery and diarrhea, and also for the treatment of liver ailment, kidney disorder and abortifacient (Sofowora, 2008).

2.6.1 Phytochemical constituents of *Borreria verticillata*

Studies of *B. verticillata* by research groups showed the presence of terpenoid indole alkaloids (Ferreria *et al.,* 1978; Balde *et al.,* 1991), and the structure of a new iridoid named borreriagenin isolated from a Methanol extract of the flowers of *B. verticillata* (Vieira *et al.,* 1999). The phytochemical investigation of *B. verticillata*, reported the structural characterization of two new simple indole alkaloids, 6-methoxy-4-(3-methylbut-2-en-1-yl)-1H-indole, named verticillatine A1) (1), and 1-(1H-indol-6-yl)-3-methylbutan-1-one, named verticillatine B1) (2), and of a new iridoid, 6'-O-(2-glyceryl) scandoside methyl ester1) (3), from the methanol extract of *B. verticillata* roots, isolated besides two known iridoids, asperuloside (4) and scandoside methyl ester (5), a mixture of aliphatic acids, a mixture of tri-Oacylglycerols, sucrose, and a mixture of glucose and sucrose were identified after preparation of the corresponding acetyl derivatives (Moreria *et al*.,2010).

2.7 The Plant: *Adenium obesum*

Adenium obesum (Forssk) Roem and Schult with synonyms: *Adenium somalense* Balf.f (1888); *Adenium socotranum* Vierh, (1904); *Adenium arabicum* Balf.f; *Adenium coetaneum* Stapf; *Adenium honghel* A.DC, *Nerium obesum* Forssk, belongs to the Family Apocynaceae (McLaughlin and Garofalo, 2002; Arbonnier, 2004; Anon, 2008; Oyen, 2008; Kanchanpoom *et al.,* 2010).

The plant was first discovered and described in Kenya in 1752 by a German Scientist, P. Forsskal (Anon, 2008). Adenium was derived from the Arabic name of the plant, *Oddaejn*, which means Aden, the former name of Yemen (McLaughlin and Garofalo, 2002) while obesum was derived from the swelling of the basal part of the plant stem (Plaizeier, 1980).

However, *A. obesum* is known locally as "Kariya" amongst the Hausa ethnic group of northern Nigeria (Dalziel, 1956; Adamu *et al.*, 2005) just as it is also called "Akpalataa" amongst the Igbo ethnic group of south-eastern Nigeria.

2.7.1 Phytochemical constituents of *Adenium obesum*

Several cardiac glycosides have been reported in *A. obesum* (Mettam, 1941).The main cardiac glycoside in the plant is Oleandrigenin β- gentiobiosyl (1→ 4) β-D- thevetoside (Yamauchi and Abe, 1990; Ahmad and Basha, 2007). In addition, Oleandrigenin-β-D-glucosyl (1→4)-β-D- digitalose was also isolated from the chloroform fraction of the plant (Kiyohara *et al*., 2012). Hoffman and Cole (1977) equally reported the presence of other active cardenolides (Somalin, honghelosi de A, 16-acetylstrospeside and honghelin) and an active flavonol (3,3-bis[o-methyl] quercetin) from the ethanol extract of *A. Obesum*. However, an inactive triterpene (dihydroifflaionic acid) and an inactive flavonol

(3-0-methylkaempferol) were also reported from the ethanol extract of *A. obesum* (Hoffman and Cole, 1977). The methanol extract of *A. obesum* stem bark has been reported to contain some alkaloids, flavonoids, saponins, tanins, glycosides, anthroquinones and steroids (Tijjani *et al.,* 2011a). However, only saponins, tannins, steroids and glycosides were reported from the petroleum spirit extract of *Adenium obesum* stem bark (Tijjani *et al.,* 2011b). Similarly, a triterpenoid named botulin (Lup-20(29)-ene-3,28-diol) was reportedly isolated from the stem bark of the plant (Tijjani *et al.,* 2012).

2.7.2 The Clinical manifestations of *Adenium obesum* toxicity

Fish showed various signs of toxicity ranging from uncoordinated movements, repeated attempts to jump out of reconstituted extracts and excessive mucous secretions to increased opercula movements, exposed snouts, adoption of different postures and sudden darts (Abalaka *et al.,* 2015).

2.7.3 The pathologic presentation of *Adenium obesum* toxicity in fish

Acute exposure of *Clarias gariepinus* to ethanolic extract of *Adenium obesum* stem bark showed vacuolation of hepatocyte,fatty degeneration,congestion of central vein,mononuclear cellular infiltration,hepatocyte necrosis,and degenerated hepatocytes in the liver; mononuclear cellular infiltration,fusion of the secondary lamellar,lamellar oedema,lamellar fusion,lamellar haemorrhage and epithelial detachment in the gill; proliferated mucous cells,hypertrophied mucous cells,eroded epithelial surface,and thickened epidermal layer in the skin (Abalaka *et al*., 2015).

2.7.4 The mechanisms of the cardio-toxicity in *Adenium Obesum* toxicity

The pharmacological relevance of cardiac glycosides is via their effect on the myocardial contractility (Boor, 1999). Cardiac glycosides exert their pharmacological or ionotropic effect by inhibiting the membrane-bound Na^+ , K^+ - ATPase pump thereby increasing intracellular Na^+ and extracellular K^+ resulting in the activation of the sodium-calcium pump (Na^+- Ca^+ pump) that increases intracellular Ca^+ leading to increased myocardial contraction force (Novotny, 2005). However, excessive inhibition of the Na^+ , K^+ - ATPase pump causes a variety of severe arrhythmias resulting in blocked cardiac activity, decreased cardiac output and death (Knight and Walter, 2001),which might be observed in *A. obesum* poisoning.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection, Authentication and Processing

The aerial part of *Borreria verticillata* was collected from Bassawa area within Zaria metropolis, Kaduna State in Nigeria between November – December, 2016, and authenticated at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a specimen was deposited and a voucher number 0670 was assigned. The leaves were picked and dried under shade until constant weight was obtained. The dried leaves were crushed into fine powder using a pestle and mortar and stored for the extraction process.

The stem bark of *Adenium obesum* was collected from Samaru, Zaria, Kaduna State, Nigeria and it was also authenticated at the Herbarium section Department of Biological Sciences, Ahmadu Bello University, Zaria and a Voucher Specimen Number 01386 was given at the Herbarium for future reference.

3.2 Extraction Process

The stem bark of *Adenium obesum* was dried under shade until constant weight was obtained, and were crushed into coarse powder using a pestle and mortar and stored for the extraction process. The fresh leaves of *Borreria verticillata* was shade-dried and ground into a fine powder. The fine powder was added into distilled water and shaken gently for ten minutes using a shaker to make a homogenous mixture. The mixture was left for 24 hours and then filtered (Saravanan *et al*. (2010).The filtrate was used for the study. The same extraction process was applied to the *Adenium obesum* bark stem as

described earlier. The extractive yield (%w/w) of the process was calculated as described by Zhang *et al.* (2007).

3.3 Determination of the Phytochemical Constituents of the Aqueous Extracts

Phytochemical screenings was carried out on the aqueous extract of *Borerria verticillata* aerial and aqueous extract *Adenium obesum* stem bark in order to confirm the presence of phytochemical constituents following the methods described by Sofowora, (2008) and Evans (2009).

3.3.1 Qualitative Phytochemical Screening

The aqueous extracts of both adenium obesum stem bark and *Borreria verticillata* aerial part was screened quantitatively for the presence of metabolites.

3.3.1.1 Anthraquinones: Borntrager's test

Five milliliters of the aqueous solution of the extract was shaken with 10 mL of benzene and filtered. Then 5 mL of 10% ammonia solution was added to the filtrate and stirred. The appearance of a pink – red or violet colour was noted (Trease and Evans,1983).

3.3.1.2 Glycosides: Fehling's solution test

To 5 ml of the aqueous solution of the extract, 5 ml of diluted sulphuric acid was added and boiled in water bath for 15 minutes before allowing it to cool. This was then neutralized with 20% potassium hydroxide before 5 ml of Fehling's solution A and B were addede.The formation of brick red precipitate was noted (Trease and Evans,1983).

3.3.1.3 Cardiac glycoside: Kella-killam test

Five milliliters of the aqueous solution of the extract was evaporated to dryness on o water bath upon which,it was dissolve in glacial acetic acid containing trace of ferric chloride solution in a dry test tube. This was then underplayed with 1 mL of concentrated sulphuric acid by allowing it to run down the inside wall of the test tube held at 45° angle without agitation or shaking.The appearance of a purple-brown " ring" at the interphase was noted. A violet ring may also appear below the brown ring while in the acetic acid layer, a greenish ring, which gradually spread throughout the layer may form above the brown ring (Evans,2005).

3.3.1.4 Saponins: Frothing test

Five milliliters of the aqueous solution of the extract was made,shaken vigorously for 30 seconds and allowed to stand for 30 minutes. The persistence of honey-comb froth was noted (Trans and Evans, 1983).

3.3.1.5 Steriods and tripertenes: Liberman-Buchard's Test

This test was performed according to Trans and Evans, 1983.

Two mililitres of ethanol was added to 3 ml of the extract solution and then, chloroform before being dehydrated with anhydrous sodium sulphate. The formation of reddish-brown colour at the interface when 2 ml of acetic anhydride was added prior to the addition of ffew drops of concentrated sulphuric acid down the side of the test tube was noted (Trease and Evans,1983).

3.3.2 Quantitative Phytochemical Screening

The aqueous extract of both *Adenium obesum* stem and *Borreria verticillata* aerial part was screened quantitatively for phenols, saponins, flavonoids alkaloids, and tannins as follows.

3.3.2.1 Phenols

The Folin-Ciocalteau method as described by Singleton and Rossi (1965) was used to determine the total phenolic contents in both plants. Up to100 µL of properly diluted sample in an organic water-miscible solvent with at least 6 mL of water + 0.5 mL of Folin-Ciocalteau reagent (Sigma); wait between 1 - 8 min, add 1.5 mL of Na2CO3; mix and bring to 10 mL total volume with water; then measure absorbance 0.5 – 2 hr later at 760 nm against a reagent blank and standards. The volume can be scaled down to conserve reagents.

3.3.2.2 Alkaloids

The gravimetric method of Harborne (1980) was used for this test.To 5g of the sample in a 250 ml beaker, 200 ml of 10% acetic acd in ethanl was added, covered and allowed to sand for 4 hours .This was filtered and concentrated on a water bath to one- quarter of the original volume and concentrated am monium hydroxide was then added in a drop-wise manner. The mixture was allowed to settle prior to filtering the precipate in a pre-weighed filter paper and washed with 1 % ammonium hydroxide solution. The precipate in the filter paper was then dried in the oven at 60°C for 30 minutes and re-weighed.The alkaloid content was calculated from the weight differences and expressed as a percentage as follows:

$$\% \text{ alkaloid} = \frac{W2 - W1}{W} \times 100$$

Where:

W : weight of sample

W1: weight of filter paper and precipitate

W2 : weight of filter paper and precipitate

3.3.2.3 Saponins

The method of obadoni and Ochuko (2001) was used where an aqueous solution of the ethanol extract was made and then reduced to 40 ml over water bath at 90 °C. The concentrate was then transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously.The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added and the mixture was washed twice with 10 ml of 5% aqueous sodium chloride.The remaining solution was heated in a water bath to evaporation after which,the sample was dried in an oven to a constant weight.The saponin content was then calculated and expressed as a percentage.

3.3.1.4 Flavonoids

The method of Boham and Kocipai-Abyazan (1994) was used where an aqueous solution of the ethanol extract was made and then filtered through whatman paper No.42 (125mm) repeatedly. The filterate was then transferred into a crucible and evaporated to dryness over a water bath and then weighed to a constant weight.The weight was the flavonoids.

3.3.1.5 Tannins

Folin-Dennis spectrophotometric method as described by Pearson (1976) was used.One gramme of the sample was dissolved in 100 ml distilled water, agitated and allowed to stand for 30 minutes at room temperature prior to centrifugation to obtain the supernatant. Then 25 ml of the supernatant was dispensed into another 50 ml volumetric flasks followed by 2.5 ml of standard tannic acid was also dispensed into another 50 ml

volumetric flasks followed by 2.5 ml of saturated sodium carbonate solution and made up to the mark in the flasks prior to incubation for 90 minutes at room temperature. The absorbance of each was measured at 250 nm with the reagent blank at zero. The tannin content was calculated and express as a percentage as follows:

$$\% \text{ tannin} = \frac{An}{As} \times \frac{C}{1} \times \frac{100}{W} \times \frac{Vf}{Va}$$

Where: An is absorbance of test sample

As: Absorbance of standard solution

C: Concentration of standard solution

W = Weight of sample used

Vf: Total volume of extract

Va: Volume of extract analysed

3.4 Study Design

3.4.1 Experimental Animal

Ethical clearance approval was given by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/014 for this study. The live juvenile African-Sharp-Tooth catfish , *C. gariepinus* (*N* = 450, average weight of (21.48±3.32) g and length (11.37±1.23) cm respectively were purchased from a commercial catfish farm of reputable standing and authenticated at the Fishery Section, Department of Biological Sciences, A. B.U., Zaria, Nigeria.

3.4.2 Toxicity Bioassay

Fish acclimatization lasted for 21 days under natural day and night photo-periods (12/12-h) with complete changing of pond water once in every three days. The fish were fed to their satisfaction (*ad libitum*) twice daily with 2mm Coppens® fish feed for aquaculture (Coppens® International by Helmond, Holland). A range finding test to determine the extract concentrations for the definitive test, as described by Fafioye (2001) was performed for the extracts of *Borreria verticillata* and *Adenium obesum*. Mortality was used as an end point of toxicity and this was determined as described in the OECD Guideline No. 423 (2001), presented in appendices VII,VIII, and IX.

3.4.3 Experimental design

The experimental design was based on ten groupings (Table 3.1) as follows:

Group One (G1), represents the control group, 0.5 litre of tank water was taken and replaced with 0.5 litre unchlorinated water on daily basis, the whole water was refreshed every three days.

Group Two (G2),represents the group where 25mg/l sub-lethal dose of *Borreria verticillata* extract (BVE) was introduced every 24 hour into the tank, in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group three (G3),represents the group where 50mg/l sub lethal dose of BVE was introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group four (G4), this represents the group where 100mg/l sub-lethal dose of BVE is introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group Five (G5),this represents the group where 200mg/l sub lethal dose of BVE was introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group Six (G6),this group contains 10% of LC_{50} AOE which represents the sub lethal concentration of AOE was 0.838mg/l, this is introduced every day into the tank after effectively dissolving the AOE in 10mls of water taken from the G6 tank,this is introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group Seven (G7), this represents the group combining the treatment of G2 and G6 (10% LC_{50} AOE + 20mg/l BVE),this is introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group eight (G8), this represents the group combining the treatment of G3 and G6 (10% LC_{50} AOE + 50mg/l BVE), this was introduced every 24 hour into the tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group nine (G9),this represents the group combining the treatment of G4 and G6 (10% LC_{50} AOE 100mg/l BVE), this was introduced every 24 hour into the tank after effectively dissolving the dried BVE in 10mls of water taken from the group tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

Group ten (G10), this represents the group combining the treatment of G5 and G6 (10% LC_{50} AOE 200mg/l + BVE), this is introduced every 24 hour into the tank after effectively dissolving the dried BVE in 10mls of water taken from the group tank in order to maintain a constant concentration of the extract in the tank and the whole water in the tank was refreshed completely every 3 days to refresh the system.

In the groups 7- 10,where the extracts were administered together,the *Adenium obesum* was administered first followed by the *Borreria verticillata* extract after an interval of 30 minutes. Fish were fed twice daily (9am and 4pm), extracts were administered into the fish tank after morning feeding daily for 28 days, and water were refreshed every three days. Samples were collected on days 0, 7,14,21 and 28.

Table 3.1: The experimental design 28 days sub lethal treatment

TREATMENTS	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
0.5L water	+	-	-	-	-	-	-	-	-	-
25mg/l BE	-	+	-	-	-	-	+	-	-	-
50mg/l BE	-	-	+	-	-	-	-	+	-	-
100mg/l BE	-	-	-	+	-	-	-	-	+	-
200mg/l BE	-	-	-	-	+	-	-	-	-	+
0.838 mg/l AOE	-	-	-	-	-	+	+	+	+	+

BVE = Aqueous Extract *Borreria verticillata* Aerial Part, AOE = Aqueous Extract *Adenium obesum* stem bark. G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE; G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.

3.5 Physicochemical Analyses

The temperature, pH, electrical conductivity and the total dissolved solids of the fish culture water were monitored with Hanna "Combo" portable hand instrument (Hi 98129, Hanna Instruments, Mauritius), while the dissolved oxygen content was monitored using the modified method of Winkler-Azide (Lind, 1979; APHA, 1985). This is the standard test for dissolved oxygen .It uses a burret and 0.025N sodium thiosulfate,the solution is acidified and the manganese (IV) is reduced by iodide to produce free iodine in proportion to the oxygen concentration.

3.6 Haematological Studies

Two (2) ml of blood was collected from each sampled fish via caudal veno-puncture, from the lateral approach, a 25G needle was introduced through the lateral line laterally to the body of the fish, until contact was made with the spine, needle was gently withdrawn with aspiration at the same time, as the blood flow into the needle the gentle backward withdrawal was stopped and more blood was drawn to the required volume needed and divided into two portions, 1 ml of the collected blood was put into a bottle containing ethylene diamine tetra acetic acid (EDTA) anticoagulant for heamatological evaluations. The remaining 1ml of blood was collected without anticoagulant for biochemical analyses.

3.6.1 Packed Cell Volume Determination

The packed cell volume (PCV) was determined using standard technique as described by Rehman *et al*. (2003). Non-heparinized capillary tube was filled up to about ¾ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tube was then centrifuged for 5 minutes at 4,383 x *g* using the

Saitexiangyi TG12MX® Micro-haematocrit centrifuge machine. Then the proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley® Micro-haematocrit Reader.

3.6.2 Red Blood Cells and Total White Blood Cell count

Red blood cells (RBC) and total white count were determined with the Natt-Herrick solution (1:200 dilution) and the Improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer.

The heparinised blood samples were slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will cause capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip was properly placed on the haemocytometer. The mixture was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, then left for 5 minutes for cells to settle down.

The light microscope (Olympus-XSZ-107BN), at low power magnification (X40) was used to view the cells and counting was done using the tally counter.

For white blood cell count, the white in the four outer large squares of the haemocytometer were counted and calculated using the formula below:

$$N/20 = \text{white} \times 10^9 /l$$

Where N = Number of white counted in the four outer large squares (or in 64 small squares)

For RBC count, the cells contained in the four corner and central squares in the mid-section of the haemocytometer were counted. Following the "**L**" rule: cells that touch the centre triple lines of the ruling on the left and the bottom sides were counted but cells that touch the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the formula below:

$$N/100 = \text{RBC} \times 10^{12} /l$$

Where N = Number of RBC counted in the 5 squares in the mid section of the haemocytometer (or in 160 squares). Note that both charged sides of the haemocytometer were counted for both the RBC and white and the average calculated.

3.6.3 Heamoglobin concentration

Blood haemoglobin concentration was assayed colorimettrically using cyanmethaemoglo bin method described by Feldmann *et al*.,(2000) . Five millilitre of haemoglobincyanide (HICN) (Drabkin) solution were measured using a 5ml syringe into plastic test tubes. Twenty microlitres (20 µl) of blood was measured using a micropipette and added to the drabkin solution in the test tube and properly mixed by gently shaking the test tube.It was centrifuged at 1,509 x g for 15 minutes to separate the empty RBC from interfering with the reading.The supernatant eas separated into a sample bottle.The mixture was absorbed into the haemoglobin meter (XF-C,China).After the weighing pump stops working,the value displayed on the screen was recorded in g/dl as haemoglobin concentration.

3.6.4 Differential white blood cell count

The differential white blood cell counts were determined using the method as described by Kemal (2014). A small drop (about 2 µl) of blood was immediately used for the preparation of blood smears each using the standard slide-to-slide technique. The air-dried smears were properly labelled using a pencil on the frosted end of the slide. The dried blood smears were placed in a coplin jar containing absolute methyl alcohol for 3 minute to fix the smear. The fixed slides were removed and allowed to air-dry. The slides were transferred to a second coplin jar containing fresh Wright-Giemsa stain and an equal amount of Sørensen's buffer (pH of 6.8) and allowed to stay for 20 to 30 minutes. These were neatly packed into a slide box until examination.

Examination of the blood smears was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion (X1, 000). One hundred white blood cells (WBC) were counted and classified based on their morphologic features (Hawkey and Dennet, 1989). The counting was done using the Marble® Blood Cell Calculator. The differential white count was then expressed as a percentage of the white blood cell series.

3.6.5 Haematological indices

Heamatological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined as described by Stockham and Scott (2002).

3.7 Serum Biochemical Studies

Blood samples collected without anticoagulant were allowed to clot and then centrifuged at 1006g for 10 minutes to obtain the serum.

3.7.1 Serum Aminotransferases and Alkaline Phosphatase

3.7.1.1 Serum Aspartate Aminotransferase (AST) Determination

AST activity was determined with the aid of manual kits following the principle described by Reitman and Frankel (1957).

Procedure

0.1mL of serum was mixed with Reagent 1(phosphate buffer, 100mmol/l, pH 7.4; L-aspartate, 100mmol/l; and α-ketoglutarate, 2mmol/l) and the mixture was incubated for exactly 30 minutes at 37^{o}C. 0.5ml of reagent 2 (2,4-dinitrophenylhydrazine (2mmol/l) was added to the reaction mixture and allowed to stand for exactly 20minutes at 25^{o}C. Then 5.0ml of Sodium hydroxide (0.4mol/l) was added and the absorbance read against the reagent blank after 5 minutes at 546nm. Reagent blank was prepared as described above replacing serum with 0.1ml of distilled water.

3.7.1.2 Serum Alanine Aminotransferase (ALT) Determination

ALT activity was determined following the principle described by Reitman and Frankel (1957).

Procedure

0.1ml of diluted serum mixed with phosphate buffer (100mmol/L, pH 7.4), L-alanine (100mmol/L), and α-oxoglutarate (2mmol/L) and the mixture incubated for exactly 30min at 37oC. 0.5ml of 2, 4-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 250C. Then 5.0ml of NaOH (0.4mol/L) was added and the absorbance read against the reagent blank after 5 min at 546nm.

3.7.1.3 Serum Alkaline Phosphatase (ALP) Determination

ALP activity was determined with the aid of manual kits following the principle described by Rec. (1972).

0.02ml of serum sample and 1.0ml of reagent was mixed together in the cuvette. After mixing sample and R1, the initial absorbance was read at 405nm and a timer started simultaneously. Absorbance was read again after 1, 2 and 3 minutes. ALP activity was calculated using the formula:

U/L = 2760 x $\triangle$A 405 nm/min

3.7.2 Antioxidants

3.7.2.1 Estimation of serum reduced glutathione (GSH) level

The method of Beutler *et al*. (1963) was followed in estimating the level of reduced glutathione.

3.7.2.2 Determination of serum superoxide dismutase activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972).

0.2 ml of serum was diluted in 0.8 ml of distilled water to make a 1 in 5 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Increase in absorbance per minute = $\frac{A_3 - A_0}{2.5}$

Where A_0 = absorbance after 30 seconds

A_3=absorbance after 150 seconds

% inhibition = 100 X $\frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance for blank}}$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

3.7.3 Determination Serum Total Protein

Total proteins were determined by the direct Biuret method (Hrubec, *et. al*, 2001), for the in-vitro determination of total proteins in serum or plasma using the total proteins test kit. The serum sample (0.02 mL) was mixed and reacted with Cu^{2+} contained in 1 mL of Biuret reagent in a clean test tube, to form a stable coloured complex. The mixture was allowed to stand for 10 minutes at room temperature. A standard was prepared by adding 1mL of the Biuret reagent and 0.02 mL of the standard into a clean test tube. This was mixed properly and allowed to stand for 10 minutes. After this, the absorbances of both the sample and standard were read at 540 nm against the working reagent blank using a spectrophotometer (SpectrumLab 750, Sweden).

The total proteins concentration was obtained with the formula:

$$\text{Absorbance of sample}/\text{Absorbance of standard} \times 5 = \text{Total protein (g/dl)}$$

3.7.4 Determination of Serum Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the levels of Malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale (1990).

Procedure

An aliquot of 400µl of the sample was mixed with 1.6ml of tris-KCl buffer to which 500 µl of 30% TCA was added. Then 500 µl of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^0C. This was then cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected and absorbance measured against a reference blank of distlled water at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ Cm^{-1}$

$$\text{LPO (MDA formed/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \text{ x volume of sample x mg protein}}$$

MDA FORMED = mmol/mg protein

3.8 Histopathological Studies

After the collection of blood for haematological evaluation the fish so harvested was observed for gross leisons, thereafter the fish was euthanized with 4% ethyl alcohol as described by Fafioye (2005). The gill, liver, intestine, and kidney, from each fish were harvested-fixed in 10% neutral buffered formalin,processed,paraffin embedded, sectioned at 5µm and stained with haematoxylin and eosin as described by Roberts (1978), and Bancroft and Cook (1994) for histopathological examination.An Olympus light

microscope was used to view the tissue slides and a Samsung digital camera was used to capture the photomicrographs.

3.8.1 Tissue processing

The formalin fixed tissues samples were then subjected to the following protocols for the production of wax block for histopathological studies.

Tissues were dehydrated in ascending grades of alcohol (50.0 % for 30 minutes; 80.0 % for 45 minutes; 90.0 % for 45 minutes). Then passed through two changes in absolute alcohol (100.0 % alcohol) for one hour each; these tissues were then placed in a bath of xylene for a process termed clearing, until they appeared translucent, then they were transferred to molten paraffin wax for one hour to completely remove the xylene.

Tissues were then embedded in paraffin wax of melting point 60^0C to 62^0C. Paraffin blocks were then cut in a rotary microtome. Cut sections (of 4 µm to 6 µm thickness) were collected on clean gelatinized microscope slides to prepare tissue sections for staining.

3.8.2 Haematoxylin and Eosin staining technique

Slides bearing the tissue samples were rinsed twice (for 5 minutes in xylene); then transferred for 1 minute into a mixture of xylene: absolute ethanol (1:1 v:v). Slides were then passed for 1 minutes each, through absolute alcohol (100%), 80% alcohol and 50% alcohol; washed for 5 minutes in running tap water. Then stained for 2 minutes in haematoxylin, washed for 5 minutes in running tap water; dipped once in acid : absolute alcohol mixture (0.5 : 100); washed for 5 minutes in running tap water. And then stained for 1 minute in Eosin. The stained slides were sequentially dehydrated through 50%, 80% and absolute (100%) alcohol for 1 minute each; then dipped once in running tap water, blotted dry with filter paper and rinsed till clear in xylene and finally mounted in D.P.X.

3.8.3 Microscopic evaluations

Mounted slides were viewed in a high power research grade Olympus (BH-2) and images captured with a samsung digital camera.

3.9 Data Analyses

Data was expressed as mean ± SEM and then subjected to Two-way Analysis of Variance (ANOVA) for statistical significance at $p<0.05$. Tukey's multiple comparison tests for means was used to compare differences between the various means using SPSS version 20.

CHAPTER FOUR

4.0 RESULTS

4.1 The Extractive Yield of Aqueous Extracts of *Adenium obesum* Stem Bark and *Borreria verticillata* Aerial Part

The aqueous extraction process on *Adenium obesum* stem Bark fine powder with dry weight of 3kg yielded 207.53g and an extractive yield of 6.91% w/w. Similarly, the aqueous extraction carried out on *Borreria verticillata* with dry weight of 3kg yielded 304.11g and had an extractive yield of 10.13w/w.

4.2 Phytochemical constituents of aqueous extracts of *Adenium obesum* stem bark and *Borreria verticillata* aerial part

The physicochemical analyses revealed similar constituents in aqueous extracts of *Adenium obesum* and *Borreria verticillata* (Table 4.1).The quantities of various constituents appeared to be higher in *Adenium obesum* than in *Borreria verticillata* extract (Table 4.2), except for phenols which amounted to 1% in the former and 9% in the latter.

Table 4.1: Phytochemical constituents of aqueous extract of *Adenium obesum* stem bark and aqueous extract of *Borreria verticillata* aerial part

Constituents	Inferences	
	AOE	BVE
Carbohydrates	+	+
Anthraquinones	-	-
Cardiac glycosides	+	-
Saponins	+	+
Steroids and Triterpenes	+	+
Tanins	+	+
Flavonoids	+	+
Alkaloids	-	+

Keys: + = present, - = absent, AOE = Aqueous extract *Adenium obesum* stem bark, BVE = Aqueous extract *Borreria verticillata* aerial part

Table 4.2: Percentage of aqueous extract of *Adenium obesum* stem bark and aqueous extract of *Borreria verticillata* aerial part

	Percentage (%)	
Constituents	AOE	BVE
Phenols	1	9
Saponins	13.3	3.1
Tanins	11.6	1.9
Flavonoids	4.9	2.1
Alkaloids	2.6	0.2

Keys: AOE = aqueous extract *Adenium obesum* stem bark, BVE = aqueous extract *Borreria verticillata* aerial part

4.3 Clinical signs in *Clarias gariepinus* exposed to aqueous extract of A*denium obesum* stem bark

Clarias gariepinus exposed to varying concentrations of aqueous extract of *A. obesum* stem bark over the 96-hr period exhibited signs that were divided into initial contact phase (0-hr to 48-hr) and a later exertive phase (72-hr to 96-hr) of the toxicity bioassay. Some of the clinical signs displayed by the fish during the 96-hr acute bioassay exposure were, repeated attempt to jump out of water, erratic movements,aggression, excessive mucus secretion,frequent opercula movements, air gulping, exposed snout, motionless, different postures (vertical,angular,flat), sudden dart,swirling/sluggish movements, and loss of balance (Appendices VII and VIII).

4.4 Median lethal concentration of aqueous extracts of *Adenium obesum* stem bark and *Borreria verticillata* in *Clarias gariepinus* juveniles

4.4.1 The median lethal concentration of aqueous extract *Adenium obesum* stem bark

The median lethal concentration (LC_{50}) of aqueous extract of *Adenium obesum* stem bark extract was determined to be 8.38 mg/l using the probit analyzing tool in SPSS version 20(Appendix VII),this was after a performance of a range finding test to determine the five extract concentration as described by Fafioye *et al.* (2004).

4.4.2 The median lethal concentration of aqueous extract *Borreria verticillata* aerial part

A range limit test conducted using five (5) fish per concentration showed no mortality at varying concentrations from 0-1200mg/l (Table 4.3).

Table 4.3: Behavioral Display of *Clarias gariepinus* juveniles *Borreria verticillata* in Range Finding Test

	RANGE FINDING TEST 1st TRIAL				RANGE FINDING TEST 2nd TRIAL			
Exposure time (HR)	**24**	**24**	**24**	**24**	**24**	**24**	**24**	**24**
Concentration(mg/l)	0	1	5	10	15	20	25	50
Loss of balance	-	-	-	-	-	-	-	-
Molting	-	-	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-
Mortality	0	0	0	0	0	0	0	0
	RANGE FINDING TEST 3rd TRIAL				**RANGE FINDING TEST 4th TRIAL**			
Exposure time (HR)	**24**	**24**	**24**	**24**	**24**	**24**	**24**	**24**
Concentration (mg/l)	50	100	150	200	400	600	800	1200
Loss of balance	-	-	-	-	-	-	-	-
Molting	-	-	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-
Mortality	0	0	0	0	0	0	0	0

4.5.1 Mean Red Blood Cell Count Sub-Lethal Treatment

The mean red blood cell (RBC) counts difference between the groups on day 0 was not significant (P>0.05).The mean red blood cell count did not vary significantly (P>0.05) in groups 2-5 when compared to the values obtained for group 1 throughout the 28 days period.On days 7 to 28, the mean RBC counts declined significantly (P<0.05) from 2.56±0.01 $x10^{12}$/L to 2.00±0.00 $x10^{12}$/L in group 6 (Table 4.4). However, the mean values of RBC counts showed singnificant (P<0.05) increase in groups 7-10 on days 14 and 28 compared to group 6.

4.5.2 Mean Hemoglobin Concentration Sub-Lethal Treatment

The mean hemoglobin concentration difference between the groups on day 0 was not significant (P>0.05). The mean haemoglobin concentration decreased significantly (P<0.05) from 11.23±0.12 g/dl on day 0 to 10.72±0.01g/dl on day 28 in group 6 unlike in groups 1-5 where the values varied insignificantly (P>0.05).Groups 7 – 10, treated with increasing concentration of *Borreria verticillata* aqueous extract had significant improvement in the mean haemoglobin concentration on days 7, 14 and 28, during the study (Table 4.5).

4.5.3 Mean Packed Cell Volume (PVC) Sub-lethal treatment

On day 0, prior to exposure, the differences in mean packed cell olume (PCV) between the groups (Table 4.6) were not significantly (P>0.05) different from one another. Following exposure, there was a significant (P<0.05) and progressive decrease in mean PCV values, from 30.97±0.00% (day 7) to 29.37±0.27% (day 28) in group 6. The mean values of PCV obtained for groups 2-5 appeared to varied insignificantly (P<0.05) during the 28 days period compared to group 1(CONTROL).However,it was observed that the

mean PCV values in groups 7 – 10 varied significantly (P<0.05) on day 14 and improved on days 21 and 28 of the study.

4.5.4 Mean Erythrocytic Corpuscular Values Sub-Lethal Treatment

The difference in the mean corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values between the groups on day 0 and day 7 were not significant (P>0.05).

The mean corpuscular volume (Table 4.7),mean corpuscular haemoglobin (Table 4.8) and mean corpuscular haemoglobin concentration (Table 4.9) of the fish all had a similar pattern of progressive significant (P<0.05) decline from days 7 to 28 in group 6. Mean values of the indices increased significantly (P<0.05) in groups 7 – 10, from days 7 – 28.

Table 4.4: Mean Red Blood Cell Count (RBC) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part

TREATMENT GROUPS	RED BLOOD CEL COUNT L($x10^{12}$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	2.69±0.00	2.71±0.01^{f}	2.73±0.00	2.73±0.00^{1}	2.73±0.02
G2	2.68±0.01	2.70±0.02	2.70±0.03	2.71±0.03	2.73±0.03
G3	2.67±0.01	2.69±0.00	2.71±0.01	2.74±0.03	2.75±0.04
G4	2.71±0.02	2.72±0.01	2.70±0.00	2.71±0.00	2.70±0.00
G5	2.73±0.03	2.70±0.02	2.71±0.02	2.70±0.01	2.70±0.00
G6	2.77±0.03	2.56±0.01^{bcde}	2.30±0.00^{ahij}	2.15±0.00^{aghij}	2.00±0.00^{aghij}
G7	2.72±0.01	2.57±0.01	2.37±0.01^{hij}	2.22±0.01	2.13±0.01^{fhij}
G8	2.70±0.00	2.59±0.00	2.46±0.01^{fgj}	2.38±0.00	2.29±0.00^{fgij}
G9	2.70±0.01	2.60±0.00^{h}	2.51±0.01^{fgj}	2.52±0.01	2.49±0.00^{fghj}
G10	2.69±0.01	2.65±0.00	2.69±0.00^{fghi}	2.63±0.02	2.68±0.01^{fghi}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5 = 200mg/l BVE; G6 = 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part.

Table 4.5: Mean Hemoglobin Concentration (Hb) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part

TREATMENT GROUPS	HEMOGLOBIN CONCENTRATION (g/dl)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	11.36±0.03	11.38±0.03	11.39±0.03	11.40±0.03	11.41±0.04
G2	11.35±0.03	11.37±0.03	11.38±0.03	11.40±0.04	11.41±0.03
G3	11.34±0.04	11.35±0.04	11.36±0.04	11.37±0.04	11.38±0.04
G4	11.38±0.03	11.39±0.02	11.40±0.02	11.41±0.02	11.42±0.02
G5	11.36±0.03	11.37±0.04	11.37±0.04	11.38±0.04	11.39±0.04
G6	11.23±0.12	11.00±0.00^{bcde}	10.85±0.00 ^{ghij}	10.72±0.01	10.65±0.00^{ghij}
G7	11.34±0.03	11.05±0.00	11.00±0.00 ^{fij}	11.07±0.00	11.17±0.00^{fij}
G8	11.35±0.05	11.10±0.03^{f}	11.10±0.00 ^{fj}	11.08±0.00	11.23±0.01^{fj}
G9	11.29±0.00	11.14±0.00	11.20±0.00 ^{fg}	11.09±0.00	11.30±0.00^{fg}
G10	11.31±0.01	11.20±0.00^{fghi}	11.30±0.00 ^{fgh}	11.12±0.00	11.38±0.00^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; G1=Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.6: Mean Packed Cell Volume (PCV) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum* stem bark and treated with *Borreria verticillata* aqueous extract aerial part

TREATMENT GROUPS	PACKED CELL VOLUME (%)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	32.35±0.66	32.36±0.66	32.71±0.33	32.72 ± 0.33^{f}	32.73±0.33
G2	32.35±0.66	32.37±0.66	32.72±0.33	33.08±0.01	33.09±0.00
G3	32.34±0.67	32.36±0.67	32.71±0.34	33.05±0.00	33.06±0.01
G4	32.40±0.67	32.41±0.66	32.42±0.66	32.76±0.33	32.77±0.33
G5	32.42±0.67	32.42±0.67	32.43±0.67	32.44±0.67	32.47±0.67
G6	32.36±0.67	30.97 ± 0.00^{abc}	29.93 ± 0.33^{ahij}	29.60 ± 0.20^{ghij}	29.37 ± 0.27^{aghij}
G7	32.37±0.67	31.04±0.00	30.04 ± 0.00^{hij}	30.20±0.06	30.94 ± 0.00^{fij}
G8	32.34±0.66	31.14±0.00	31.47 ± 0.03^{fg}	30.72±0.01	31.27 ± 0.12^{fij}
G9	32.38±0.67	31.19±0.00	31.73 ± 0.03^{fg}	30.94±0.00	31.83 ± 0.03^{fj}
G10	33.00±0.00	31.31±0.00	32.00 ± 0.00^{fg}	32.00±0.00	32.20 ± 0.05^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

TABLE 4.7: Mean Corpuscular Volume (MCV) of *Clarias gariepinus* juveniles exposed aqueous extract *Adenium obesum stem* bark treated with *Borreria verticillata* aerial part

TREATMENT GROUPS	MEAN CORUSCULAR VOLUME (%)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	134.97±0.12	135.31±0.47	135.64±0.79	135.08±0.12	135.10±0.25
G2	135.62±0.61	134.98±0.14	134.96±0.15	134.97±0.13	135.02±0.15
G3	135.01±0.16	134.96±0.13	134.96±0.16	134.98±0.13	135.09±0.21
G4	135.00±0.15	134.98±0.14	134.96±0.09	134.96±0.13	134.98±0.14
G5	134.96±0.12	134.98±0.15	134.96±0.15	134.96±0.13	134.94±0.15
G6	134.99±0.16	133.31±0.00^{a}	130.23±0.00^{agjij}	130.02±0.13^{ghij}	128.79±0.01^{aghij}
G7	135.03±0.19	133.35±0.00	131.35±0.00^{fhij}	132.35±0.00	130.35±0.01^{fhij}
G8	135.00±0.16	133.38±0.00	132.48±0.00^{fgij}	132.48±0.00	131.48±0.01^{fg}
G9	135.03±0.19	133.39±0.00	133.54±0.02^{fgh}	132.58±0.00	131.68±0.01^{fg}
G10	134.99±0.16	133.41±0.00	133.66±0.01^{fgh}	133.60±0.00	131.77±0.01^{fg}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.8: Mean Corpuscular Hemoglobin (MCH) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	MEAN CORPUSCULAR HEMOGLOBIN (pg)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	40.29±0.48	40.30±0.48	40.31±0.48	40.31±0.48	40.33±0.48
G2	40.32±0.48	40.32±0.50	40.32±0.50	40.33±0.50	40.34±0.48
G3	40.32±0.49	40.31±0.49	40.32±0.48	40.31±0.50	40.31±0.48
G4	40.28±0.49	40.29±0.49	40.30±0.49	40.31±0.49	40.32±0.48
G5	40.30±0.47	40.30±0.48	40.31±0.48	40.32±0.48	40.33±0.48
G6	40.30±0.46	37.47±0.11^{aghij}	36.50±0.05^{aij}	35.57±0.12^{ahij}	34.67±0.14^{aghij}
G7	40.32±0.47	37.37±0.25^{a}	37.01±0.01^{ij}	37.04±0.00^{ij}	37.37±0.25^{fj}
G8	40.28±0.50	38.64±0.30	38.01±0.01^{j}	38.64±0.30^{f}	38.02±0.01^{fj}
G9	40.28±0.49	39.00±0.00	39.04±0.00^{g}	39.00±0.00^{f}	39.00±0.00^{f}
G10	40.29±0.48	40.52±0.06	39.95±0.02^{fgh}	39.95±0.02^{fg}	39.95±0.02^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.9: Mean Corpuscular Hemoglobin Concentration (MCHC) of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (%)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	32.63±0.33	33.28±0.57	33.29±0.57	33.30±0.57	33.31±0.57
G2	32.27±0.58	33.27±0.57	33.25±0.56	33.25±0.58	33.24±0.56
G3	31.93±0.89	33.28±0.57	33.01±0.58	33.24±0.58	33.27±0.57
G4	33.26±0.57	33.27±0.57	33.04±0.57	33.25±0.57	33.28±0.57
G5	33.27±0.57	33.25±0.59	33.03±0.03	33.20±0.59	33.27±0.57
G6	33.29±0.57	27.47±0.33^{ghij}	26.47±0.03^{ghij}	25.57±0.28^{aghij}	24.26±0.00^{aghij}
G7	33.28±0.57	28.24±0.00	29.00±0.00^{fj}	29.00±0.00^{fj}	29.07±0.06^{fj}
G8	33.29±0.57	29.29±0.00	29.29±0.00^{f}	29.20±0.00^{f}	29.24±0.00^{fj}
G9	32.17±0.58	29.34±0.00	29.34±0.00^{f}	29.74±0.00^{f}	30.57±0.00^{f}
G10	32.26±0.57	30.26±0.13	30.89±0.09^{f}	31.12±0.13^{fg}	31.39±0.00^{fgh}

Superscripts,^{a,b,c,d,e,f,g,h,I,j,} indicates statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively; G1=Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/l BVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

4.5.5 Mean Total White Blood Cell Count Sub-lethal Treatment

The total white blood cell count of the juvenile *C. gariepinus* fish in group 6 increased significantly ($P<0.05$) from $17.98\pm0.04x10^9$ cells/L on day 7 to $18.80\pm0.06 \times 10^9$ cells/L on day 28.These values were higher than those of groups 1-5 and 7 -10. However, there was a dose and time dependent decrease in the total white blood cell counts in group 7 – 10 (Table 4.10). Similarly, there were significant ($P<0.05$) increase in differential counts of heterophils (Table 4.11), eosinophils (Table 4.12), monocytes (Table 4.13) and lymphocytes (Table 4.14) in group 6. For all the white blood cell types herein evaluated,the increase was first observed on day 7,except for monocyte counts,the increase of which was delayed until day 14.The mean values of differential white blood cell counts also decreased significantly ($P<0.05$) with increase in the dose of BVE and time.

4.5.6 Mean Serum Total Protein Sub-lethal Treatment

The serum total protein (TP) of the fish in group 6 decreased significantly ($P<0.05$) from 4.73±0.12g/dl on day 7 to 3.03±0.00g/dl on day 28.These values were lower than those of groups 1-5 and 7 -10.There was a dose and time dependent increase in the mean serum total protein values in group 7 – 10 (Table 4.15).

Table 4.10: Mean Total White Blood Cell Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	TOTAL WHITE BLOOD CELL COUNT ($x10^9$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	17.96±0.03	17.99±0.01^{fghij}	18.00±0.02^{fghij}	17.99±0.03^{fghij}	18.00±0.04^{fghij}
G2	17.96±0.04	17.98±0.02	17.99±0.03	18.00±0.03	18.01±0.03
G3	17.93±0.07	17.96±0.04	17.97±0.06	17.98±0.03	17.98±0.06
G4	17.90±0.11	18.00±0.02	18.01±0.03	18.02±0.03	18.02±0.04
G5	17.87±0.14	18.00±0.02	17.98±0.06	17.92±0.04	17.92±0.13
G6	17.98±0.04	18.14±0.01^{ghij}	18.22±0.00^{ghij}	18.50±0.06^{ghij}	18.80±0.06^{ghij}
G7	17.98±0.05	18.10±0.00^{f}	18.16±0.00^{fhij}	18.27±0.01^{fj}	18.37±0.01^{fij}
G8	17.98±0.06	18.07±0.00^{f}	18.12±0.00^{fgj}	18.22±0.01^{f}	18.32±0.00^{fj}
G9	17.98±0.06	18.08±0.01^{fj}	18.10±0.00^{fgj}	18.19±0.00^{f}	18.26±0.01^{fgj}
G10	17.98±0.07	18.04±0.00^{fgh}	18.08±0.00^{fgh}	18.13±0.01^{fg}	18.09±0.01^{fghi}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE = Aqueous extract *Borreria verticillata* aerial part

Table 4.11: Mean Heterophil Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	HETEROPHIL COUNT ($x10^9$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	0.93±0.03	0.93 ± 0.03^{f}	0.93 ± 0.03^{f}	0.93 ± 0.03^{f}	0.94 ± 0.03^{f}
G2	0.93±0.03	0.93±0.02	0.93±0.02	0.93±0.02	0.94±0.02
G3	0.93±0.04	0.93 ± 0.04^{f}	0.93±0.03	0.93±0.04	0.94±0.04
G4	0.93±0.04	0.93 ± 0.03^{f}	0.93±0.02	0.93±0.03	0.93±0.02
G5	0.92±0.03	0.93 ± 0.03^{f}	0.93±0.03	0.93±0.00	0.94±0.03
G6	0.93±0.02	1.02 ± 0.00^{ghij}	1.05 ± 0.00^{ghij}	1.09 ± 0.01^{ghij}	1.12 ± 0.00^{ghij}
G7	0.93±0.03	1.00 ± 0.01^{b}	1.01 ± 0.00^{fj}	1.02 ± 0.01^{f}	1.07 ± 0.01^{fij}
G8	0.93±0.02	0.98±0.00	0.96 ± 0.00^{f}	0.98 ± 0.03^{f}	1.04 ± 0.03^{ij}
G9	0.93±0.01	0.96±0.00	0.94 ± 0.00^{f}	0.96 ± 0.00^{f}	1.00 ± 0.03^{fgj}
G10	0.93±0.01	0.94±0.00	0.92 ± 0.00^{fg}	0.91 ± 0.01^{f}	0.92 ± 0.01^{fghi}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE = Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.12: Mean Eosinophil Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	EOSINOPHIL COUNT ($x10^9$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	0.37±0.03	$0.38±0.04^{fgh}$	$0.38±0.04^{fgh}$	$0.38±0.04^{fgh}$	$0.37±0.01^{fgh}$
G2	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03
G3	0.37±0.02	0.37±0.02	0.37±0.03	0.38±0.03	0.38±0.02
G4	0.36±0.03	0.37±0.03	0.37±0.03	0.37±0.03	0.37±0.03
G5	0.37±0.02	0.37±0.02	0.38±0.02	0.38±0.02	0.38±0.01
G6	0.37±0.02	$0.48±0.00^{ghij}$	$0.50±0.02^{ghij}$	$0.52±0.02^{fghij}$	$0.56±0.00^{ghij}$
G7	0.37±0.03	$0.43±0.03^{ij}$	$0.44±0.03^{fij}$	$0.45±0.03^{fij}$	$0.46±0.03^{fij}$
G8	0.37±0.02	$0.41±0.03^{fj}$	$0.42±0.03^{fj}$	$0.43±0.03^{fj}$	$0.44±0.03^{fj}$
G9	0.37±0.01	$0.39±0.03^{fg}$	$0.40±0.03^{fg}$	$0.41±0.03^{fg}$	$0.42±0.03^{fg}$
G10	0.38±0.01	$0.37±0.03^{fgh}$	$0.38±0.03^{fgh}$	$0.39±0.03^{fgh}$	$0.39±0.03^{fgh}$

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.13: Mean Monocyte Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	MONOCYTE COUNT ($x10^9$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	2.54±0.03	2.55±0.02	2.55±0.03^{f}	2.55±0.01^{f}	2.55±0.01^{f}
G2	2.53±0.00	2.54±0.01	2.54±0.01	2.54±0.01	2.54±0.02
G3	2.52±0.01	2.53±0.02	2.53±0.02	2.54±0.02	2.54±0.02
G4	2.53±0.00	2.54±0.01	2.55±0.01	2.55±0.01	2.55±0.01
G5	2.53±0.03	2.54±0.00	2.54±0.00	2.53±0.01	2.54±0.00
G6	2.54±0.03	2.58±0.00	2.65±0.01^{ghij}	2.79±0.34^{ij}	2.94±0.33^{ghij}
G7	2.55±0.06	2.57±0.01	2.60±0.00^{f}	2.68±0.00^{f}	2.70±0.00^{f}
G8	2.55±0.05	2.56±0.00	2.58±0.00^{f}	2.65±0.00	2.67±0.00^{f}
G9	2.55±0.07	2.55±0.00	2.58±0.00^{f}	2.63±0.00	2.64±0.00^{f}
G10	2.55±0.06	2.55±0.00	2.56±0.01^{f}	2.60±0.00^{f}	2.60 ±0.00^{f}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicates statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.14: Mean Lymphocyte Count of *Clarias gariepinus* juveniles exposed to aqueous extract *Adenium Obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	LYMPHOCYTE COUNT ($x10^9$/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	13.24±2.18	13.44±1.66^{f}	13.45±1.66^{f}	13.44±1.66^{f}	13.45±1.66^{f}
G2	13.42±1.66	13.43±1.66	13.45±1.66	13.45±1.66	13.46±1.66
G3	13.34±1.33	13.36±1.32	13.37±1.34	13.38±1.34	13.38±1.34
G4	13.38±1.65	13.45±1.65	13.46±1.65	13.46±1.65	13.46±1.66
G5	13.30±1.32	13.39±1.34	13.37±1.33	13.33±1.33	13.33±1.33
G6	13.44±1.65	14.26±0.01^{ghij}	14.51±0.00^{ghij}	15.18±0.00^{ghij}	15.41±0.02^{ghij}
G7	13.43±1.68	14.17±0.00^{fhij}	14.23±0.00^{f}	14.51±0.00^{fj}	14.78±0.01^{hij}
G8	13.38±1.84	13.95±0.00^{fgij}	14.00±0.00^{f}	13.90±0.00^{f}	14.54±0.00^{fg}
G9	13.38±1.88	13.46±1.33^{fghi}	13.60±0.00^{f}	13.68±0.00^{f}	14.29±0.00^{fg}
G10	13.38±1.83	13.18±0.00^{fghi}	13.39±0.00^{f}	13.38±0.00^{fg}	13.97±0.00^{fg}

K-eys: Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1=Control; G2=25mg/l BVE; G3 = 50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6; AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part.

Table 4.15: Mean Serum Total Protein (TP) level of *Clarias gariepinus* juveniles exposed to sub lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	SERUM TOTAL PROTEIN (g/dl)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	4.70±0.06	4.71±0.06^{f}	4.72±0.06^{f}	4.73±0.06^{f}	4.74±0.06^{f}
G2	4.70±0.05	4.72±0.05	4.74±0.05	4.76±0.05	4.78±0.05
G3	4.71±0.05	4.72±0.06	4.73±0.06	4.74±0.05	4.75±0.06
G4	4.73±0.06	4.74±0.06	4.75±0.06	4.76±0.06	4.77±0.06
G5	4.73±0.04	4.74±0.05	4.74±0.06	4.75±0.07	4.75±0.07
G6	4.73±0.12	4.46±0.03	3.27±0.03^{ghij}	3.10±0.02^{ghij}	3.03±0.00^{ghij}
G7	4.64±0.11	4.46±0.01	4.06±0.01^{fj}	4.10±0.00^{fj}	4.15±0.00^{f}
G8	4.65±0.10	4.48±0.00	4.10±0.00^{fghi}	4.15±0.00^{fg}	4.21±0.01^{f}
G9	4.69±0.06	4.50±0.00	4.23±0.01^{fj}	4.23±0.01^{f}	4.27±0.01^{f}
G10	4.72±0.06	4.54±0.00	4.45±0.00 ^{fgh}	4.32±0.02^{fg}	4.31±0.01^{f}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/l BVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

4.6 Mean Serum Levels of Aminotransferases and Alkaline phosphatase of *Clarias gariepinus* Juveniles Sub-Lethal Treatment

4.6.1 Mean Serum Aspartate Aminotransferase levels Sub-Lethal Treatment

The mean serum levels of aspartate amino transferase (AST) in groups 2 – 5 did not vary significantly ($P<0.05$) when compared to group 1 during the period (Table 4.16). There was a significant increase in the level of AST in group 6 from day 7 to 28. Mean values of the enzyme in groups 7 – 8, were significantly ($P<0.05$) higher than those of groups 1 – 5,but were significantly ($P<0.05$) lower than those obtained in group 6 from days 14 to 28.

4.6.2 Mean Serum Alanine Aminotransferase Levels Sub-Lethal Treatment

There was a progressive increase in mean serum levels of alanine amino transferase (ALT) of the fish in group 6,observed from days 7 to 28 (Table 4.17).The values in this group were significantly ($P<0.05$) higher than those recorded for groups 1 – 5. On the other hand, the mean ALT values of groupd 7 – 10 were significantly ($P<0.05$) lower than those of group 6, from days 14 to 28.

4.6.3 Mean Serum Alkaline Phosphatase Levels Sub-Lethal Treatment

The mean serum levels of alkaline phosphatase (ALP) of the fish in group 6 increased significantly ($P<0.05$) from day 14 and remained higher than the values in groups 1 – 5 (Table 4.18). The juvenile fish in groups 7 – 10 exposed to AOE and treated with BVE, had mean serum ALP values that were significantly ($P<0.05$) lower than those of groups 6, but were higher ($P<0.05$) than those observed in groups 1 – 5.

Table 4.16: Mean Serum Aspartate Aminotransferase (AST) Level of *Clarias gariepinus* juveniles exposed to Sub-Lethal Treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

	ASPARTATE AMINOTRANSFERASE (IU/L)				
TREATMENT GROUPS	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	56.76±0.48	56.73±0.50^{f}	56.76±0.48^{f}	60.76±2.90^{f}	56.76±0.48
G2	56.77±0.48	56.77±0.48	56.78±0.48	56.79±0.49	56.76±0.48
G3	56.77±0.49	56.75±0.48^{a}	56.75±0.48	56.74±0.49	56.76±0.48
G4	56.77±0.49	56.76±0.48	56.75±0.49	56.74±0.49	56.76±0.48
G5	56.77±0.48	56.76±0.49	56.74±0.48	56.73±0.48	56.76±0.48
G6	56.77±0.49	58.62±0.33^{a}	59.96±0.01^{a}	60.33±0.33^{aghij}	60.98±0.01^{aghij}
G7	56.73±0.49	58.81±0.01	58.85±0.00	58.84±0.00^{f}	58.79±0.00^{f}
G8	56.74±0.49	58.70±0.00	58.74±0.00	58.81±0.00^{f}	58.74±0.00^{f}
G9	56.75±0.48	58.61±0.01	58.64±0.00	58.71±0.01^{f}	58.71±0.00^{f}
G10	56.78±0.49	58.53±0.02	58.34±0.01	58.43±0.01^{f}	58.64±0.01^{f}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference (P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; G1=Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.17: Mean Serum Alanine Aminotransferase (ALT) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	SERUM ALANINE AMINO TRANSFERASE (IU/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	33.39±0.67	33.36±0.67	33.37±0.67^{f}	33.38±0.67^{f}	33.39±0.68^{f}
G2	33.35±0.67	33.35±0.67	33.36±0.66	33.37±0.67	33.38±0.66
G3	33.40±0.66	33.36±0.67	33.36±0.67	33.37±0.67	33.37±0.67
G4	33.45±0.65	33.35±0.67	33.39±0.67^{a}	33.37±0.67	33.39±0.68
G5	33.48±0.67	33.39±0.67	33.33±0.68	33.39±0.68	33.39±0.67
G6	33.41±0.66	34.19±0.67	36.24±0.33	36.8±0.33^{ghij}	38.04±0.34^{hij}
G7	33.42±0.67	34.70±0.00	34.74±0.01	34.26±0.01^{f}	35.67±0.01
G8	33.44±0.67	34.60±0.00	34.64±0.01	34.22±0.01^{f}	35.51±0.01^{f}
G9	33.46±0.67	34.50±0.00	34.52±0.01	34.20±0.00^{f}	34.47±0.01^{f}
G10	33.39±0.67	34.40±0.00	34.41±0.01	33.45±0.33^{f}	33.69±0.34^{f}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicates statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.18: Mean Serum Alanine Phosphatase (ALP) Levels of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	SERUM ALKALINE PHOSPHATASE (IU/L)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	68.67±0.42	69.67±0.42	69.67±0.43^{f}	69.68±0.42^{f}	69.68±0.43^{f}
G2	69.66±0.41	69.67±0.42	69.67±0.42	69.68±0.42	69.68±0.43
G3	69.67±0.42	69.67±0.42	69.67±0.43	69.68±0.43	69.68±0.43
G4	69.66±0.41	69.66±0.42	69.67±0.42	69.68±0.42	69.68±0.42
G5	69.67±0.42	69.67±0.42	69.68±0.42	69.68±0.43	69.69±0.42
G6	69.65±0.42	70.65±0.66	73.22±0.33^{ij}	72.95±0.00^{j}	73.86±0.34^{ghij}
G7	69.68±0.42	70.30±0.33	71.80±0.00	71.82±0.01	71.48±0.00^{f}
G8	69.66±0.41	70.26±0.34	71.70±0.00	71.72±0.00	71.34±0.01^{f}
G9	69.67±0.41	70.24±0.33	71.60±0.33^{f}	71.61±0.33	71.23±0.01^{f}
G10	69.67±0.42	70.13±0.33	71.46±0.01^{f}	71.42±0.01^{f}	71.02±0.01^{f}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicates statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1=Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

4.7 Effect of aqueous extract *Borreria verticillata* aerial part on serum oxidative stress markers in *Clarias Gariepinus* Juveniles

4.7.1 Mean Serum levels of Malondialdehyde

There were no significant (P>0.05) alterations in the mean serum MDA levels of the fish between all the groups on day 0 (Table 4.19). On day 7, the mean serum MDA level in group 6 increased from 0.15±0.01 mmol/mg protein to 0.48±0.01 mmol/mg protein on day 28, these mean values were significantly higher (P<0.05) than those in corresponding days of sampling in groups 1 -5. A significant (P<0.05) dose dependent increase in serum levels of MDA were observed on each day of sampling from groups 7 - 10, compared to group 1. However, the statistical analysis showed a significant (P<0.05) decline in the MDA level of these groups in comparison to group 6.

4.7.2 Mean Serum levels of reduced glutathione

There was a significant (P<0.05) rise in mean serum level of reduced glutathione in fish exposed to *Adenium obesum* (group 6), from 10.40±0.03µg/ml on day 7 to 10.56±0.01 µg/ml on day 28 (Table 4.20).These mean values of GSH declined significantly (P<0.05) in groups 7 – 10,with increase in the dose of BVE, on the respective days of sampling. It should be noted that mean levels of GSH in groups 6 – 10 were significantly (P<0.05) higher than those of group 1, on the same days of sampling.

4.7.3 Mean Serum levels of Superoxide Dismutase

The mean serum levels of superoxide dismutase (SOD) in group 6 (1.86±0.01- 1.98±0.00 µmol/min) were significantly (P<0.05) higher than the 1.75 ± 0.01 µmol/min recorded for group 1 during the period (Table 4.21). Groups 7 to 10 also had significantly (P<0.05) higher values compared to groups 1 – 5. However, these values declined significantly

(P<0.05) with increase in the dose of BVE administered to the fish, as observed on each day of sampling,compared to group 6,but significantly (P<0.05) higher than G1.

Table 4.19: Mean Serum Malondialdehyde (MDA) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	SERUM MALONDIALDEHYDE (mmol/mg protein)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	0.09±0.01	0.09±0.00^{f}	0.09±0.01^{f}	0.08±0.01^{f}	0.09±0.01^{f}
G2	0.08±0.00	0.08±0.01	0.09±0.01	0.06±0.00	0.05±0.01
G3	0.08±0.01	0.07±0.00	0.09±0.01	0.05±0.00	0.06±0.01
G4	0.08±0.02	0.08±0.01	0.09±0.01	0.08±0.01	0.09±0.01
G5	0.08±0.01	0.08±0.00	0.09±0.01	0.06±0.00	0.07±0.00
G6	0.08±0.01	0.15±0.01^{hij}	0.39±0.00^{ghij}	0.41±0.01^{ghij}	0.48±0.01^{ghij}
G7	0.08±0.01	0.13±0.00^{f}	0.17±0.00^{f}	0.23±0.00^{fhij}	0.29±0.00^{fj}
G8	0.08±0.00	0.11±0.00^{f}	0.16±0.00^{f}	0.20±0.00^{fghj}	0.28±0.01^{fj}
G9	0.09±0.00	0.11±0.00^{f}	0.15±0.00^{f}	0.18±0.00^{fg}	0.26±0.01
G10	0.08±0.01	0.10±0.00^{f}	0.14±0.00^{fgh}	0.17±0.00^{fgh}	0.23±0.00^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/lBVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.20: Mean Serum Reduced Glutathione (GSH) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

	SERUM REDUCED GLUTATHIONE (µg/ml)				
TREATMENT GROUPS	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	10.13±0.01	10.13±0.00^{f}	10.11±0.01^{f}	10.10±0.00^{f}	10.10±0.00^{f}
G2	10.12±0.01	10.10±0.00	10.11±0.00	10.13±0.01	10.12±0.01
G3	10.12±0.01	10.13±0.01	10.12±0.00	10.13±0.00	10.13±0.01
G4	10.13±0.00	10.12±0.00	10.13±0.00	10.14±0.00	10.10±0.01
G5	10.12±0.00	10.13±0.00	10.12±0.01	10.14±0.00	10.12±0.01
G6	10.12±0.01	10.40±0.03^{ghij}	10.43±0.04^{ghij}	10.50±0.02^{ghij}	10.56±0.01^{ghij}
G7	10.11±0.00	10.15±0.00^{fij}	10.30±0.00^{fij}	10.31±0.00^{fij}	10.47±0.00^{fij}
G8	10.12±0.01	10.14±0.00^{fj}	10.28±0.01^{fj}	10.26±0.00^{fj}	10.36±0.00^{fj}
G9	10.12±0.01	10.13±0.00^{fg}	10.26±0.00^{fghj}	10.21±0.00^{fgj}	10.31±0.00^{fgj}
G10	10.11±0.02	10.12±0.00^{fgh}	10.15±0.00^{fghi}	10.16±0.01^{fghi}	10.20±0.00^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; G1=Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/l BVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

Table 4.21: Mean Serum Superoxide Dismutase (SOD) level of *Clarias gariepinus* juveniles exposed to Sub-Lethal treatment of aqueous extract *Adenium obesum stem* bark and treated with aqueous extract *Borreria verticillata* aerial part

TREATMENT GROUPS	SUPEROXIDE DISMUTASE (µmolSOD/min)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	1.75±0.01	1.74±0.01^{f}	1.73±0.01^{f}	1.72±0.01^{f}	1.73±0.01^{f}
G2	1.74±0.00	1.74±0.00	1.72±0.02	1.71±0.01	1.71±0.02
G3	1.74±0.01	1.72±0.01	1.72±0.00	1.73±0.01	1.73±0.00
G4	1.73±0.00	1.69±0.01	1.69±0.04	1.69±0.01	1.70±0.03
G5	1.73±0.00	1.68±0.01	1.68±0.05	1.68±0.01	1.67±0.06
G6	1.73±0.01	1.96±0.01^{ij}	1.97±0.01^{j}	1.98±0.01^{hij}	1.98±0.01^{hij}
G7	1.72±0.00	1.93±0.00	1.93±0.00^{j}	1.93±0.00^{ij}	1.90±0.00^{ij}
G8	1.72±0.01	1.92±0.00	1.92±0.00	1.91±0.00^{f}	1.80±0.00^{fj}
G9	1.72±0.02	1.92±0.00^{f}	1.91±0.00	1.91±0.01^{fg}	1.83±0.01^{fg}
G10	1.71±0.01	1.90±0.01^{f}	1.90±0.00^{fg}	1.90±0.00^{fg}	1.78±0.01^{fgh}

Superscripts, ^{a,b,c,d,e,f,g,h,I,j,} indicate statistical difference(P<0.05) when compared to groups 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; G1 = Control; G2=25mg/l BVE; G3=50mg/l BVE; G4=100mg/l BVE; G5=200mg/l BVE;G6= 10% LC50 AOE; G7= G2+G6; G8= G3+G6; G9=G4+G6; G10=G5+G6.Keys: AOE=Aqueous extract *Adenium obesum* stem bark; BVE= Aqueous extract *Borreria verticillata* aerial part

4.8 Histopathlogical Studies

4.8.1 Histopathological changes in fish gills during Sub-Lethal Treatment

Sections of gills from juvenile *Clarias gariepinus* in group 1 (control) showed no alterations in the primary and secondary lamellae (Plate I) on days 0 to 28. Similarly, there were no microscopic changes on the gills of Group 5 during the period (Plate II) on day 14,but denuded and atrophied secondary lamellae was observed on day 28 (Plate V). On the other hand, fusion of secondary lamellae, atrophied primary lamellae and congested blood vessels were observed in gills from Group 6 on day 14 (Plate III). The sections of gills obtained from group 6 on day 28 had fusion of secondary lamellae, club-shaped primary lamella, complete fusion of the secondary lamella and congested blood vessel (Plate VI).The gills in group 10 had one-sided fusion, curled secondary lamellae and telangiectasia in primary lamellae as at day 14 (Plate IV) and cellular infiltration on day 28 (Plate VII) of the study.

4.8.2 Histopathological changes in fish Liver during Sub-Lethal Treatment

Histopathology revealed normal liver in Group 1 (Plate VIII) and Group 5 (Plate IX) during the period. The liver sections in Group 6 had hepatocellular vacuolation and aggregation of mononuclear cells (Plate X). The vacuolation was more severe and widespread on day 28 (Plate XIII).These changes were also seen in liver samples obtained on days 14 (Plate XI) and 28 (Plate XIV) in Group 10, but less severe.

4.8.3 Histopathological changes in fish Intestine during Sub-lethal treatment

Sections of posterior segments of the intestines showed normal histology in Groups 1 (Plate XV) and 5 (Plate XVI and Plate XIX) during the study. The intestinal sections in

Group 6 were characterized by hyperplasia of goblet cells (Plate XVII and Plate XX), similar to those in Group 10 (Plate XVI and Plate XXI) on day 14 and day 28 respectively.

4.8.4 Histopathological changes in fish kidney with Sub-Lethal Treatment

There were normal kidney structures in Groups 1 (Plate XXII) and group 5 (Plates XXIII and VI) throughout the four weeks of this study. Tubular degeneration, congestion of blood vessels and hypercellularity of glomeruli were observed in Groups 6 (Plates XXIV and XXVII) from days 21 to 28. Most of the kidneys in group 10 similarly showed a near normal architecture and hypercellularity glomeruli (Plates XXV and XXVIII).

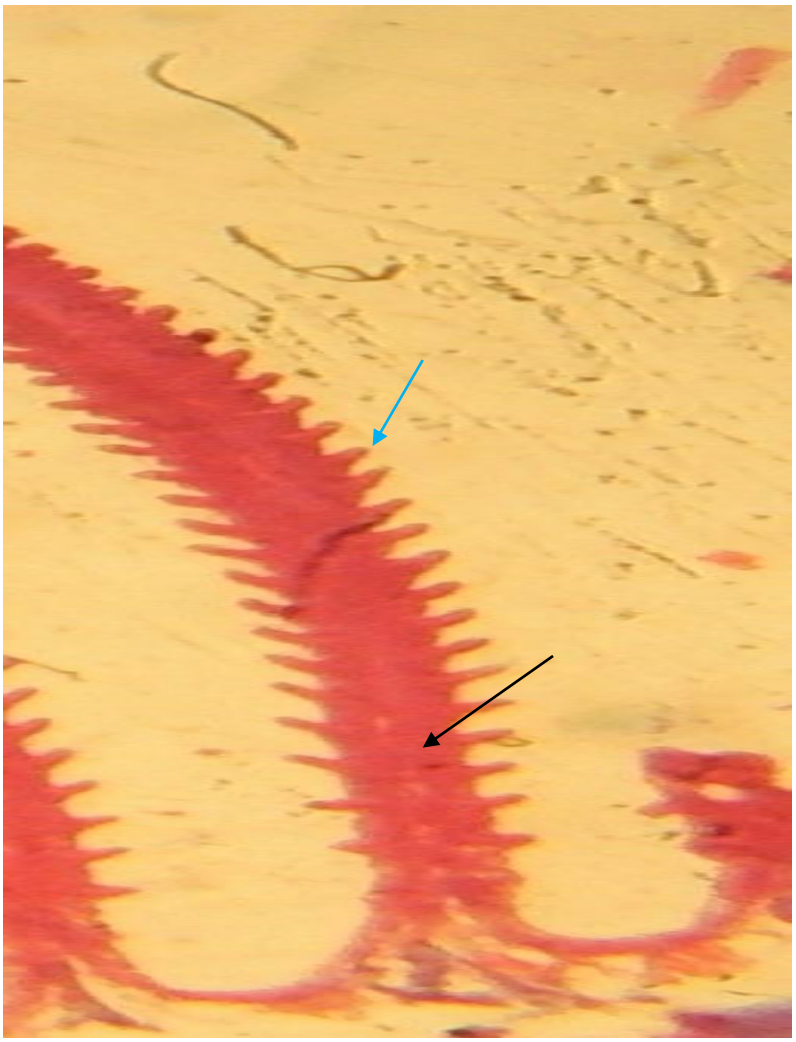


Plate I : Photomicrograph of the gill in G1 (CONTROL) on D14, showing a normal primary lamella (Black arrow) and a normal secondary lamellae (blue arrow)(H & E, X200).

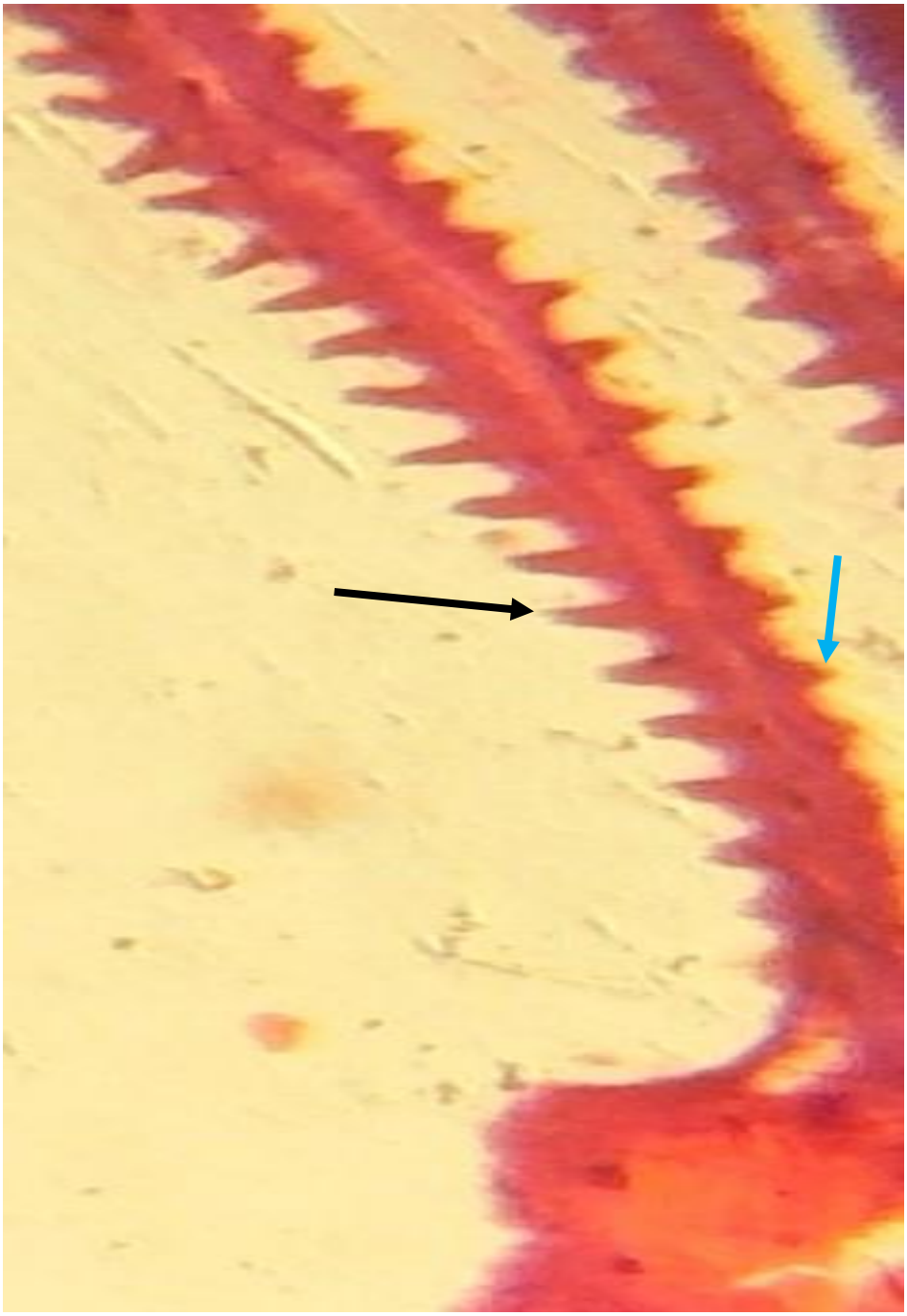


Plate II: Photomicrograph of the gill in G5 on day 14, showing secondary lamellae (black arrow), atrophied secondary lamellae (blue arrow) (H & E, X200).



Plate III: Photomicrograph of the gill in G6 on day 14, showing vacuolated primary lamella (yellow arrow), hypertrophy secondary lamella (red arrow).(H & E, X400)

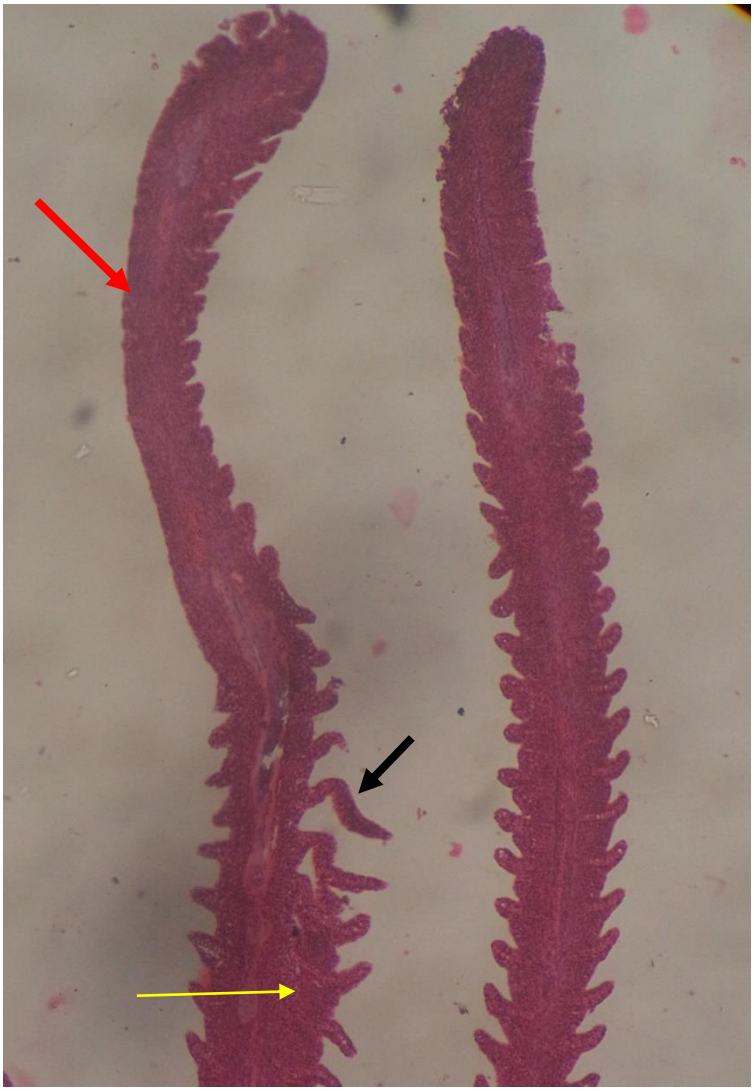
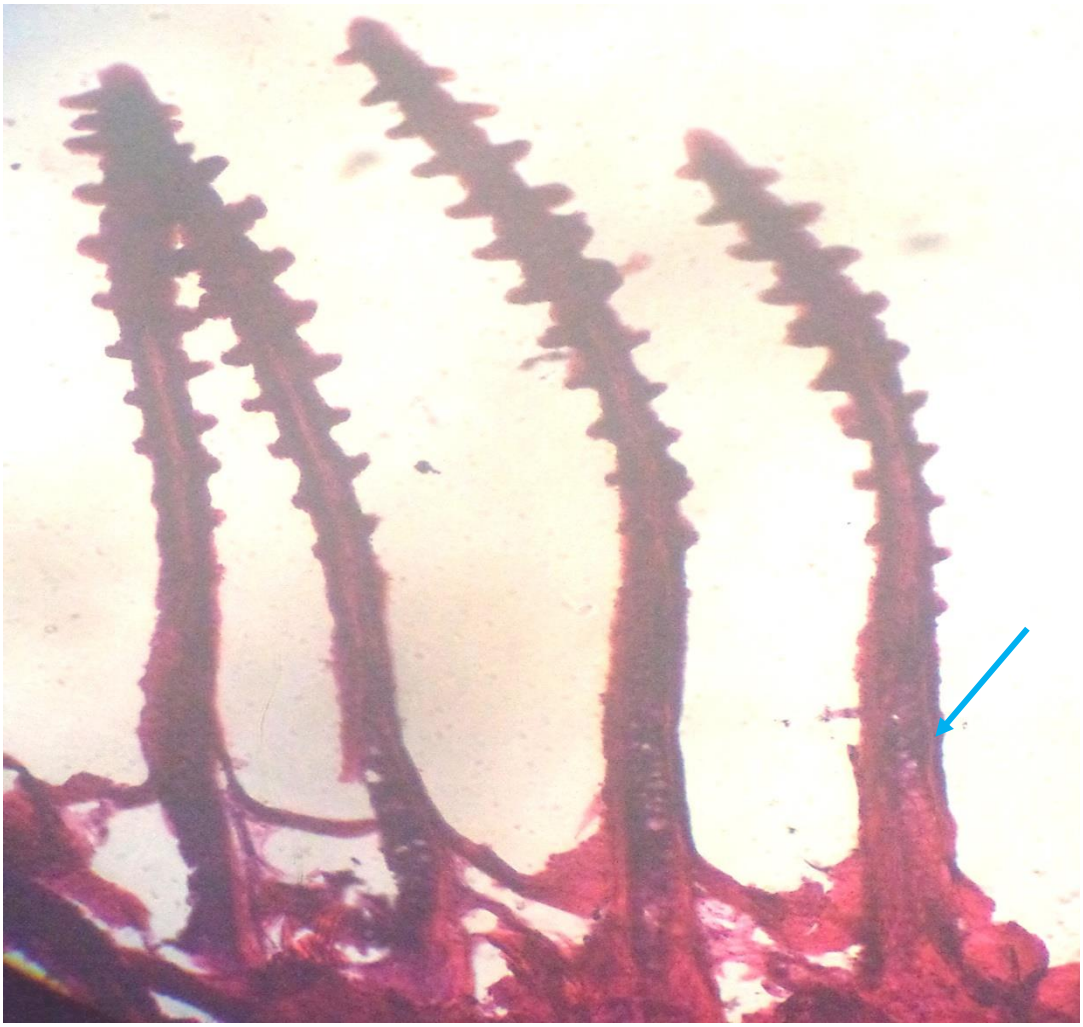


Plate IV: Photomicrograph of the gill in G10 on day 14,showing a one-sided fusion of the secondary lamella(red arrow),mis-shapened secondary lamella (black arrow), Telangiectasia within the primary lamella (yellow arrow) (H&E, X100).



PlateV: Photomicrograph of the gill G5 on day 28, showing,denuded ,fused,and atrophied secondary lamellae (blue arrow). (H&E, X400).



Plate VI: Photomicrograph of the gill in G6 on day 28, showing fusion of secondary lamella (Yellow arrow),clubbed-shaped primary lamella with complete fusion of the secondary lamella (Black arrow) and congested blood vessel (Red arrow) (H&E, X200).

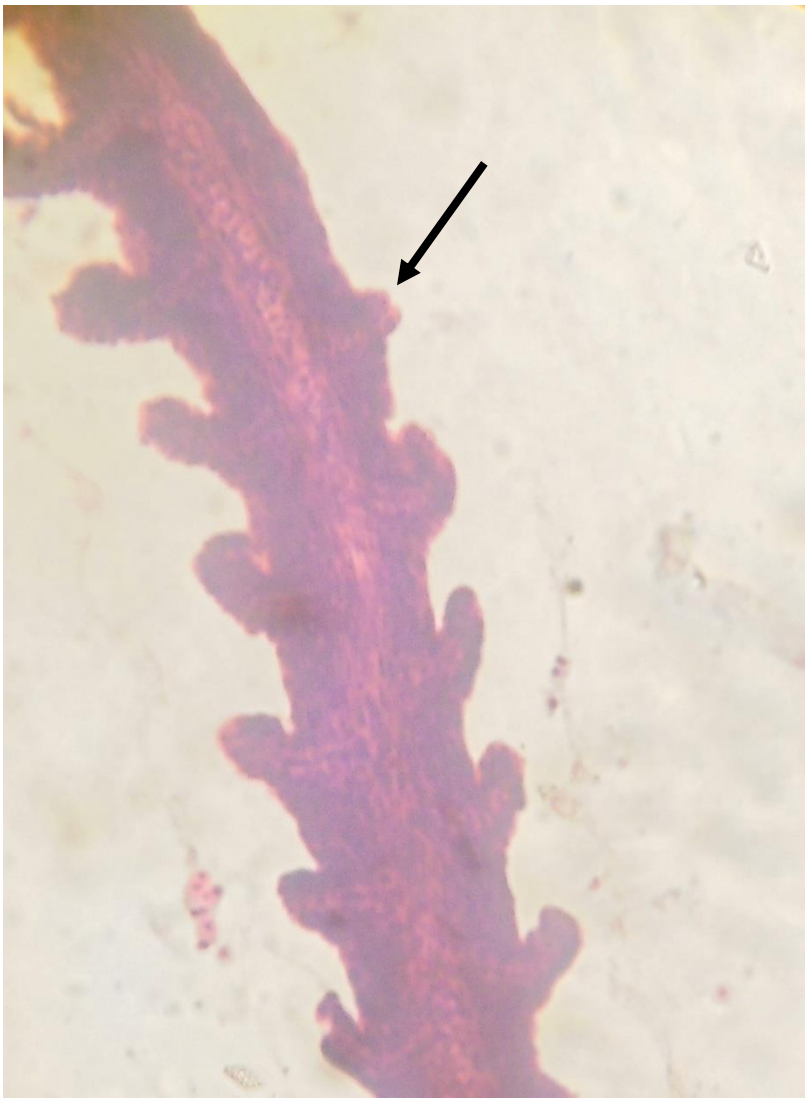


Plate VII: Photomicrograh of the gill in G10 on day 28; showing atrophied secondary lamelae(black arrow). (H & E, X100).



Plate VIII: photomicrograph of the liver G1; showing a normal parenchyma of the liver tissue, bile duct (Black arrow),central vein (yellow arrow),melanomacrophage centre (blue arrow). (H & E, X200).

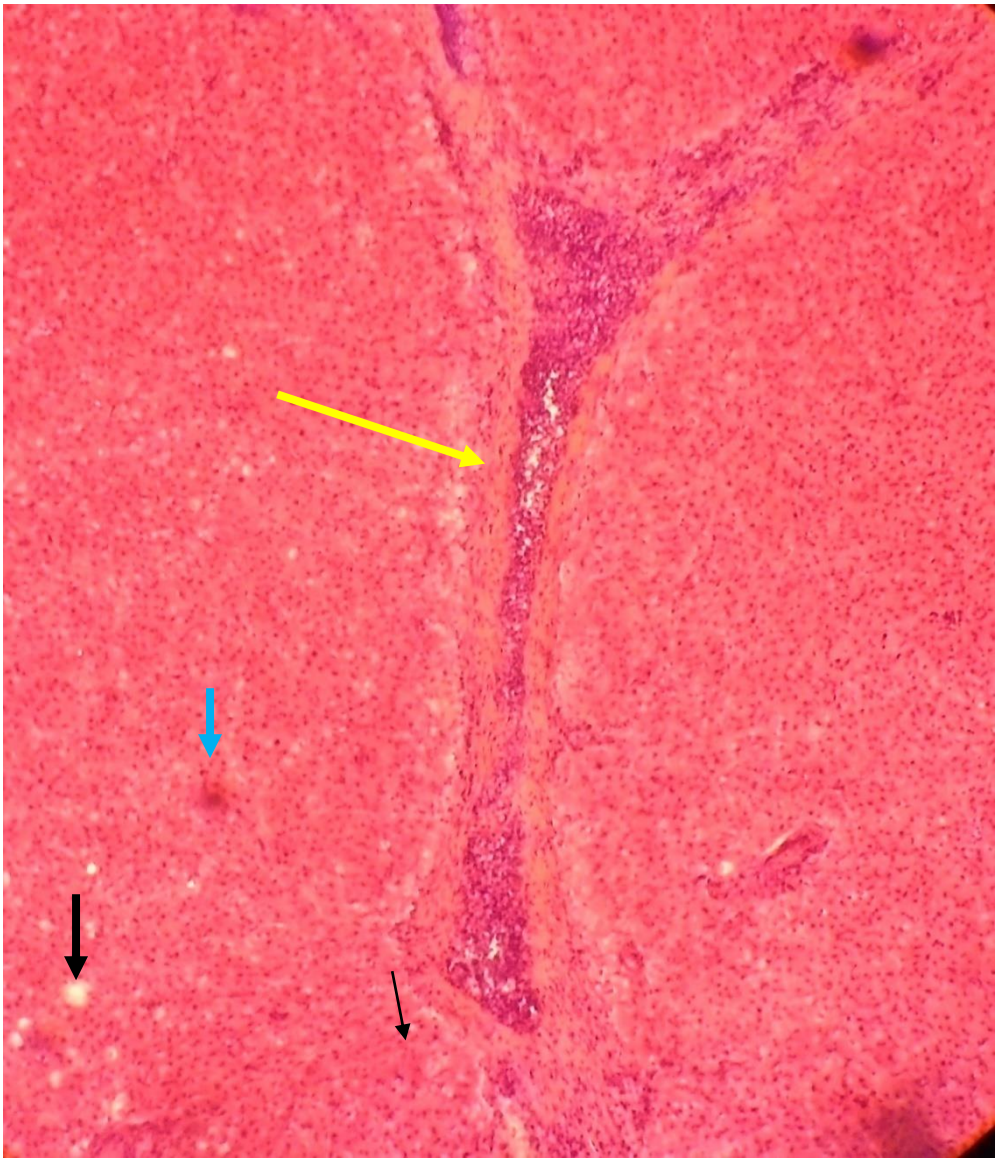


Plate IX: Photomicrograph of the liver in G5 on day 14, hepatopancrease (yellow arrow), melanomacrophage (blue arrow), diffuse areas of vacuolar degeneration of hepatocytes (black arrow). (H&E,X400).

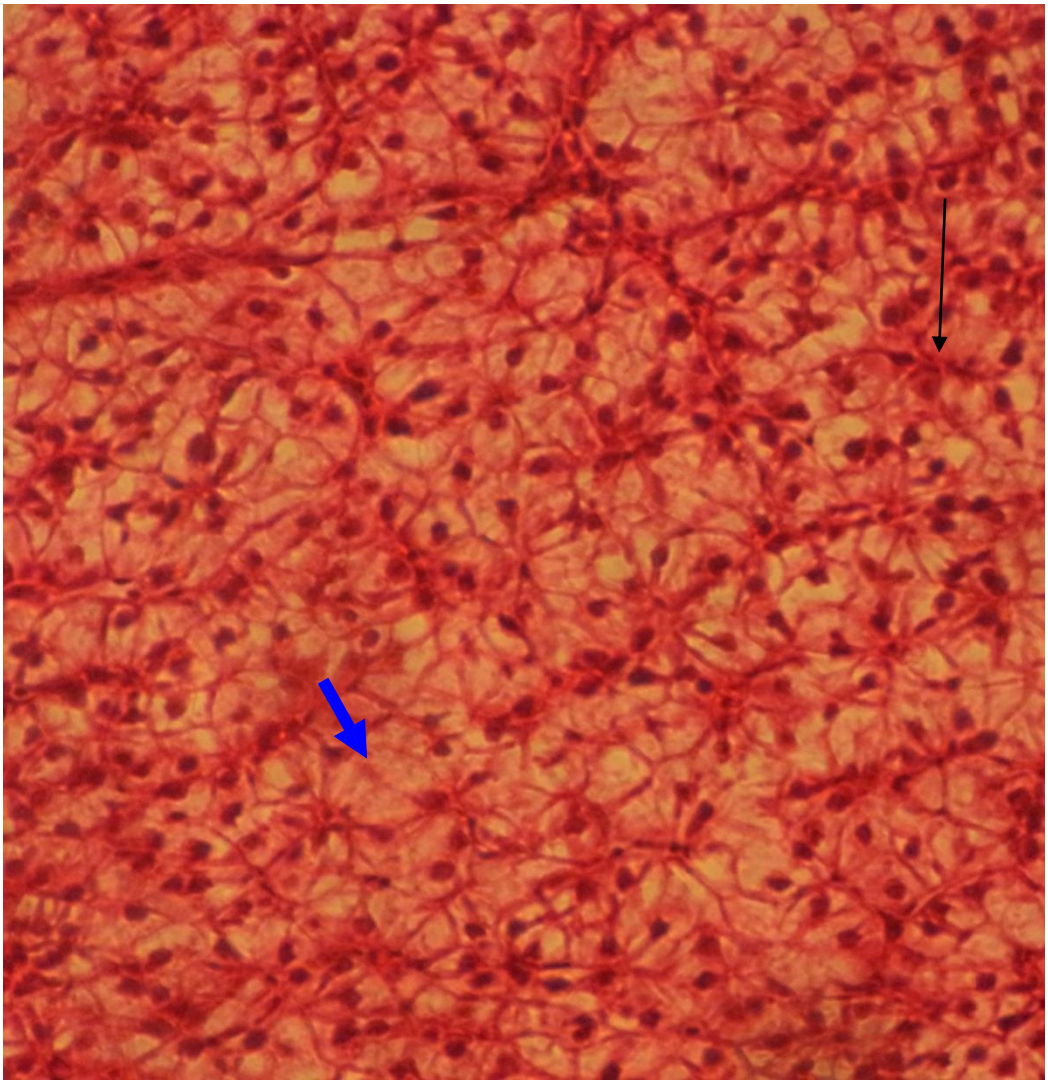


Plate X: Photomicrograph of the liver in G6 on day 14, showing severe vacuolation (blue arrow) (H & E, X2400).

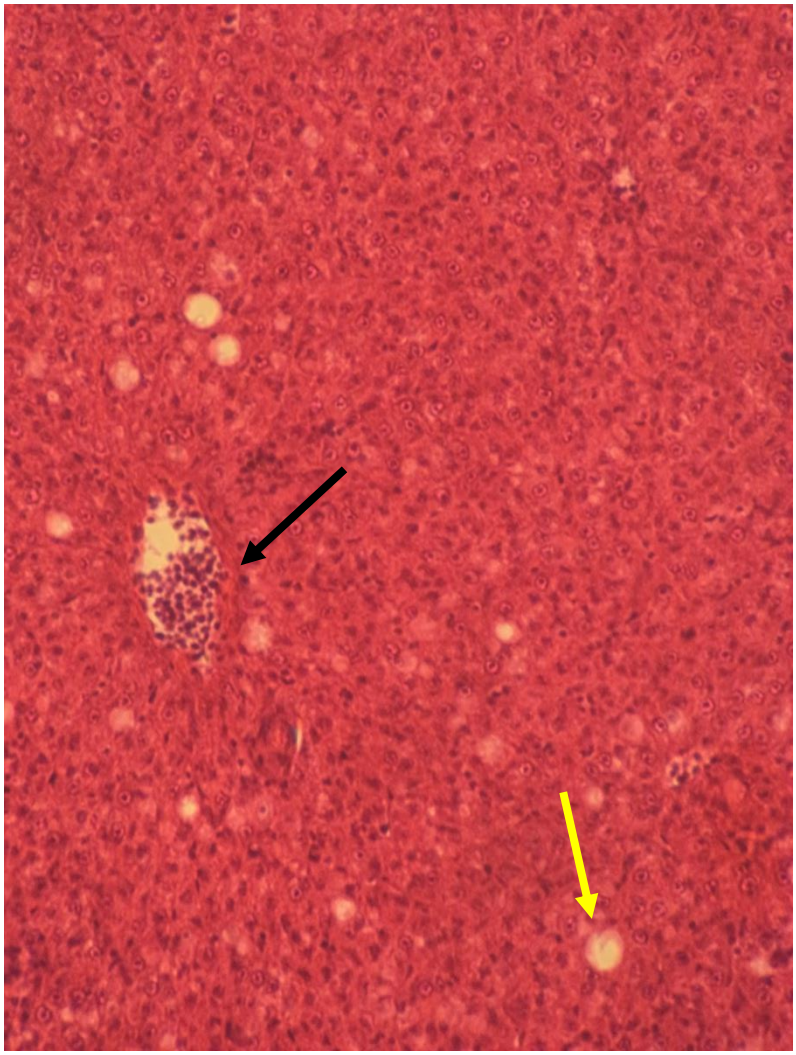


Plate XI: Photomicrograph of the liver in G10 on day 14, showing;centrilobular mononuclear cell aggregations(black arrow), hepatocyte vacuolation (yellow arrow).(H & E, X1360).

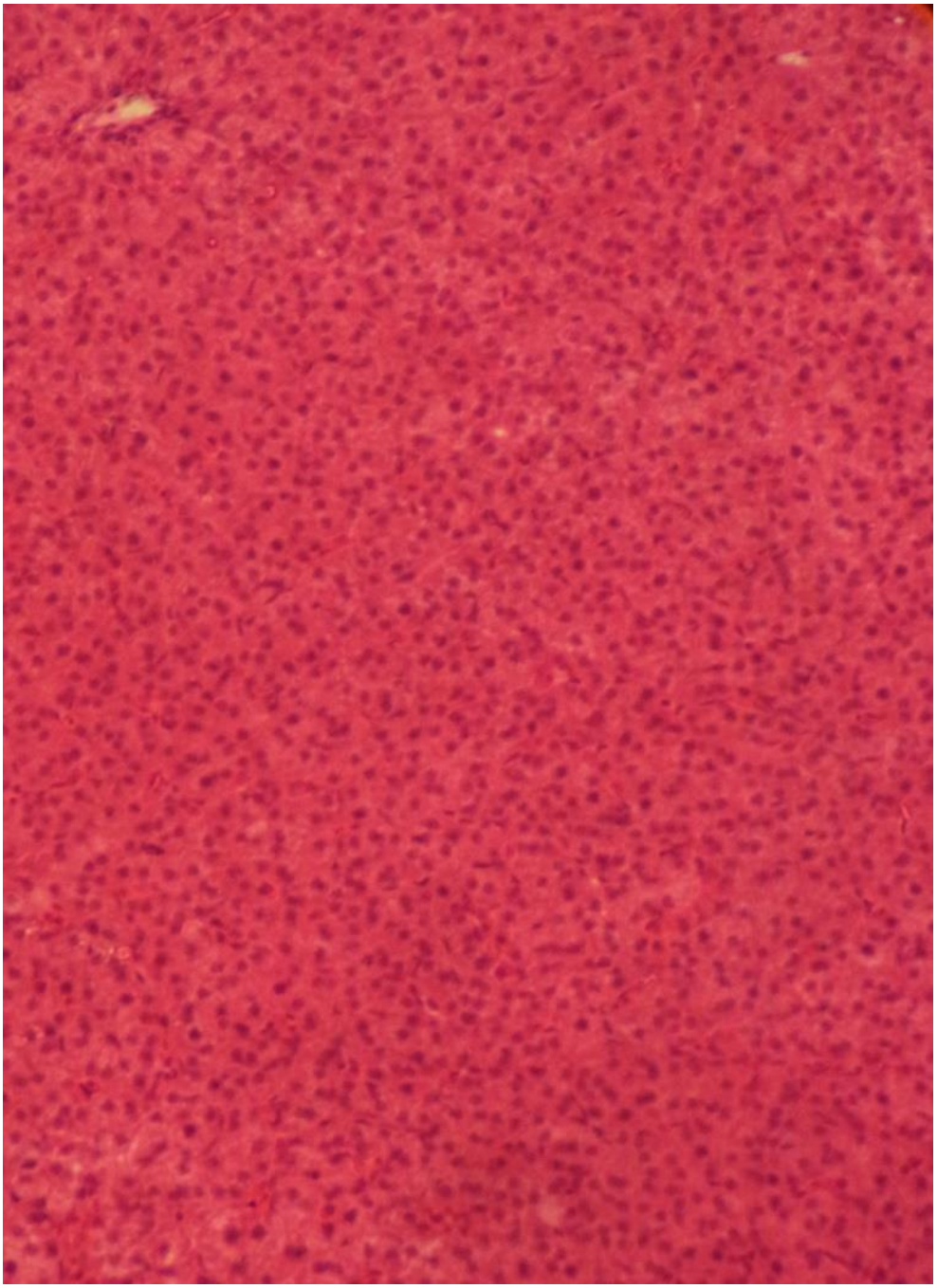


Plate XII: Photomicrograph of the liver in G5 on day 28; showing normal liver parenchyma. (H & E, X40).

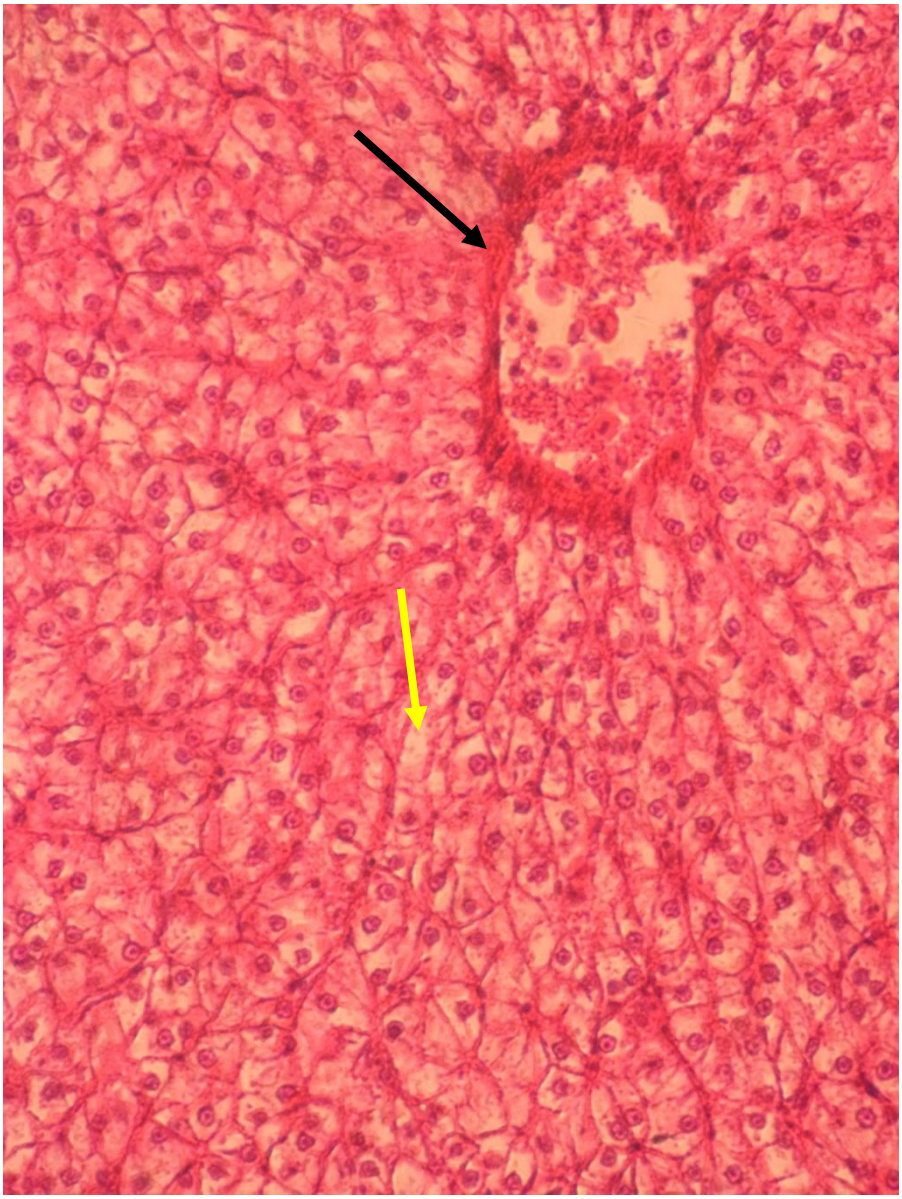


Plate XIII: Photomicrograph of the liver in G6 on day 28; showing congested central vein (black arrow),severe widespread hepatocellular vacuolation (yellow arrow). (H & E, X2400).

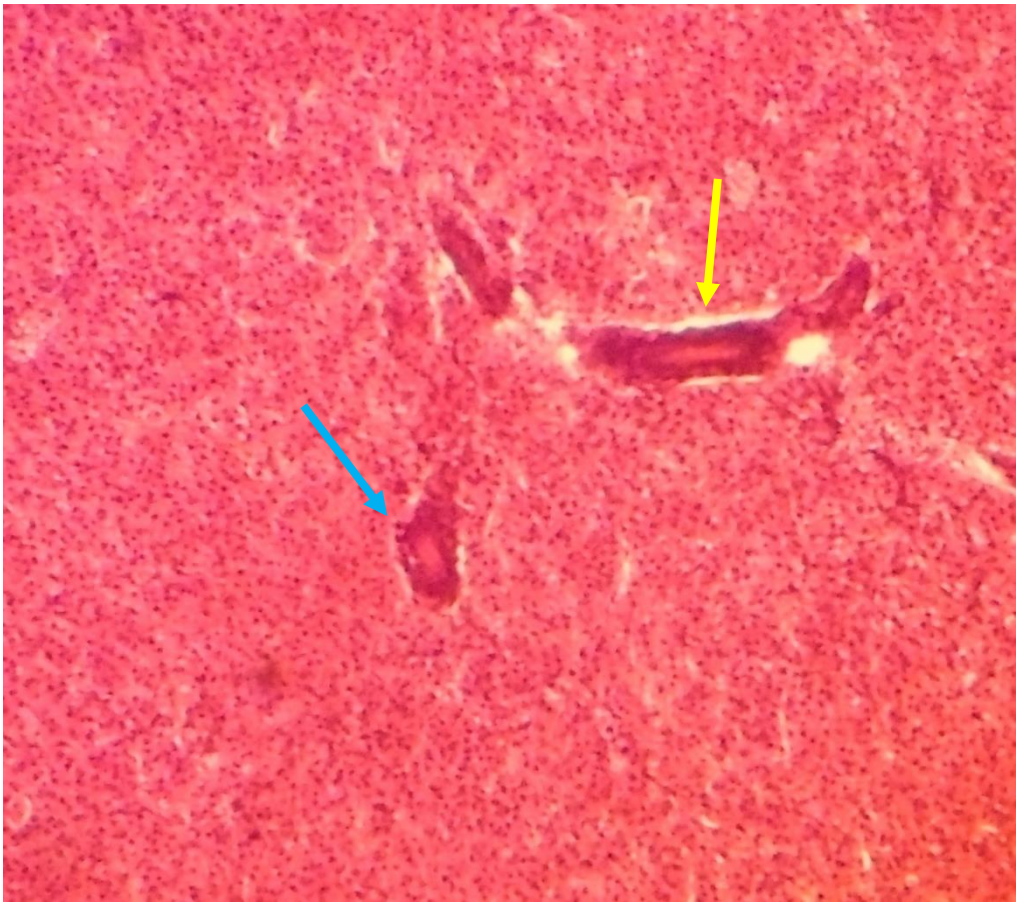


Plate XIV: Photomicrograph of the liver G10 on day 28; atrophied hepatopancrease (yellow arrow),blood vessel (blue arrow)(H & E, X200).

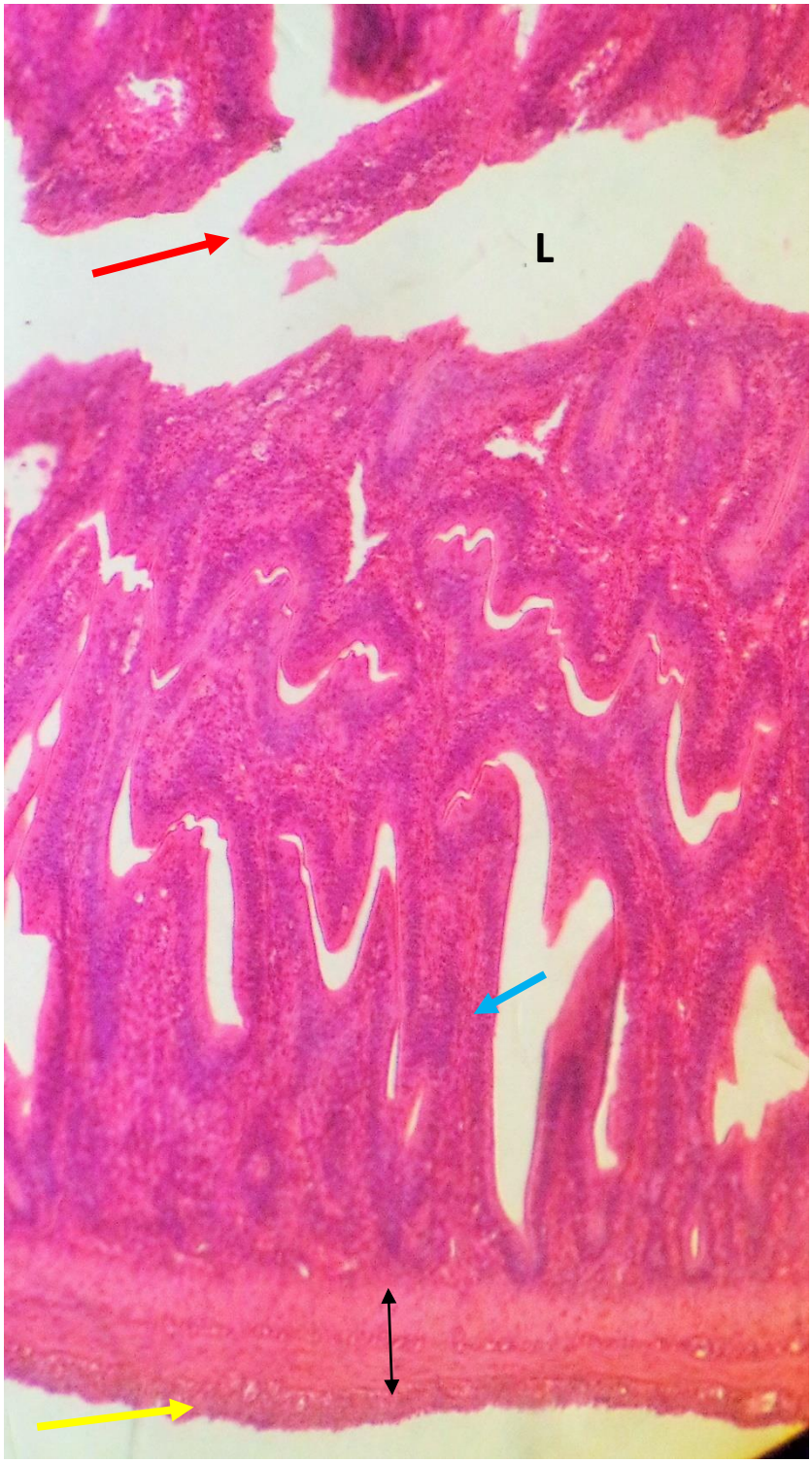


Plate XV: Photomicrograph of the posterior intestine G1(Normal); showing,intestinal lumen (L), intestinal villi (red arrow), lamina propria (blue arrow), muscularis mucosa (double arrow), serosa (yellow arrow) (H & E, X1440).

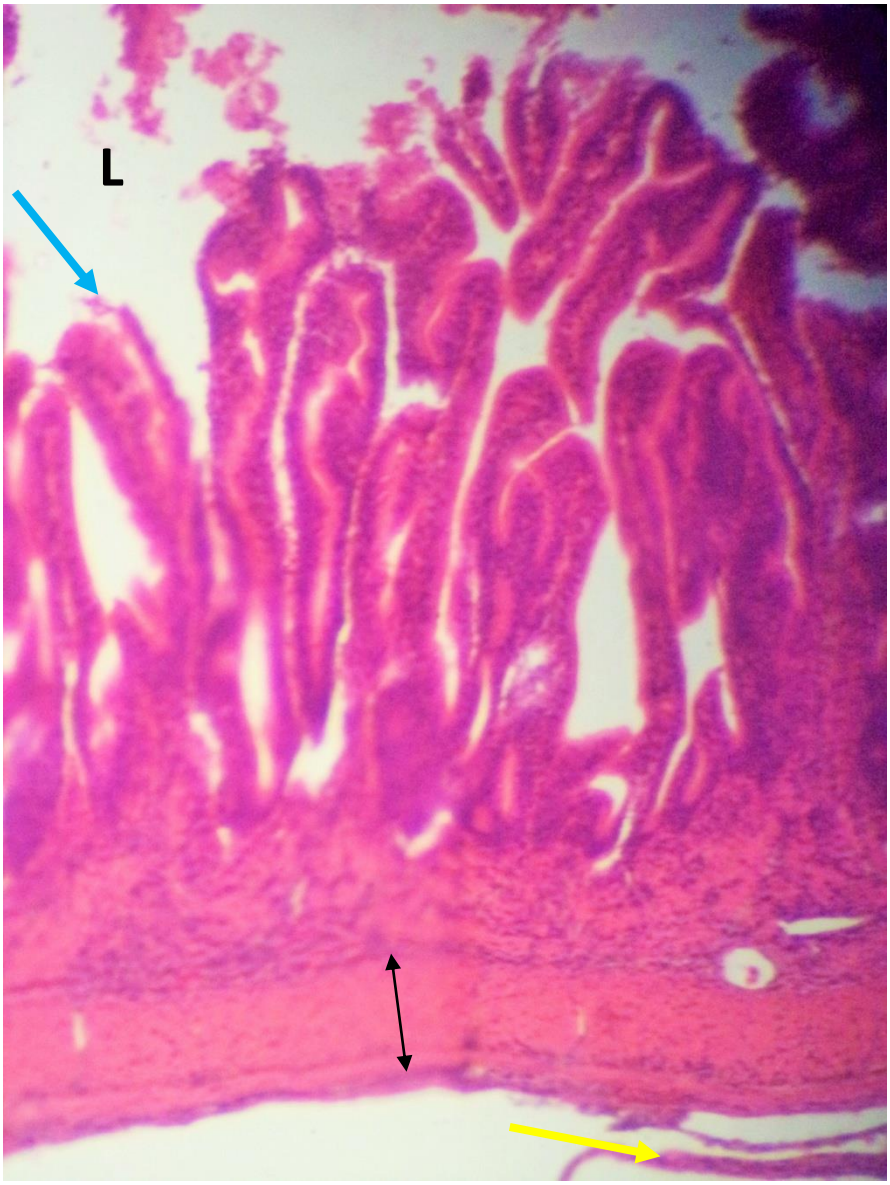


Plate XVI: Photomicrograph of the posterior intestine G5 on day 14; showing intestinal lumen (L),atrophy of villi (green arrow), muscularis mucosa (double arrow), erosa (yellow arrow). (H & E, X1440).

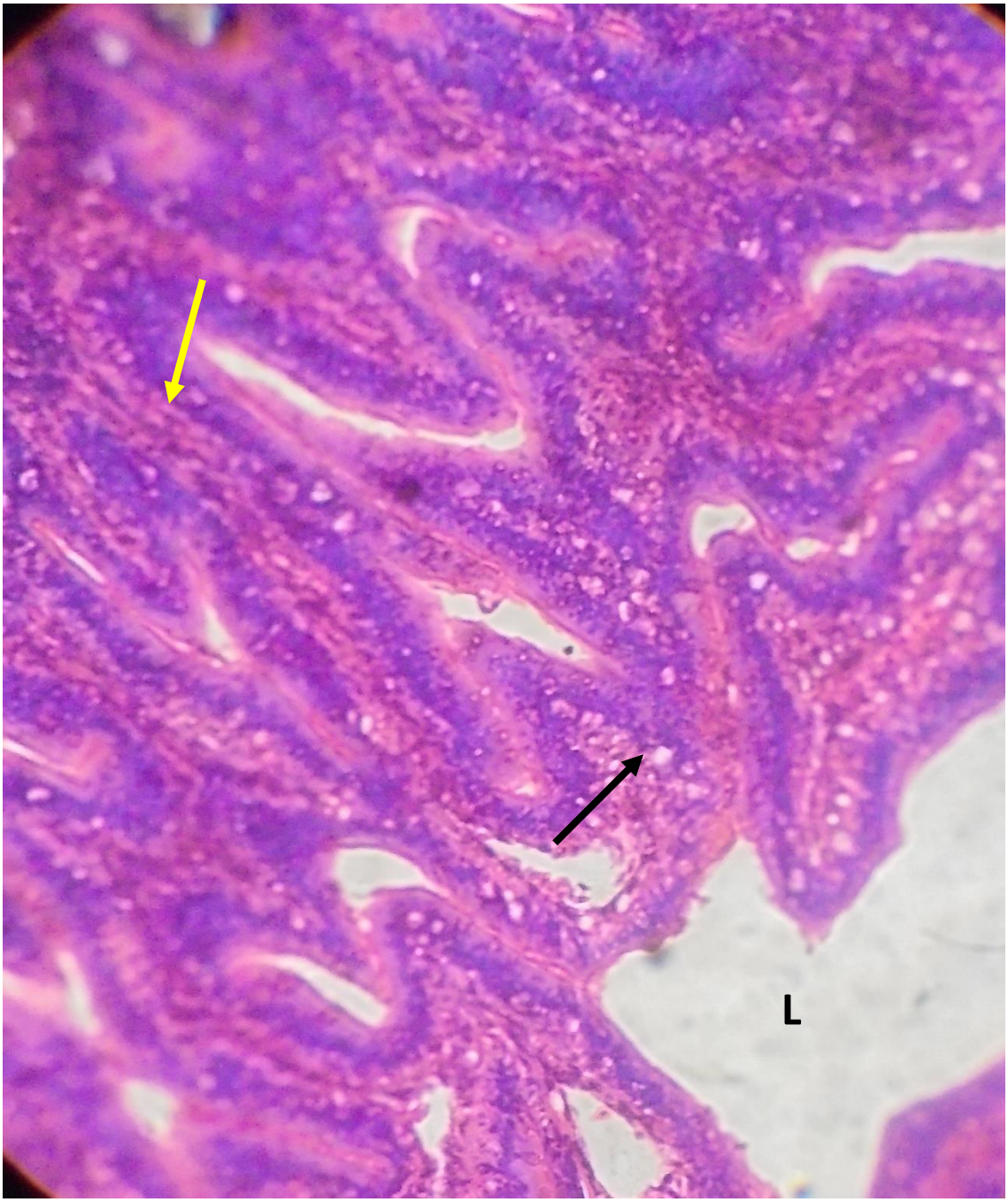


Plate XVII: Photomicrograph of the proximal intestine in G6 of day 14; showing hyperplasia of goblet cells (black arrow), lumen (L). (H & E, X400).

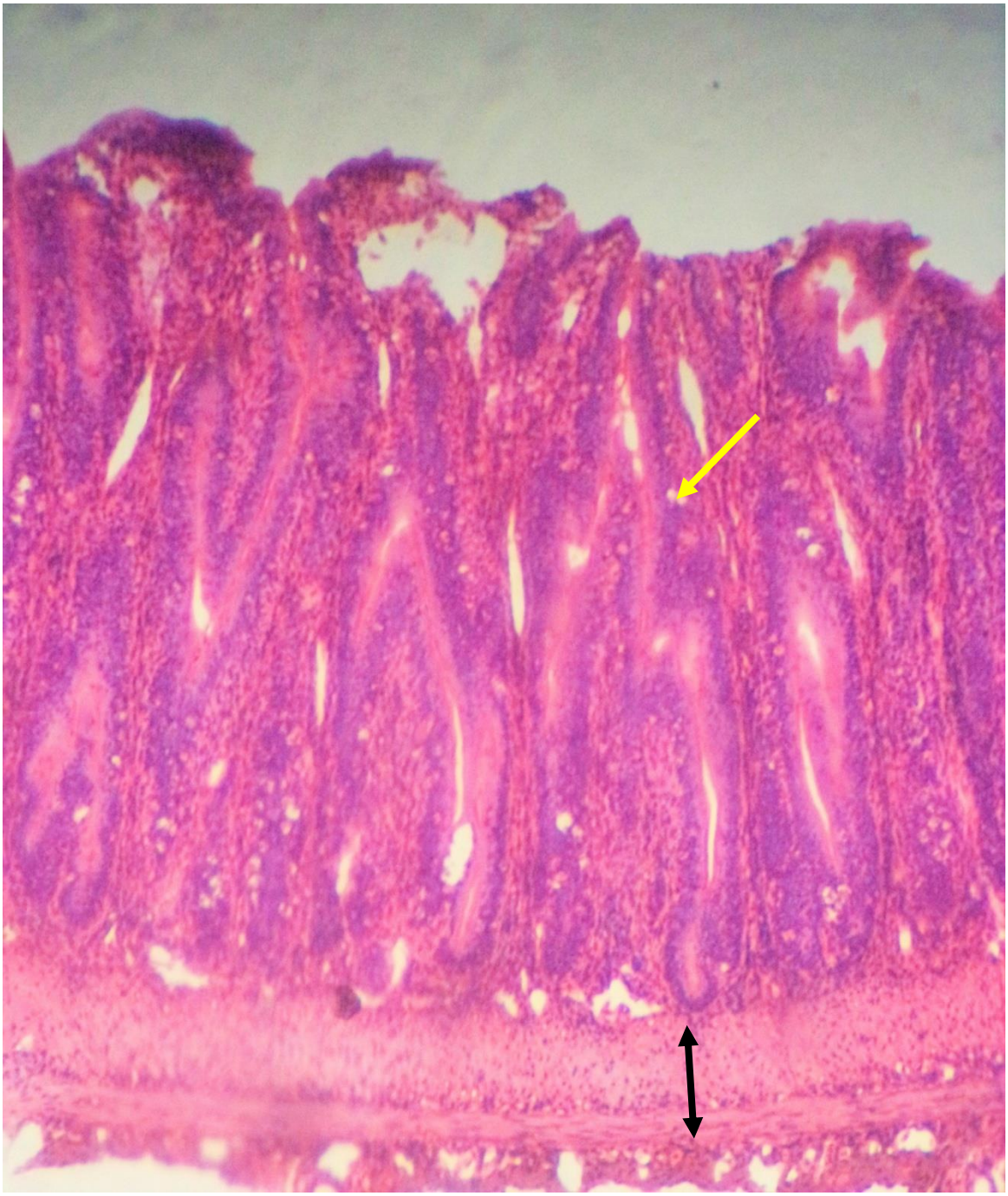


Plate XVIII:Photomicrograph of the posterior intestine G10 on day 14; howing,intestinal lumen (L),Goblet cell hyperplasia (yellow arrow), muscularis mucusa (double arrow). (H & E, X1280).

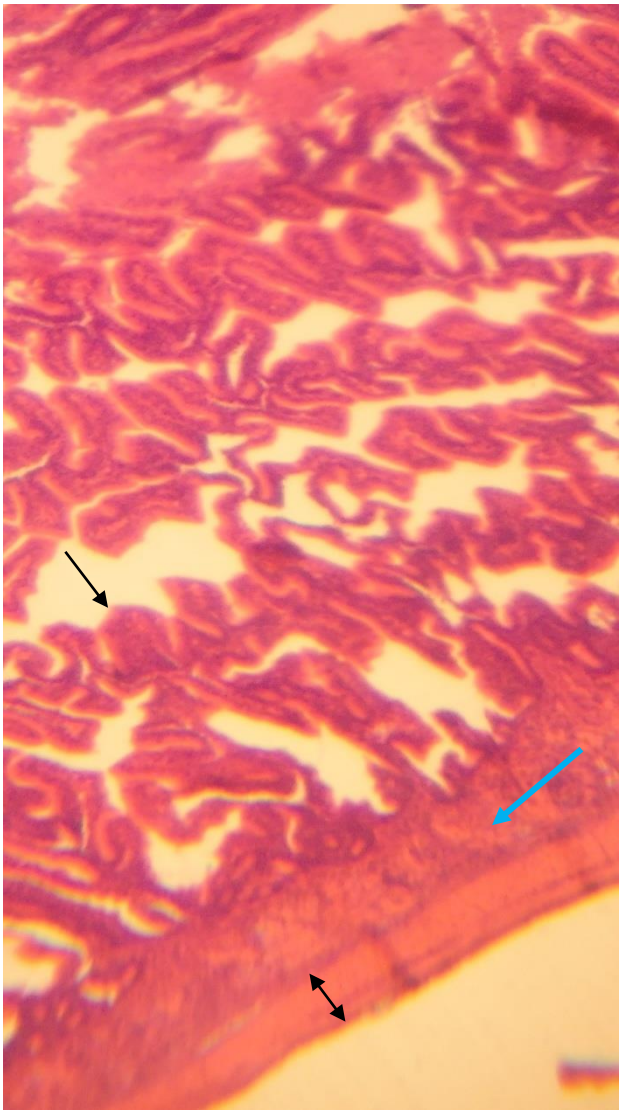


Plate XIX: Photomicrograph of the sagittal section of the posterior intestine G5 day 28, showing, normal secondary villus (black arrow), submucosa (blue arrow), muscularis mucosa (double black arrow), (H & E, X1280).

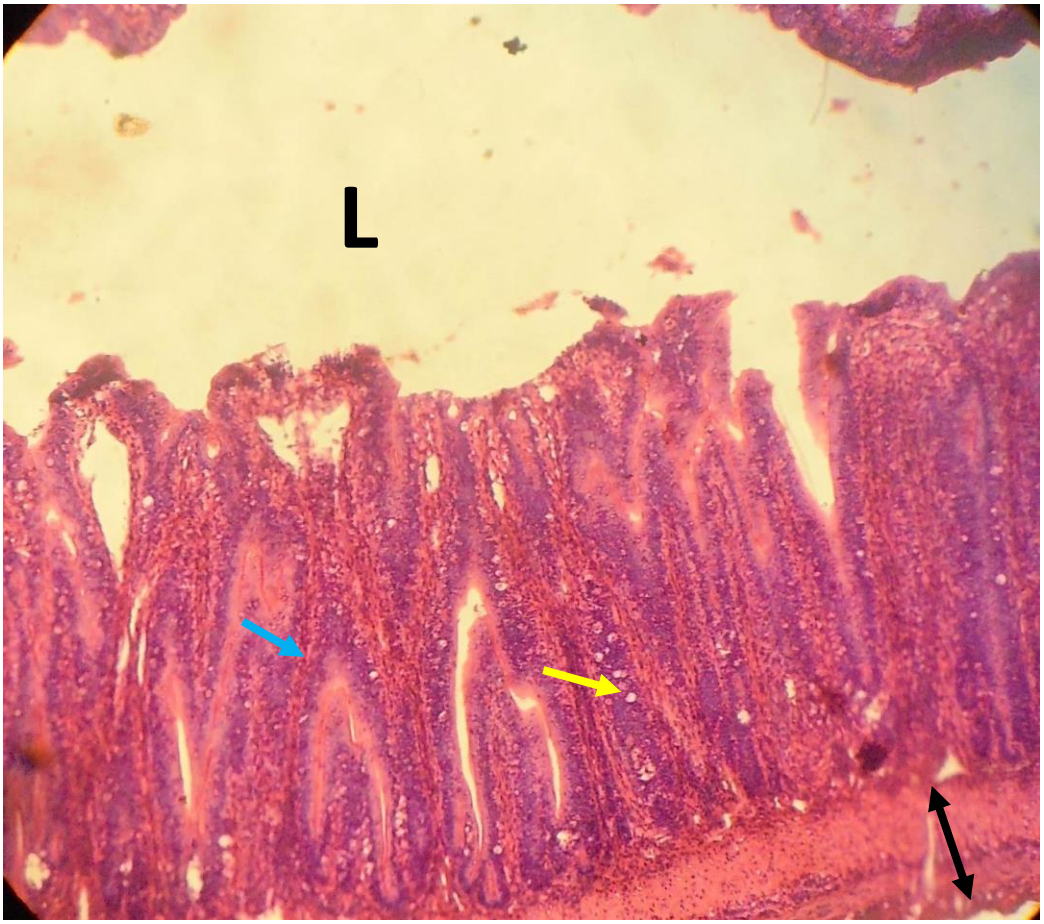


Plate XX: Photomicrograph of the sagittal section of the posterior intestine in G6 on day 28; showing, intestinal lumen (L), hyperplasia of the goblet cells (yellow arrow), lamina propria (blue arrow),muscularis mucosa (double arrow) (H & E, X1280).

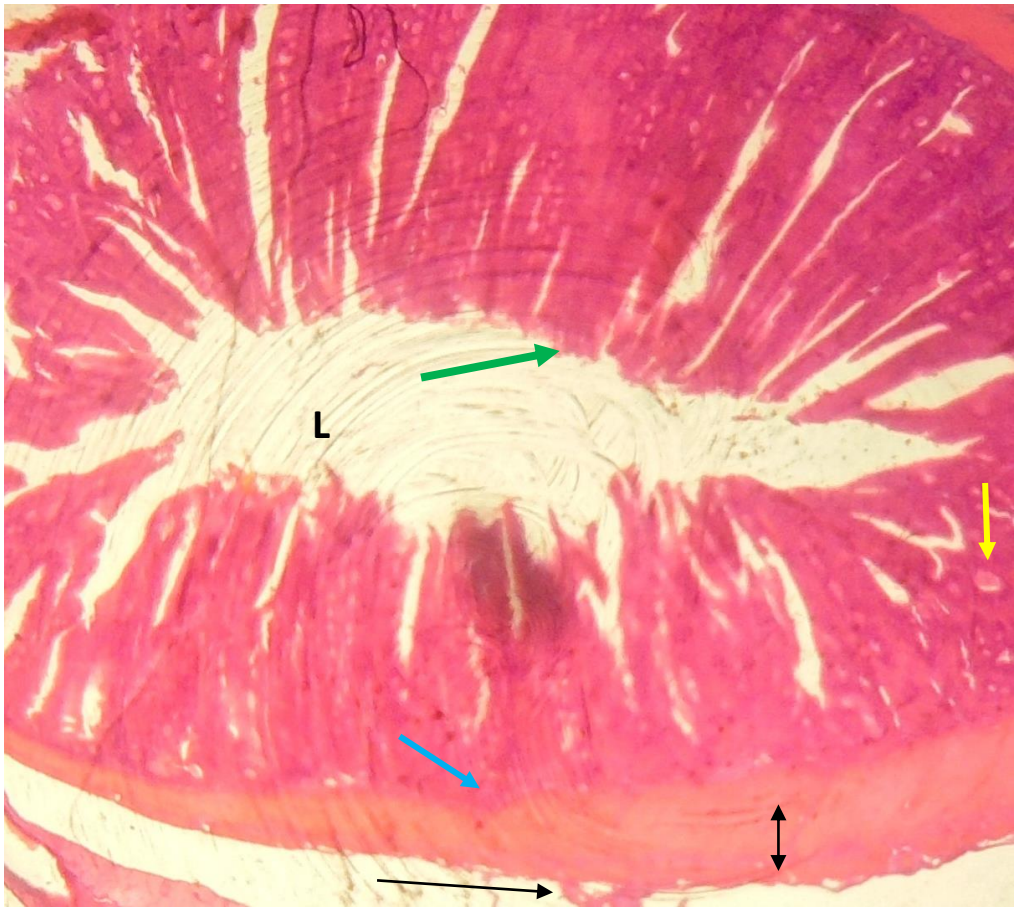


Plate XXI: Photomicrograph of the sagittal section of the posterior intestine in G10 on day 28; showing, intestinal lumen (L), villi (green arrow), goblet cell (yellow arrow), sub mucosa (blue arrow), muscularis mucosa(double arrow),serosa (black arrow),(H & E, X1280).

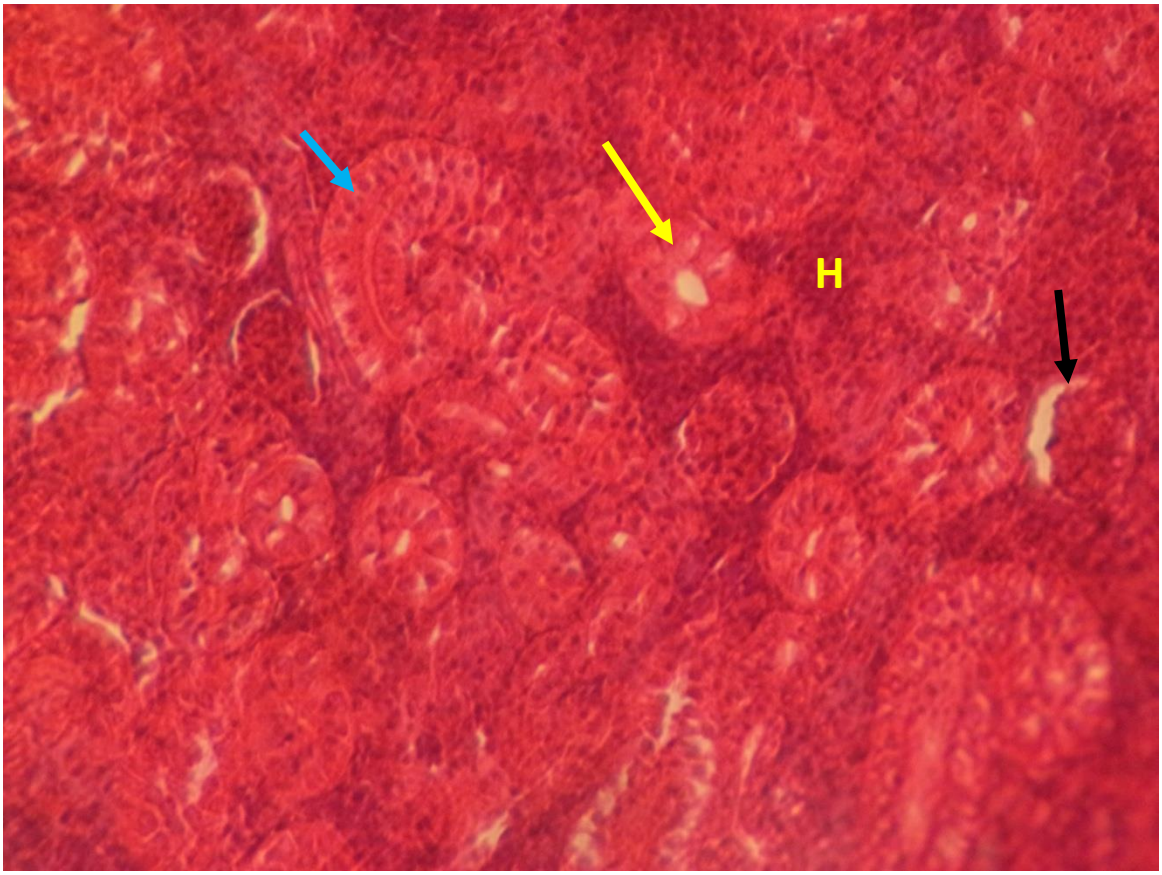


Plate XXII: Photomicrograph of the transverse section of the kidney G1(Normal), showing; distal convoluted tubule (yellow arrow), proximal convoluted tubule (blue arrow), hematopoietic tissue (H) , glomerulus (black arrow), (H & E, X1280).

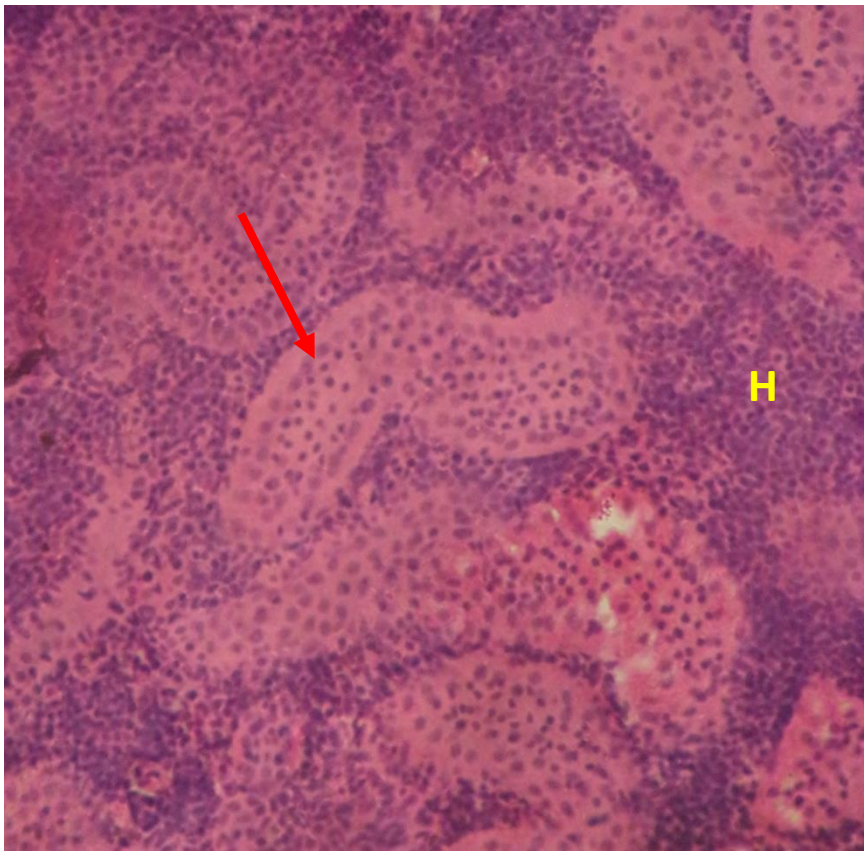


Plate XXIII: Photomicrograph of the transverse section of the kidney in G5 day 14; showing, heamatopoietic tissue (H), proximal convuluted tubule (red arrow).(H & E, X1680).

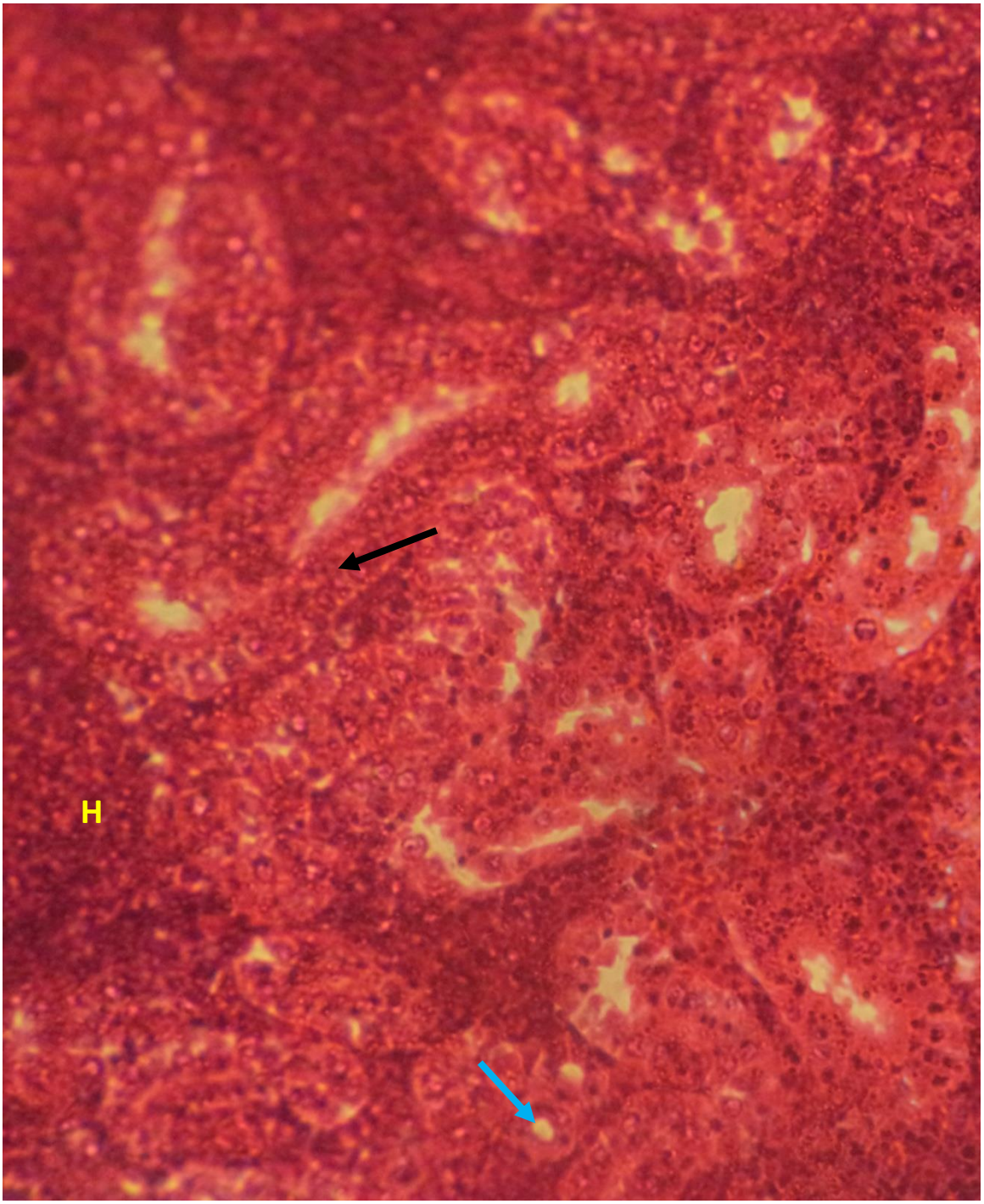


Plate XXIV: Photomicrograph of the transverse of the kidney in G6 on day 14; showing, depleted hematopoietic tissue (H), tubular degeneration (black arrow), distal convoluted tubules (blue arrow). (H & E, X1680).

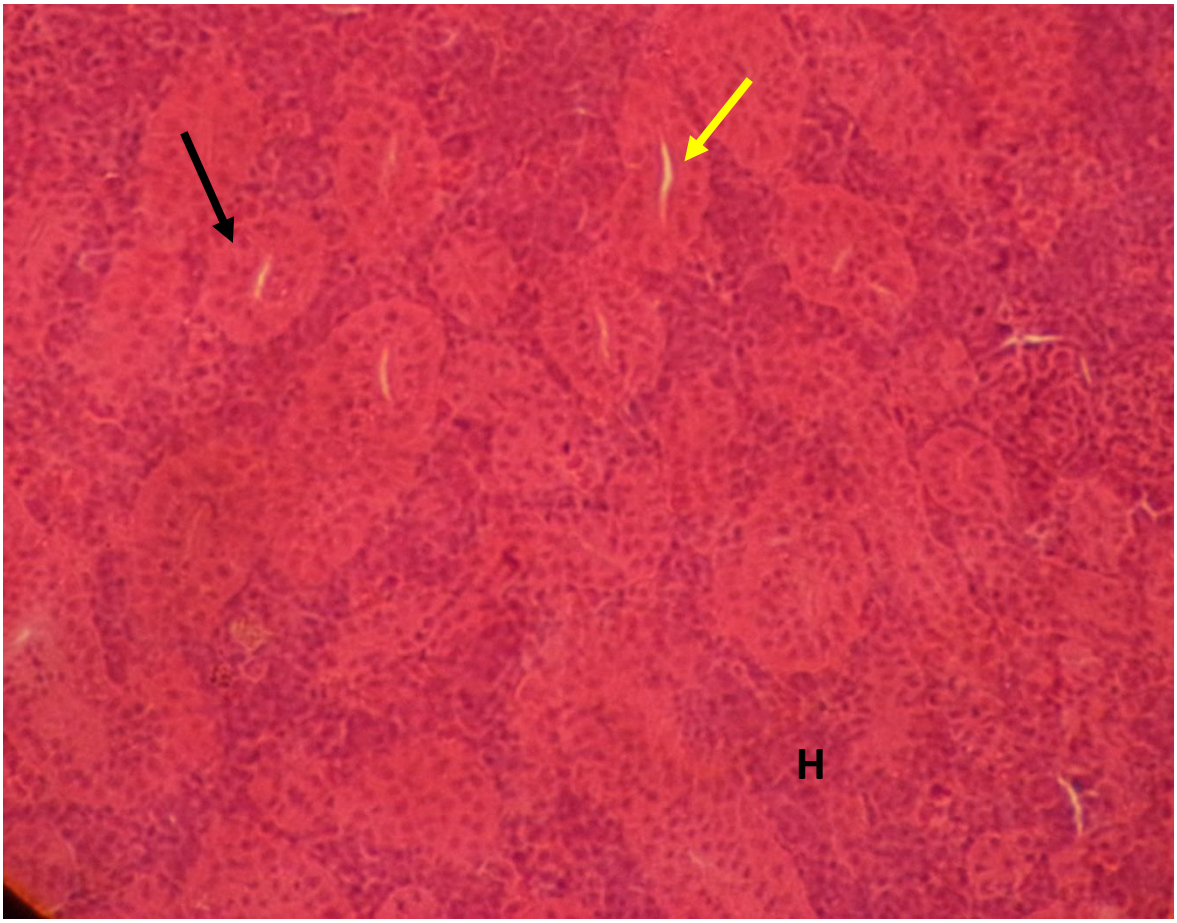


Plate XXV: Photomicrograph of the transverse section of the kidney in G10 on day 14, showing,proximal convuluted tubule (yellow arrow), hematopoietic tissue (H), distal convoluted tubule (black arrow). (H & E, X1120).

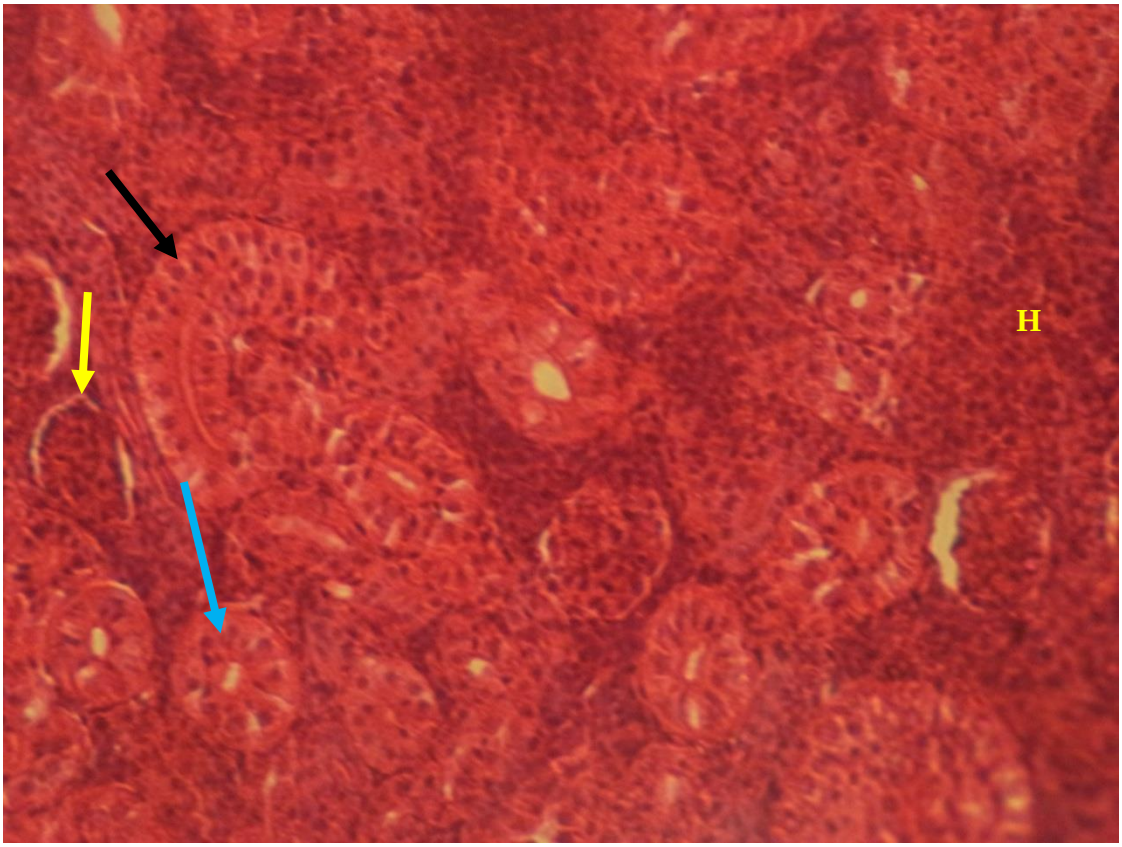


Plate XXVI: Photomicrograph of the transverse section of the kidney in G5 on day 28; showing, hematopoietic tissue (H),glomerulus (yellow arrow), proximal convuluted tissue (black arrow), distal convuluted tubule (blue arrow) (H & E,X1680).

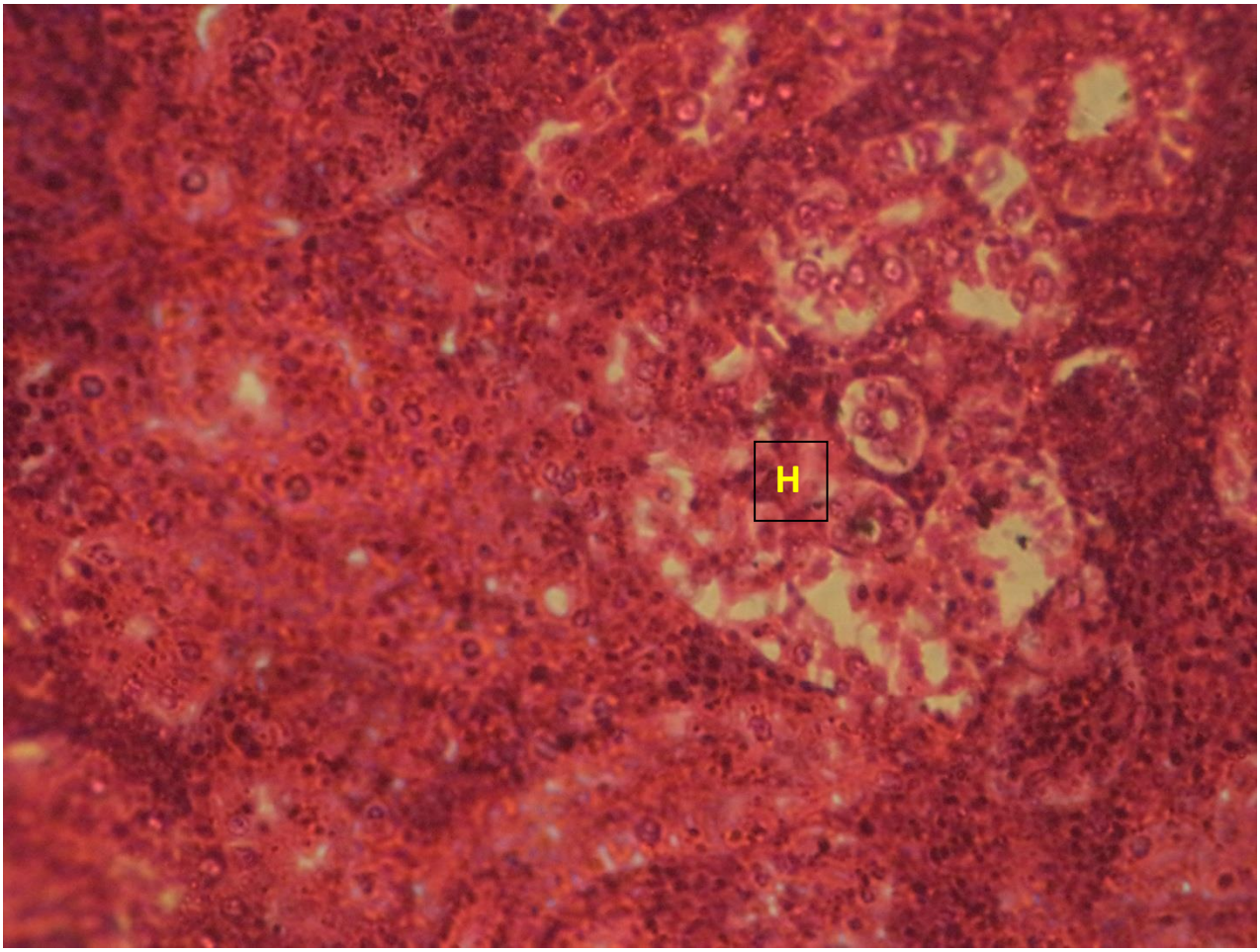


Plate XXVII: Photomicrograph of the transverse section of the kidney in G6 on day28; showing, generalized tubular degeneration and necrosis (H) (H & E, X1680).

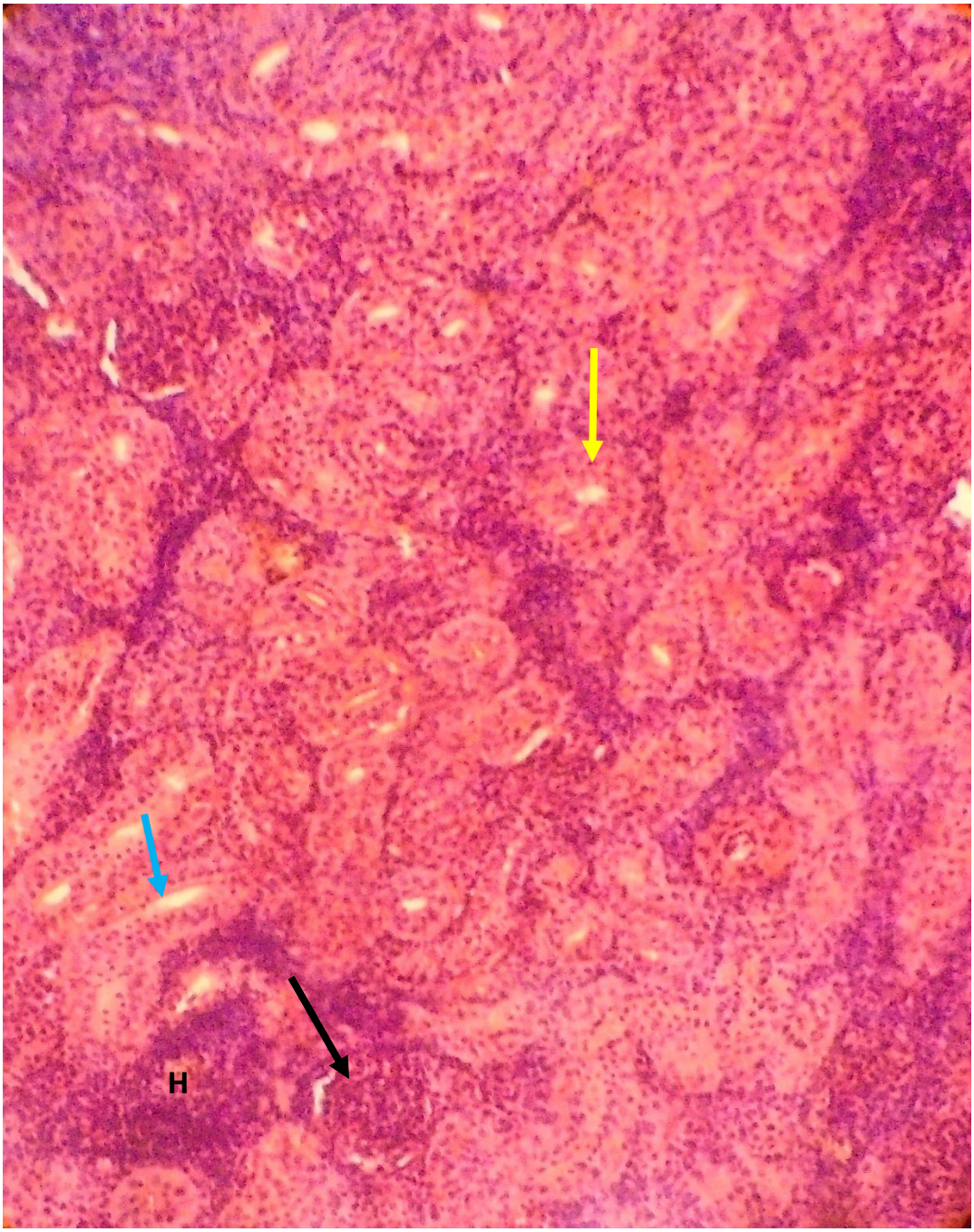


Plate XXVIII: Photomicrograph of the transverse section of the kidney in G10 on day 28; showing, dilated proximal convuluted tubule (blue arrow),distal convuluted tubule (yellow arrow), hematopoietic tissue (H), hypercellularity of the glomerulus (black arrow). (H & E, X1120).

CHAPTER FIVE

5.0 DISCUSSION

The Sub-Lethal study revealed that fish exposed to aqueous extract *Adenium obesum* (G6) alone during the twenty eight days showed a decrease in Red Blood Cell Count (RBC),Haemoglobin Concentration (Hb), Packed Cell Volume (PCV), and serum total protein levels,while there was an increase in aspartate aminotransaminase,alanine aminotransaminase, alanine phosphatase, reduced glutathione, superoxide dismutase, and malondialdehyde levels, compared to those exposed to aqueous extract *Adenium obesum* stem bark with different sub lethal doses of aqueous extract of *Borreria verticillata* (G7, G8, G9, G10),where it was noticed that the values were improved.

The phytochemical findings in this study agreed with the works of Mgbojikwe, (2000) and Abalaka *et al*. (2015) who reported that the ethanol extract and aqueous extract of *Adenium obesum* stem bark, respectively contained carbohydrates, glycosides, tannins, cardiac glycosides, saponins and flavonoids, steroids and triterpenes in addition to resin reported by Mgbojikwe (2000). This study also agreed with the works of Tijjani *et al.* (2011a, 2011b, 2012) who reported the presence of alkaloids, saponins, tannins, glycosides, anthraquinones and steroids in addition to triterpenoid, betulin from the methanol, petroleum spirit and the petroleum ether extracts of *A. obesum* stem bark.The difference observed in the phytochemical constituents, especially as it relates to the presence/absence of resins, botulin and anthraquinones reported in these studies and others may be due to the extraction methods used. This might also be due to the age and parts of the plants used, genetic variability between species, climatic conditions and the

nature of the soil profile upon which the plant was cultivated (Norton, 1975; Sellapan *et al.,* 2002).

The preliminary phytochemical screening of the aqueous extract of *Borreria verticillata* revealed the presence of flavonoids, alkaloids, saponins, sterols, tannins and glycosides, while anthraquinone and cardiac glycoside were absent. This finding is in agreement with a previous reports of Abdullahi *et al*.(2014) and Murtala *et al*.(2015) in which ethanol and aqueous extract of *Borreria verticillata*, respectively were used.These compounds are known to have analgesic and anti-inflammatory activities as well as hepatocurative effect (Perez, 2001; Lucia and Jesu, 2012). A number of flavonoids as well as tannins isolated from plants have been discovered to have significant analgesic and/or anti-inflammatory effects (Duke, 1992; Ahmadi ani *et al*., 1998, 2000). The inhibitory effect of flavonoids on eicosanoids synthesis has been documented (Damas *et al.,* 1985; Raj *et al.,* 2001; Danjuma *et al.,* 2011). Flavonoids inhibit a wide array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2, phosphodiesterases among others have been reported (Middleton, 1998). Alkaloids and saponins possess analgesic and antispasmodic effects, while the healing of wounds and inflamed mucus membranes are hastened by tannins (Okwu, 2005). Flavonoids are potent water soluble antioxidants which prevent oxidative cell damage (Okwu, 2005; Akinmoladun *et al*., 2007a; Akinmoladun *et al*., 2007b). Thus, the presence of these constituents may be contributory to the analgesic and anti-inflammatory effects of the plant (Abdullahi *et al.,* 2014; Silva *et al.,* 2017).

During the acute bioassay of the aqueous extract of *Adenium obesum* stem bark,the temperature (23 – 32°C), pH (7.30-7.44) and dissolved oxygen (4.69 – 4.87 mg/l) of the fish culture were within the normal ranges with tolerable changes for growing of *C*

.*gariepinus* and survival of fish in general (Alabaster and Lloyd, 1980; Swann,1992; Byod and Tucker,1998; Chapman, 2000).The changes in behavior,tissues and haemato-biochemical parameters herein being recorded are therefore not likely to be the responses of the fish to the physicochemical properties of the water.It has been established that changes in dissolved oxygen content of the culture water does not adversely affect the survival of *C. gariepinus* since it has fully developed accessory breathing organs (ABOs) which can utilize atmospheric oxygen (Solomon and Taruwa, 2011; Petri *et al*., 1992; Okechi, 2004). Solomon and Taruwa (2011) had stated that decrease in dissolved oxygen content of fish culture water overtime may be occasioned by increasing organic wastes load in the presence of bacteria decomposition. However, the regular change of water during this experiment herein being reported here could not have allowed significant alterations in the physicochemical properties as observed by earlier investigators (Viveen *et al.,* 1985; Janseen, 1987; 1992; Hogendoom, 1992; Petri *et al.,* 1992; Swann, 1990; Chapman, 2000; Lawson, 2011).Thus, it is most probable that the changes and clinical signs of the fish in this study were due to the toxicity of *A. obesum* extract administered.

The appearance and intensity of clinical signs in the contact phase of the fish toxicity study of juvenile *Clarias gariepinus* exposed to aqueous *Adenium obesum* extract was concentration-dependent as these increased with increasing extract concentrations. The initial agitations and restlessness characterized by erratic movements and repeated attempts of the fish to jump out of the culture water in the contact phase of the fish toxicity study were natural avoidance response to escape from the toxic aquatic environment. This was possibly to prevent the continuous absorption of the toxicant in the water environment. Similar hyperactivity and uncoordinated movements were reported in ticks exposed to the aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000). This is a

normal adaptation response, which allows fish to survive in altered environment (Svecevieus, 2001).

There was progressive respiratory distress, characterized by increased opercula movements, air gulping and exposed snouts above water surfaces. Respiratory impairment may arise from increasing gill cellular damage and/or increasing accumulation of elaborated mucous on gill surfaces (Konar, 1970; Sambasiva Rao, 1999; Fafioye *et al.*, 2004; Abalaka and Auta, 2010).

Inhibition of pyridine-linked substrates oxidation suggestive of interference with oxidative phosphorylation have been reported in ticks exposed to the aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000). The respiratory distress might have also been caused by the reconstituted extracts containing saponin known to induce surface tension (Geidam *et al.*, 2007; Arnstrong, 2008). The observed surfacing phenomenon has been reported (Sambasiva Rao, 1999) as attempt to cope with the increasing oxygen demand. Frequent air gulping are attempts to utilize atmospheric oxygen in an effort to limit continuous gill contact with the toxic aquatic environment.

Motionlessness, sudden quick darts and adoption of different postures with swirling, sluggish movements and loss of balance or equilibrium might be due to nervous disorders caused by the toxicity of the plant extract. Similar uncoordinated movements and paralysis were reported in ticks exposed to the aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000). The appearance of neurotoxicity characterized by change in the form, frequency and/or posture of swimming movements before the occurrence or mortality in some of the exposed fish agreed with the reports of Little and Finger (1990) and Little and Brewer (2001), respectively. These nervous disorders could be due to the inhibition of brain cytochrome C oxidase leading to hypoxia, with resultant changes in the electrical

activity of the brain associated with the maintenance of equilibrium (Sambasiva and Chandrashekar, 1987). The *Adenium obesum* extract contained cyanogenic glycosides, which are known to inactivate cytochrome oxidase with subsequent inhibition of cellular respiration resulting in anoxia in the cardiovascular, respiratory and central nervous system (Brimer, 2010). The nervous signs might have also been due to acetylcholinesterase inhibition leading to acetylcholine accumulation at synaptic junctions with subsequent hyperstimulation (Halappa and David, 2009). Mgbojikwe (2000) reported the inhibition of acetylcholinesterase activity in *A. obesum* exposed ticks.

The clinical responses noticed in the acute bioassay of *Adenium obesum* extract study agreed with the findings of previous investigators on *Clarias gariepinus* juveniles exposed to different plant extracts (Yeeken and Fawole, 2011; Adeboyejo *et al.,* 2013; Orji *et al.,* 2014; Abalaka *et al.,* 2015). Similarly, Ubahe *et al*. (2012) observed irrational behaviour in *Clarias gariepinus* exposed to *Hypoestes forskalei* extract, and these include vigorous movement, fast back stroke movement, restlessness, increased opercular movement and jumping. The erratic behaviour prior to death in the present and past studies may be associated with the impact of toxicants on fish. The excessive mucus secretions observed in the exposed fish in this study has also been reported (Jothivel and Paul, 2008; Abalaka and Auta, 2010; Orji *et al*., 2014). Excessive mucus secretions is part of natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of the offending toxicant (Cagauan *et al.,* 2004). However, such excessive mucus secretions are reported to reduce respiratory activity in fishes (Konar, 1975). Several physiological and biochemical alterations have been reported in fish exposed to various botanical extracts (Devadoss *et al.,* 2013; Audu *et al.,* 2015).

The established low LC_{50} of *Adenium obesum* aqueous extractvalue showed that the extract was very toxic to the exposed fish. There was strong correlation (r = 0.949) between increase in concentrations of AOE and mortality of the exposed fish. This was similar to what was recorded by Mgbojikwe (2000) in ticks exposed to the aqueous extract of the stem bark. This present observation might be due to bio-transformation of the active toxic constituents of the plant's extract over time (Kela *et al.,* 1989; Fafioye, 2005), especially as the extract concentrations were not maintained daily throughout the exposure period. Factors such as parts of the plant, age, genetic variations within plant species and differences in the climatic conditions, including the soil profile of where the respective plants are found at each point in time affects the types and concentrations of their active toxic constituents (Norton, 1975; Sellappan *et al.*, 2002).

On the other hand the aqueous extract of *Borreria verticillata* aerial parts was found to be non toxic to the juvenile *Clarias gariepinus*, which showed no mortality or signs of distress at and beyond 1000mg/l,according to GESAMP (2002) classification for acute exposure in aquatic environment. This agrees with Abdullahi *et al.* (2014), that *Borreria verticillata* was non-toxic to rats and mice when given orally or intraperitoneally (LD_{50} > 5000mg/l). Thus *Borreria verticillata* could be classified as nontoxic when given through this route, according to the toxicity classification proposed by Loomis and Hayes (1996) which states that substances with an LD_{50} values between 500 and 5000 mg/kg and 5000 mg to15,000 mg/kg body weight are regarded as slightly toxic and practically non-toxic, respectively in rats.

The Red Blood Cell Count (RBC), Haemoglobbin Concentration (Hb), Packed Cell Volume (PCV),Total White Blood Cell Count (TWBC), Aspartate Aminotransferase (AST),Alanine Aaminotransferase (ALT), Alanine Phosphatase (ALP),

Malondialdehhyde (MDA), Total protein (TP), Reduced Glutathione (GSH) and Superoxide Dismutase (SOD) values in G2, G3, G4 and G5 showed no significant difference in their values during the course of the Sub-Lethal exposure, which showed that the sublethal doses of aqueous extract of *Borreria verticillata* treatments alone cause no detectable pathology in the blood chemistry of the fish in these groups compared to the control (G1).

The aqueous extract of *Borreria verticillata* aerial parts have been shown to contained flavonoids which are antioxidants and free radical scavengers which prevent cell damage, and protect the cells against carcinogenesis (Salah *et al.*, 1995; Okwu, 2004).This may be responsible for the absence of significant altrations on the blood indices mentioned.Abdullahi *et al.* (2014) reported the antioxidant, anti-inflammatory and analgesic effects,while Murtala *et al*., (2015) observed the hepatoprotective effect of aqueous extract *Borreria verticillata* in rats.

There was a significant decrease in the values of RBC, PCV, Hb,TWBC, in fish exposed to the aqueous extract of *Adenium obesum* stem bark acute bioassay, in contrast to the report of Abalaka *et al*. (2015), in which an insignificant decrease was observed in fish exposed to ethanol extract of *Adenium obesum* stem bark. On the contrary Agwu *et al.,* (2016), observed significant decrease in RBC, PCV and Hb of *Clarias gariepinus* fingerlings exposed to the roots of *Telfairia occidentalis* extract. White tilapia, *oreochromis niloticus,* had also showed a decrease in the levels of RBC, Hb, and PCV on exposure to almond (*Terminalia catappa),* pawpaw (*Carica papaya),* Neem (*Azadirachta indica),* Tobacco (*Nicotiana tobacum)* and Cassava (*Manihot esculenta*) extracts (Fafioye, 2012). Tobacco leaf extract have been shown to induce significant decrease in

all blood indices except MCHC (Musa and Omoregie, 1999; Omoregie *et al*., 1994; Omoniyi *et al.,* 2002).

Many other investigators have demonstrated significant reduction of RBC, Hb, PCV and erythrocytic indices due to extracts with toxic constituents (Nounou *et al.,* 1997; Ezzat *et al*., 1998; Das and Mukherjee, 2000; Devi and Benerjee, 2007; Singh *et al.,* 2010; Obiezue *et al.,* 2011; Olusegun and Adebayo, 2014; Okwogu *et al.,* 2015; Nwani *et al.,* 2016; Jegede *et al.,* 2017), as being reported in this study.The significant decrease in PCV is suggestive of anemia as it is the variable that is normally used to assess the basic status of the erythron (Kerr, 2005). The observed relative decrease in the PCV, Hb and RBC counts of the exposed fish may either be due to loss or destruction of RBCs (Payne, 2005).

Reductions in hemoglobin, packed cell volume and red blood counts were reported in *Clarias batrachus* exposed to toxicants (Masheswaran *et al.,* 2008). This implies that the extract of the aqueous extract of *Adenium obesum* could cause anemia, possibly through a decreased rate of production of red blood cells or an increased loss of the red blood cells. Gill and Epple (1993) have attributed anemia to impaired erythropoiesis due to a direct effect of a toxicant on hematopoietic centers (kidney/spleen), accelerated erythroclasia due to altered membrane permeability and/ or increased mechanical fragility, and defective iron metabolism or impaired intestinal uptake of iron due to mucosal lesions.

However, the *Borreria verticillata* extract had in-significant effect on the total red blood cell count, packed cell volume, heamoglobin concentration and erythrocytic indices of juveniles' *clarias gariepinus* in this study.

In this present investigation, there was significant increase in total and differential white blood cell counts possibly in response to tissue damage caused by aqueous extract

Adenium obesum stem bark. This similar observation has been reported in fish exposed to plant extracts (Olusegun and Adedayo, 2014; Agwu *et al.,* 2016).White blood cells play a major role in the defense mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Wedemeyer and Mcleay, 1981).

There were also significant decrease in the values of MCV, MCH, and MCHC, in the acute exposure of aqueous extract *Adenium obesum* stem bark, in agreement with Fafioye (2012) and Baki *et al* (2015), who reported a significant decrease in all the erythrocytic indices in acute exposure of white tilapia (*Oreochromis niloticus*) to almond (*Terminalia catappa),* pawpaw (*Carica papaya),* Neem (*Azadirachta indica),*tobacco (*Nicotiana tobacum)* and cassava (*Manihot esculenta*) extract.The reduction in levels of these indices may indicate a microcytic hypochromic anemia, the non-responsive nature of the anemia may be due to compromised haematopoietic tissues in the fish such as kidney and spleen.

Such may also arise from osmoregulatory imbalances and inhibition in pathways as in the biosynthesis of Hb or inhibiting the utilization of delta-amino levulinic acid (Haux *et al*., 1985; Nussey *et al.,* 1995). The values of erythrocytic indices among the fish treated with AOE were found to decrease with increasing toxicant concentration.Similar reduction has been observed by Okomoda *et al*. (2010).

The values of erythrocytic indices improved with concurrent treatment using BVE in G7, G8 G9 and G10.This can be attributed to a possible ameliorative effect of *Borreira verticillata* in preventing damage to the cell walls of RBCs through its anti-inflammatory action ,in a dose dependent manner (Abdullahi *et al.,* 2014).

The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased significantly in the *C. gariepinus* juveniles in group 6 exposed to AOE, in this study. This agrees with earlier reports of toxicity in rainbow trouts exposed to paremethrin and adult *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* (Mozhdeganloo *et al.,* 2015).

The significant increase of AST may be indicative of cellular damages in the heart, skeletal muscles, liver, brains, kidney, pancreas, lungs, whites and RBCs where it is primarily found (Pratt and Kaplan, 2000). Similarly, elevated ALT activity could be suggestive of hepatic and renal injuries where it is mostly found (Wroblewski, 1958; Jung *et al.,* 1981; Adolph and Lorenz, 1982; Sherman, 1999). It has been well known that AST, ALT and ALP are non-serum enzymes, thus an increase in their serum activities signifies cellular damages (Wells *et al.,* 1986; Sallie *et al.,* 1991). However, the ALT is more hepato-specific compared to AST activity (Herfindal and Gourley, 2000).

The liver damage might not be unconnected with its function as a detoxification centre, for the metabolism of xenobiotics where morphological alterations do always occur in the process (Rocha and Monteiro, 1999; Yamawaki *et al.,* 1986).Hepatic damage might have also been due to the tannins in the aqeuous extract of *A. obesum* stem bark. Tannins are hepatotoxic agents (Nellis, 1997; Evans, 2005) with ability to precipitate exogenous and endogenous proteins, including digestive enzymes (Temmink *et al.,* 1989; Bagepallis *et al.,* 1992; Abara, 2003; Bele *et al.,* 2010).

Several physiological and biochemical alterations have been reported in fish exposed to various botanical extracts (Ercal *et al.,* 2001; Dabas *et al.,* 2012; Devadoss *et al.,* 2013; Audu *et al.,* 2015). The level of malondialdehyde significantly increased in the exposed fish during the course of the acute toxicity study for the aqueous extract *Adenium obesum*

study compared to the control reflecting oxidative stress and lipoperoxidation. The elevated values of LPO obtained are in agreement with previous reports in fish exposed to different ichthyotoxic botanicals (Piner and Uner, 2013; Thadani and Salunke, (2014). Lipid peroxidation, the metabolic process in which reactive oxygen specie (ROS) result in the oxidative deterioration of lipids,is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues (Gutteridge, 1995). Lipid peroxidation and antioxidants have been employed as biomarkers of tissue damage (Gutteridge, 1995; Marnett, 1999; Davies, 2000; Marnett, 2000; West and Marnett, 2006).

The *Borreria verticillata* extract had insignificant effect on the malondialdehyde level of juveniles *Clarias gariepinus* exposed to the extract alone in this study, thus *Borreria verticillata* extract did not induce oxidative stress in juveniles *Clarias gariepinus* while administered alone in this study.

The decreased protein content as observed in *C. gariepinus* exposed to the aqeuous extract of *Adenium obesum* stem bark alone may be due to impairment of liver synthesis of protein by alkaloid bioactive molecule emetine (Moller *et al.,* 2007). Similar results were obtained in *Euphorbia royleana* and *Jatropha gossypifolia* lattices (Singh and Singh, 2002) and in *Cyprinus carpio* exposed to *Moringa oleifera* seed extract (Kavitha *et al.,* 2012).

The extract might have possibly caused protein loosing enteropathy in the exposed fish. It may also be due to the fact that under stress condition fish directly utilized protein to meet their increasing energy demand for sustainable metabolic activity (Martinez *et al.,* 2004). It is established that AST and ALT do catalyze the transfer of amino groups from aspartate and alanine to ketoglutaric acid so as to generate oxaloacetic acid and pyruvic

acid that are involved in mitochondrial energy production in the presence of oxygen (Limdi and Hyde, 2003; Burger-Mendonca, 2008). The levels of AST and ALT were shown to have increased significantly in the exposed fish. In addition, the level of ALP known to be involved in protein synthesis was found to have decreased (Pilo *et al.*, 1972). The ALP has a strong affinity for inorganic pyrophosphate (PP_i) at a more physiologic pH and low Mg^{2+} concentration (Moss *et al.*, 1967). The hydrolysis of the ALP increases the available concentrations of amino acyl-tRNA for protein synthesis while providing inorganic phosphate for re-synthesis of ATP and bone mineralization via the abolition of the known inhibitory effect of PP_j on bone mineralization (Fleisher *et al.*, 1977). Significant decrease in total protein concentrations, that were significant, was reported in ticks exposed to the aqueous extract of the stem bark of the plant (Mgbojikwe, 2000) and Nwani *et al.* (2016).

The antioxidant levels of GSH significantly decreased in the *C gariepinus* juveniles exposed to AOE acutely in agreement with the observations of Sayeed *et al.* (2003) and Sentürk *et al.* (2009) after exposure to pyrethroids. These antioxidants are likely use up while scavenging for free radicals during the oxidative process in the AOE-exposed fish. It is known that GSH is an important intracellular antioxidant that protects cells against endogenous and exogenous oxidants (Parke and Piotrowski, 1996), functioning as a catalyst for disulfide exchange reactions, and contributes in H_2O_2 detoxification. It plays a major role in antagonizing the oxidative action of the herbicides, insecticides or other toxicants (Parke and Piotrowski, 1996). In a study, Nwani *et al.* (2016) reported an increase in SOD, but with a decrease in reduced glutathione level and explained that depletion of GSH by oxidants may alter the redox status of the cell and present a stressful and toxic situation.

The pan leukocytosis induced in G6, the high white blood cell counts recorded in this study could be due to an attempt by the fishes to fight against toxic constituents, with possible production of antibodies to improve the health status of the fish. Similar findings were reported by Ates *et al*. (2008) had speculated that the increase in white blood cell counts during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defense mechanism of the fish to tolerate the toxicity. The increase in lymphocytes count indicates the stimulatory effects of the toxicant on the immune system. Enhancement in the total white blood cell count following exposure in G6 could be possible due to leucocytosis which is an outcome of proliferation of haemopoietic cells, leading to progressive increase in the white blood cell counts in the peripheral blood (Tariq, 2007; Sharma *et.al*., 2007) .Changes in TWBC and differential count have been reported to play important roles in the assessment of the state of health of fishes, while leucopenia and leukocytosis in *Clarias gariepinus* occur during exposure to pathogens, heavy metals and chemotherapeutics (Van Vuren *et al.*, 1994; Ezeri, 2001; Omoregie and Oyebanji, 2002).

Various fish species exposed to mercury have shown significant increase in total and differential leucocytes counts ((Ribeiro *et al.*, 2006; Maheswaran *et al.*, 2008; Ibrahim, 2011), similar to the present observation. It can be explained that the stimulation of the immune system causes an elevation in lymphocytes by damages to lymphoid tissues (Shah and Altindag, 2005; Ibrahim, 2011). Kumar *et al.* (2004) had found that exposure to mercuric chloride induced changes in the differential white blood cell counts and caused lymphocytosis, heterophillia, monocytosis, eosinophilia and thrombocytopenia in *Anabas testudineus*.

Treatment of fish, with extract of *Borreria verticillata* in Groups 7 – 10, led to decrease in MDA levels. This might possibly have been mediated by antioxidant activity of BE which investigators have reported (Abdullahi *et al.*, 2014) which helps to avoid tissue damage (Avci *et al.*, 2005; Atli *et al.*, 2006). However, the detailed mechanism of this seeming tissue healing or protection by BE, seen as decrease in MDA levels may need further sstudies.

The exposure to AOE for 28 days in G6 led to elevation of GSH compared to the control (G1) unlike in the acute study.The GSH level may increase as an adaptive defence mechanism by the fish in response to the induced stress by aqueous extract *Adenium obesum* stem bark. Elevation of GSH has been reported by Saliu and Bawa-Allah (2012) in Clarias gariepinus exposed to $ZnCl_{2.}$

The surge in GSH levels in *C. gariepinus* exposed to heavy metals (Saliu and Bawa-Allah, 2012) and polycyclic aromatic hydrocarbons (Olagoke, 2008), as environmental pollutants.This elevation was regarded as adaptive and protective response of the biomolecule against oxidative stress.

The groups with the combination of aqueous extract *Adenium Obesum* stem bark and different sub lethal doses of *Borreria verticillata* (G7, G8, G9 and G10) showed decreased levels of GSH in a concentration-dependent manner over the 28 days period compared to G6. This shows that the aqueous extract *Borreria verticillata* aerial part might have prevented damages that may illicit inflammatory reactions capable of increasing the activity of GSH in an adaptive response manner. It seems that the *Borreria verticillata* extract contains constituents with ability to scavenge free radicals in a concentration dependent manner as have been reported by Abdullahi *et al.* (2014).

There was an increase in the levels of SOD in G6 exposed to aqueous extract *Adenium Obesum* stem bark) observed during the 28 days in a time dependent manner compared to the control (G1); but the condition improved in groups 7-10. This learns credence to the observation of Uner *et al.* (2001) who reported significant increase in SOD activities in liver and kidney of *Oreochromis niloticus* and *Cyprinus carpio* exposed to cypermethrin when compared to the controls. The liver, kidney and gills of cyprinus carpio exposed to malathion had similarly shown significant increase in SOD activity Yonar *et al*. (2015). The increase in serum SOD may be an adaptive response by the fish to protect the fish against the free radicals generated by the toxicity induced by AOE.

Histopathological changes have been widely used as biomarkers in evaluating the health of fish exposed to contaminants, both in the laboratory (Thophon *et al.,* 2003) and field studies (Hinton and Filho, 1990). The gill, liver, intestine and kidney tissue of exposed C*larias gariepinus* juveniles showed various pathological changes in this study.

Hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae observed in AOE exposed fish in this study, might have been a defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants in this study (Mallat, 1985; Hinton and Laurén, 1990; Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003). Coutinho and Gokhale (2000) found epithelial lifting in the gills of carps (*Cyprinus carpio*) and tilapias (*Oreochromis mossambicus*) exposed to the effluents of waste water treatment plant. Engelhardt *et al*. (1981) observed such epithelial lifting along with lamellar fusion in rainbow trouts (Oncorhynchus mykiss) exposed to petroleum residues. Similar alterations in the gills have been observed in the fishes exposed to metals (Oliveira Ribeiro *et al.,* 2000; Cerqueira and Fernandes, 2002; Martinez *et al*., 2004) and organic contaminants (Rosety-

Rodríguez *et al.,* 2002; Fanta *et al.,* 2003). According to Mallat (1985) such alterations are non-specific and may be induced by different types of contaminants. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003). Most part of the gill lesions caused by sub lethal exposures affects lamellar epithelium (Hinton and Laurén, 1990).

However, some alterations in blood vessels may also occur, when fishes suffer from more severe types of stress. Damaged cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel and blood congestion (Takashima and Hibiya, 1995; Rosety-Rodríguez *et al*., 2002; Heath, 1987; Martinez *et al.*, 2004; Poleksic and Mitrovic-Tutundzic, 1994).

The observed changes, on gill sections, such as club-shaped and atrophy of primary lamellae, vacuolations and fusion of secondary lamellae as well as congestion of blood vessels in the gill arches of Group 6 appeared to be milder in Groups 7 -10 and absent in Groups 1-5. Similar alterations have been reported in fish exposed to permethrin and other toxicants (Kumaraguru *et al.,* 1982; Mallat, 1985; Poleksic and Mitrovic-Tutundzic, 1994; Van Heerden *et al.,* 2004; Abalaka and Auta, 2010).In an earlier study,hyperemia, epithelial proliferation, epithelial detachment, lamellar edema, mucous cells hypertrophy, partial lamellar fusion, epithelial cellular infiltration, hemorrhage, complete lamellar fusion, epithelial degeneration and epithelial necrosis were recorded in *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* (Abalaka *et al.,* 2015). Aqueous extract *Vernonia amygdalina* (Audu *et al*., 2017). The lamellar fusion was a defensive response to reduce brachial superficial area in contact with the toxicant (Mallat, 1985; Figueirodo-Fernandes *et al.,* 2007). The erosion and adhesion or fusion of the secondary lamellae

herein reported is in accordance with the findings of earlier investigators (Erkmen *et al.*, 2000; Daoust *et al.*, 1984).

These gill pathology might have increased the diffusional distance between gill capillaries and epithelial cells thereby increasing diffusional resistance within it, which is a limiting factor for exchanges across such gills (Tuurula, 1983), during exposure to many extracts (Onusiriuka and Ufodike, 1994; Fafioye *et al.*, 2004).

The presence of pathology in gills from Groups 7 – 10 in this study may be due to the continuos exposure of these groups to the toxicant(AOE) without a recovery period to allow for complete healing. However, the fact that the changes appeared to be milder than the group treated with AOE alone may suggest that the extract of *B. verticillata* contains constituents which have some restorative or protective effects on the gills.

The liver pathology observed in this study was indicative of the effect of *A. obesum* extract. Hepatocytes of *Clarias gariepinus* exposed to *Khaya senegalensis* have shown such changes (Matouke and Aburi, 2015). The liver is the organ most associated with the detoxification and biotransformation process, and due to its function, position and blood supply (Van der Oost *et al.*, 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998). Anomalies such as irregularly shaped hepatocytes with cytoplasmic vacuolation and nuclear changes were described in fish exposed to organophosphate pesticides (Fanta *et al.*, 2003). Vacuoles observed in the cytoplasm of hepatocytes in exposed groups in this study, may be lipids or glycogen, which are related to the normal metabolic function of the liver, especially when the animals are stressed (Hinton and Laurén, 1990; Wilhelm Filho *et al.*, 2000). The glycogen acts as a reserve of glucose to supply the energy demand occurring in such situations (Panepucci *et al.*, 2001). Pacheco and Santos (2002) described increased vacuolation of

the hepatocytes as a signal of exposure to contaminated water. Moderate to marked vacuolation in the liver of *Clarias gariepinus* fed with *Vernomia amagdylina* is said to be suggestive of increased storage of glycogen (Ajala *et al.,* 2015).

The present study showed hyperplasia of goblet cells in Groups 6 and 7 – 10 on day 14. Between days 21 and 28 ,there was also hypertrophy of the the muscular layer of the intestine, in these groups.The sections of intestine from *Oreochromis mossambicus* exposed to biopesticidal plants (*Carica papaya* and *Nerium oleander*) extracts have been shown to have vacuolar degeneration of villi cells, with massive infiltration of inflammatory cells in the sub mucusa, desquamation and thicker tunica muscularis (Tasneem *et al.,* 2014). The intestinal pathology here being reported lends support to earlier observations (Wagh *et al.,* 2009; Suchismita and Abhik Gupta, 2013).

The microscopic changes in fish kidneys from Group 6 were tubular degeneration, swollen glomeruli, congestion of blood vessels. In Groups 7 – 10, there were dilation of both distal and proximal convoluted tubules and glomerular hypercullarity. It is apparent that the kidneys were adversely affected by toxic constituents of AOE, since Groups 1 – 5 showed no pathology.

It has been established that the teleostean kidney is one of the major target organs to be affected by contaminants in water (Thophon *et al.,* 2003) and it also appears to be particularly sensitive to a variety of toxins due to the high volume of renal blood inflow, its ability to concentrate substances and biotransform some parent compounds to their toxic metabolites. Large quantity of diluted urine may be produced and many of the enzymatic reactions occurring in the liver have been known to also occur in the kidneys (Mohssen, 2001). The kidneys receive bulk of the post branchial blood flow and are important in the detoxification and elimination of aquatic contaminants in fish (Durmaz *et*

al., 2006). These explain why damage to kidney has been observed following fish exposure to toxicants (Jiraungkoorskul *et al.,* 2003).

In this study, the kidney pathology observed were consistent with what other researchers had reported earlier (Takashima and Hibiya, 1995).The changes may arise from parasitic infections (Ventura and Paperna,1984),microbial agents invading the tissue (Chang and Plumb,1996), or exposure to sewage being discharged into the water ecosystem (Kakuta and Murachi, 1997. Agricultural and industrial pollutants (Schmidt *et al*., 1999; Mohammed, 2003; Mahjooor and Loh, 2008), have also induced renal lesions similar to the present observations.

Widespread tubular degeneration with mononuclear intestinal infiltration, haemorrhage and subcapsular congestion have been variously observed in fish exposed to toxicants (Capkin *et al.*, 2006; Adeogun *et al.*, 2012; Neelima *et al.*, 2015).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the results obtained in the study, the following conclusions were drawn:

i. The aqueous extract of *Borreria verticillata* aerial part was nontoxic to C*larias gariepinus* juveniles in acute exposure.

ii. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium Obesum* stem bark on the RBC in G6 from (2.00±0.00 $x10^{12}$/L) to (2.68±0.01 $x10^{12}$/L) in G10 on day 28 of study.

iii. The aqueous extract of *Borreria verticillat*a aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the Hb in G6 from (10.65±0.00g/dl) to (11.38±0.00g/dl) in G10 compared on day 28 of study.

iv. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the PCV in G6 from (29.37±0.27%) to (32.20±0.05%) in G10 on day 28 of study.

v. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the TWBC in G6 from (18.80±0.06$x10^{9}$/L) to (18.09±0.01$x10^{9}$/L) in G10 on day 28 of study.

vi. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on

the serum aspartate amino transferase (AST) in G6 from (60.98±0.01IU/L) to (58.64±0.01IU/L) in G10 on day 28 of study.

vii. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the serum alanine amino transferase (ALT) in G6 from (38.04±0.34IU/L) to (33.69±0.34IU/L) in G10 on day 28 of study.

viii. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the serum alanine phosphatase (ALP) in G6 from (73.86±0.34IU/L) to (71.02±0.01IU/L) in G10 on day 28 of study.

ix. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium obesum* stem bark on the MDA in G6 from (0.48±0.01mmol/mg protein) to (0.23±0.00 mmol/mg protein) in G10 day 28 of study.

x. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium ob*esum stem bark on the Serum reduced glutathione in G6 from (10.56±0.01 µg/ml) to (10.20±0.00 µg/ml) in G10 on day 28 of study.

xi. The aqueous extract of *Borreria verticillata* aerial part (BVE) significantly (P<0.05) ameliorate the induced toxic effect of *Adenium ob*esum stem bark on the Serum superoxide dismutase in G6 from (1.98±0.01µmolSOD/min) to (1.78±0.01 µmolSOD/min) in G10 on day 28 of study.

xii. The pathology induced by exposure to AOE in the gills was ameliorated by the administration of BVE in *Clarias gariepinus* juveniles in terms of severity.

xiii. The pathology induced by exposure to AOE in the liver was ameliorated by the administration of BVE in *Clarias gariepinus* juveniles in terms of severity of lesions.

xiv. The histopathology lesions in the intestine of fish exposure to AOE were ameliorated by the administration of BVE in *Clarias gariepinus* juveniles in terms of severity.

xv. The pathology induced by exposure to AOE in the kidney was ameliorated by the administration of BVE in *Clarias gariepinus* juveniles in terms of severity.

6.2 Recommendations

Based on these conclusions, it is recommended that:

i. *Borreria verticillata* can serve as a potential feed additive in fish production to ameliorate the production of oxidative stress due to handling, transportation and intensive aquaculture practices.

ii. *Borreria verticillata* can serve as an immunostimulant for fish that are at risk of infection or are on treatment.

iii. Further studies that will investigate the geno-pathology due to the administration of *Borreria verticillata* in fish and other animal models should be conducted.

REFERENCES

Abalaka, S.E. and Auta, J. (2010). Toxic Effects of Aqueous and Ethanol Extracts of *Parkia biglobosa* Pods on *Clarias gariepinus* Adults. *World Journal of Biological Research,* 3: 9-1.

Abalaka, S.E., Fatihu, M.Y., Ibrahim, N.D.G. and Ambali, S. (2015). Liver histopathological changes in *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. *Journal of Morphological Science*, 32(1): 22-28.

Abara, A.E. (2003). Tannin content of Dioscorea bulbufera. *Journal of Chemical Society*. Niger, 28: 55-56

Abbas, H.H. and Ali, F.K. (2007). Study the Effect of Hexavalent Chromium on Some Biochemical, Citotoxicological and Histopathological Aspects of the *Orechromis spp*. Fish. *Pakistan Journal of Biological Sciences,*10: 3973-3982.

Abd El-Salam, M.M., Mohamed, G.A. and Lotfy, S.A. (1994). The effect of deltamethrin on the levels of certain amino acids and some enzymes in catfish heart and serum. *Journal of Egyptian-German Society of Zoology,* 13: 345-363.

Abdel-Tawwab, M., Shalaby, A.M.E., Ahmed, M.H. and Khattab, Y.A. (2001). Effect of supplement dietary L-ascorbic acid (Vitamin C) on mercury intoxication and growth performance of Nile Tilapia (*Orechromis niloiicul* L.). *Annals of Agricultural Science,* 39: 961-973.

Abdullahi, H.S., Ahmed, A., Umar, Z.A. and Hussaini, I.M. (2014). Preliminary evaluation of ethanol leaf extract of Borreria verticillata Linn (Rubiaceae) for analgesic and anti-inflammatory effects. *Journal of Medicinal Plant Research,* 8 (20): 736-747.

Abu-Darwish, M.S., Al-Fraihat, A.H., Al-Dalain, S.S., Afifi, F.U. and AlTabbal, J.A. (2011). Determination of essential oils and heavy metals accumulation in Salvia officinalis cultivated in three intra-row spacing in Ash-Shoubak, Jordan. *International Journal of Agriculture and Biology,* 13: 981-985.

Achionye-Nzeh, C.C. and Jimoh, K.O. (2010). Ultrasound evaluation of the gonads in catfish Clarias gariepinus (Teugels): an initial experience in Africa.*World Journal of* F*ish and Marine Sciences*, 2(4): 343-347.

Achuba, F.I. and Osakwe, S,A. (2003). Petroleum induced free radical toxicity in African catfish (clapasgariepinus). *Fish Physiology and Biochemistry,* 29: 97-103.

Adams, A.E., Botstein, D. and Drubin, D.G. (1989). A yeast actin-binding protein is encoded by SAC6, a gene found by suppression of an actin mutation. *Science*, 243(4888):231-3

Adams, P.J., Seinfeld, J.H., Koch, D. , Mickley, L.J. and Jacob, D.J. (2001).General circulation model assessment of direct radiative forcing by the sulfate-nitrate-ammonium-water inorganic aerosol system. *J. Geophys. Res.*, 106: 1097-1112.

Adamu, H.M., Abayeh, O.J., Agbo, M.O., Abdullahi, A.L., Uba, A., Dukku, H.U. and Wufem, B.M. (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. *Journal of Ethnopharmacology,* 99: 1-4.

Adeboyejo, O.A., Fagbenro, O.A., Adeparusi, E.O. and Clarke, E.O. (2013). Acute toxicity of industrial effluents from Agbara Environs of Ologe Lagoon on early life stages of African-Sharp-Tooth catfish *Clarias gariepinus. American Journal of Research Communication,* 1(3): 50-59.

Adedeji, A., Weller, T.M. and Gray, J.W. (2007). MRSA in children presenting to hospitals in Birmingham, UK. *Journal of Hospital Infection*, 65: 29-34.

Adeogun, A.O., Alaka, O.O., Taiwo, V.O. and Fagade, S.O. (2012). Some Pathological Effects of Sub-Lethal Concentrations of the Methanolic Extracts of *Raphia Hookeri* on *Clarias Gariepinus. African journal of biochemical research*, 105-115.

Adeyemo, O.K. (2008). Histological alterations observed in the gills and ovaries of *Clarias gariepinus* exposed to environmentally relevant lead concentrations. *Journal of Environmental Health*, 70: 48-51.

Adeyemo,O.K., Agbede, S.A., Olaniyan A and Shoaga, O.A (2003). The heametological Response of *Clarias gariepinus* to changes in acclimation temperature. *African Journal of Biomedical Research,* 6: 105-108.

Adhikari, S. and Sarkar, B. (2004). Effects of *cypermethrin* and carbofuran on certain parameters and prediction of their recovery in a fresh water Teleost *labeo rohitta. Ecotoxicology and Environmental Safety,* 58: 220-226.

Adolph, L. and Lorenzi, R. (1982). Diagnosis in Diseases of the Heart, Liver and Pancreas. S Krager, New York, USA, Pp.9-27.

Agrahari, S., Pandey, K.C. and Gopal, K. (2007). Biochemical alteration induced by monocrophos in the blood plasma of fish, Channa punctatus (Bloch). Pest. *Biochem. Physiol,* 88: 268-272.

Agwu, E.J., Odo, G.E., Ekeh, F., Attamah, G.N., Uwagbae, M. and Eze, C. (2018). A survey of the insect pests and farmers' practices in the cropping of yellow pepper Capsicum annuum Linnaeus in Enugu State of Eastern Nigeria. *African Journal of Agricultural Research,* 13(15): 742-752.

Ahmad, V.U. and Basha, A. (2007). Spectroscopic Data of Steroid Glycosides: Cardenolides and pregnanes. *Springer Science + Business Media,* 4:2612.

Ahmadiani, A., Fereidoni, M., Semnanian, S., Kamalinejad, M. and Saremi, S. (1998). Anti-nociceptive and Anti-inflammatory Effects of *Sambucus ebulus* Rhizome Extract in rats. *Journal of Ethnopharmacology,* 61: 229-235.

Ahmadiani, A., Hosseiny, J., Semnanian, S., Javan, M., Saeedi, F., Kamalinejad, M. and Saremi, S. (2000). Anti-nociceptive and anti-inflammatory Effects of *Eleagnus angustifolia* Fruit Extract. *Journal of Ethnopharmacology,*72: 287-292.

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Akinrinola, B.L., Onibon, T.R., Akinboboye, O.A., Obuotor, E.M. and Farombi, E. (2007b).Chemical Constituents and Antioxidant Activity of *Alstonia boonei*. *African Journal of Biotechnology*, 6(10): 1197-1201.

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007a). Phytochemical Constituents and Antioxidant Activity of Extract from the Leaves of *Ocimum gratissimum, Scientific Research and Essay*, 2 (5): 163-166.

Alabaster, J. S.; Lloyd, R. 1980: Finely divided solids. In: Alabaster, J. S.; Lloyd, R. ed. Water quality criteria for freshwater fish. London, Butterworth. Pp. 1-20.

Alderman, D.J and Hastings, T.S. (1998). Antibiotic use in aquaculture : development of antibiotic resistance - potential for consumer health risks. *International Journal of Food Science and Technology*, 139-155.

Allsopp, M., Johnson, P. and Santillo, D. (2008). *Challenging the aquaculture industry on sustainability: Technical overview.* 49-189.

Almroth, B.C., Sturve J., Berglund, A. and Forlin, L. (2005). Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. *Aquatic Toxicology*, 73: 171-180.

Alwan, S.F., Hadi, A.A. and Shokr, A.E. (2009). Alterations in haematological parameter of fresh water fish. *Tilapia Zilli* exposed to Aluminium. *Journal of Science and its Applications,* 3(1): 12-19.

American Public Health Association (1985). *Standard method for the examination of water and waste water* (6th ed.). Washington DC, USA: Pp.413-426.

Amminikutty, C.K. and Rege, M.S. (1977). Effects of acute and chronic exposure to pesticides, thiodan 35 EC and agallol 3 on the liver of widowwrtetra, posure to pesticides, thiodan 35 EC and agallol 3 on the liver of widowwrtetra, Gymnocorymbus ternetzi Boulenger. *Indian Journal Exploratory Biology,* 15: 197-200.

Anand, R.J.K., Arabi, M., Rana, K.S. and Kanwar, U. (2000). Role of vitamins C and E with GSH in checking the oxidative damage to human ejaculated spermatozoa. *International Journal of Urology*, 7: 1-98.

Anderson, D. (1992).Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture. *Annual Revision of Fish Diseases,* 2: 281-307.

Andreozzi,R.,Caprio,V.,Insola,A. and Sanchirico,R.(2000).Advanced oxidation processes for the treatment of mineral oil-contaminated wastewaters.*Water Research*, 34(2): 620-628.

Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones, Margraf Publishers, CIRAD, MNHN, Paris, Pp. 161.

Arima, R. and Shiba, R. (1992): Radioprotective effect of exogenous glutathione on rat parotid glands. *International Journal of Radiation Biology,* 61(5): 595-702.

Armstrong M (2008) Strategic Human Resource Management, A Guide to Action, London: Kogan, Pp.15

Ashley, P.J. (2007).Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science*, 104: 199-235.

Astalos, B., Nemesok, I., Benedesky, R., Gabriel, A., Szabo, A and O.J, Rezaike (1990).The effects of pesticides on some biochemical parameters of Carp (*Cyprinus carpio*) *Archives of Environmental Contamination and Toxicology*,19:275-282.

Asia-Pacific Fishery Commission (2000). Report of the Ad Hoc Working Group of Experts in Rural Aquaculture. Bangkok, Thailand,FAO Fish. Report No. 610, Pp.22.

Assem, H, Abo-Hegab, S. and Belal, I. (1992).Comparison of haematological effects of some toxicants on Clarias gariepinus, *Journal of Egyptian German Society of Zoology*, 9: 33-50.

Atamanalp, M. and Yanik, T. (2003). Alterations in haematological parameters of rainbow trout *(Oncorhynchus mykiss)* exposed to mancozeb. Turkey journal *Veterinary Animal Science,* 27: 1213-1217.

Ates, B., Orun, I., Talas, Z.S., Durmaz, G. and Yilmaz, I. (2008). Effects of sodium selenite on some biochemical and haematological parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaun, 1792) exposed to lead and copper. *Fish Physiology and Biochemistry*, 34: 53-59.

Audu, B.S., Ajima, M.N.O. and Ofojekwu, P.C. (2015). Enzymatic and biochemical changes in common carp, Cyprinus carpio (L) fingerlings exposed to crude leaf extract of *Cannabis sativa* (L.). *Asian Pacific Journal Tropical Disease,* 5:107-115.

Audu, B.S., Omirinde, J.O., Gosomji, I.J and Wazhi, P.E. (2017). Histopathological changes in the gill and liver of clarias gariepinus exposed to acute concentrations of vernonia amygdalina. *Animal Research International,* 14(1): 2576-2587.

Ayasutake, W.T. and Wales, J.H. (1983). Microscopic anatomy of salmonids: an atlas. Washinton DC: Fish and Wildlife Service Resource Publication. Pp. 136.

Ayensu, E.S. (1978). Medicinal plants of the West Indies. Unpublished Manuscript. Pp110.

Ayinla, O.A. (2007). Analysis of feeds and fertilizers for sustainable Aquaculture Development in Nigeria. In: Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development, Hasan, M.R., T. Hecht, S.S. De Silva and A.J.G. Tacon (Eds.). Pp. 453-470.

Ayotunde, O.E., Offem, B.O. and Ada, F.B. (2010). Evaluation of the acute and chronic toxicity of the seeds of (carica papaya Linn) it's haematological and piscicidal effects on a freshwater catfish (Clarias gariepinus) adults. *Journal of Fisheries and Aquatic science,* 2(3): 2265-2274.

Bader, A., Cioni, P. L., Flamini, G. (2010). GC-MS analysis of the essential oils of ripe fruits, roots and flowering aerial parts of Elaeoselinum asclepium subsp.meoides growing in Sicily. *Natural product communications,* 5(7): 1111-1114.

Badwen-Davis, J. (2010). Adenium species.Retrieved December 20,2016,from http//www.ehow.com/about 6721472 adenium-species.html.

Baeverfjord, G. and Krogdahl, J. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: A comparison with the intestines of fasted fish. *Journal of Fish Diseases*, 19: 375-387.

Bagepallis, S., Narasinga, R. and Tatinemi, P. (1993). Tannin contents of foods commonly consumed in India and its influence on ionisable iron.*Journal of Scientific Food and Agriculture*, 33: 89-96.

Baki, B., Sudip, P., Sabira, S., Jahid, H. and Abdus, S. (2015). Effects of perfluorooctane sulfonate on the hematology and histopathology of juvenile tilapia, *Oreochromis niloticus.Journal Biology Science,* 4(2): 37-45.

Bakkali, F., Ayerbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils, A review.*Food and Chemical Toxicology,* 46(2): 446-475.

Bakthavathsalam, R. (1991).Hematology of the fish Anabas testudineus exposed to lindane and carbofuran at submerged condition and on exposure on air. *Environmental Ecology,* 9(1): 124-127.

Balde, A.M., Pieters, L.A., Gergely, A., Wray, V., Claeys, M. and Vlietinck, A.J. (1991). *Phytochemistry,*Pp.30: 997.

Balon, E.K. (1995). Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture,* 129: 3-48.

Balouet, G. and Poder, M. (1981).Effets biologiques de la pollution par les hydrocarbures de 1'Amoco-Cadiz sur l'ostreiculture en Bretagne Nord. In: Arnoco-Cadiz. Consequences d'une pollution accidentelle par les hydrocarbures. CNEXO, Paris, Pp. 703-713.

Bancroft, J.D. and Cook, H.C. (1994). *Manual of Histological Techniques and Their Diagnostic Application.* London: Churchill Livinstone. Pp. 289-305.

Becerril, H., Alonso, D., Álvarez-Góngora, R., Cynthia, B., Sofia, C.C., Gerardo, S., Jorge, M.C., Maria, J.R., Norma, M.V., Fanny, M.B., Alejandro, O., Luis, O.C., Elizabeth, R.C., Casimiro, R.S.R. (2007). Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican Coasts. Part A, Toxic/hazardous substances and environmental engineering. *Journal of environmental science and health,* 42: 1349-63.

Bele A.A., Jandhav V.M. and Kadam V.J. (2010). Potential of Tannnins: A review.*Asian Journal of Plant Science,* 9: 209-214.

Benech, P.D., Perez, C. and Wietzerbin, J. (1993). Two cis-DNA Elements Involved in Myeloid-Cell-Specific Expression and Gamma Interferon (IFN-y) Activation of the Human High-Affinity Fcy Receptor Gene: a Novel IFN Regulatory Mechanism.*Molecular and Cellular Biology*, 2182-2192.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P. and Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases,* 22: 25-34.

Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione, *Journal of Laboratory Clinical Medicine,* 61: 882-890.

Beveridge, M. (2013). Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology*, 83(4): 1067-1084.

Blaxhall, P.C. (1972).The haematological assessment of the health of freshwater fish. Review of selected literature.*Journal Fish Biology,* 4: 593-604.

Blumenthal, M., Goldberg, A., Brinckmann, J., Foster, S. and Tyler, V.E. (2000). Herbal medmedicine. *Integrative Medicine Communications, Newton (Mass).*

Bocek,B.R.(1984).Ethnobotany of Costanoan Indians,California,based on collection by John. P. Harrington. *Economy Botany,* 38(2): 240-255.

Boham, B.A. and Kocipai-Abyazan, R. (1994). Flavonoids and condensed tannins from leaves of Haiwaiin, *Vaccinium vaticulalum* and *Vaccinium calycinium. Pacific Science,* 48: 458-463.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R.,Tan, Z. and Shariff, M. (2005). Disease and health management in Asian aquaculture.*Veterinary Parasitology,*132: 249-272.

Botha, C.J. and Penrith, M.L. (2008). Poisonous plants of veterinary and human importance in sourthern Africa. *Journal of Ethnopharmacology,* 119: 549-558.

Bouhdid, S., Abrini J., Zhiri, A., Espuny, M.J. and Manresa, A. (2009). Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. *Journal of Applied Microbiology,* 106: 1558-1568.

Brackman, G., Defoirdt, T. and Miyamoto, C. (2008). Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in Vibrio spp. by decreasing the DNA-binding activity of the quorum sensing response regulator Lux R. *BMC Microbiology,* 8: 149.

Braucbeck, T. and Appelbaum, S. (1999).Ultrastructural alterations in the liver and intestine of carp Cyprinus carpio induced orally by ultra-low doses of endosulfan.*Diseases of aquatic organisms,* 36: 183-200.

Braunbeck, T., Burkhardt-Holm, P., Gorge, G., Nagel, R., Negele, R.D., Storch, V. (1992). Rainbow trout and zebra fish, two models for continuous toxicity tests: relative sensitivity, species and organ specificity in cytopathologic reaction of liver and intestines to atrazine. *Schriftenrver-wasser-Boden-Lufthyg,* 89: 109-145.

Braunbeck, T., Starch, V. and Nagel, R. (1989). Sex-specific reaction of liver ultrastructure in zebrafish (Brochydunio rerio) after prolonged sublethal exposure to 4-nitrophenol. *Aquatic Toxicology,* 14: 185-202.

Braunbeck, T. and Volkl, A. (1993). Toxicant-induced cytological alterations in fish liver as blomarkers of environmental pollution?,a case study on hepatocellular effects of dinitro-o-cresol in golden ide (*Leuciscus idus melanotus*). In: Braunbeck T. Hanke W, Segner H (eds) *Fish ecotoxicology and ecophysiology*. VCH, Weinheim, Pp. 55.

Brimer, L. (2010). Cyanogenic glycosides in food, feeling stuffs and green medicine. In: Bernhoft, A. (Ed). Bioactive Compounds in Plants-Benefits and Risk for Man and Animals. *The Norwegian Academy of Science and Letters, Norway,* Pp. 125-143.

Bruton, M.N. (1976). On the size reached by *Clarias gariepinus*. *Journal of Limnology Soceity of South Africa,* 2: 57-58.

Bruton, M.N. (1979b). The breeding biology and early development of Clarias gariepinus (PIsces: Claridae) in lake sabiya, south africa with review of breeding in species of sub genus Clarias. *Transaction of the Zoological soceity of London*, 35: 1-45.

Bruton, M.N. (1979c). The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator ofcichlids. *Transactions of the Zoological Society of London*, 35: 47-114.

Bucke, D., Vethaak, D., Lang, T. and Mellergaar, S. (1996). Common diseases and parasites o ffis h in the North Atlantic: training guide for identification. ICES TIMES 19: 1-27.

Burger, W. and Taylor, C.M. (1993). Family rubiaceae. In: Burger, W., Ed., field museum of natural history, Chicago. Pp. 33: 1-333.

Burger-Mendonca, M., Bielavsky, M. and Barbosa, F.C.R. (2008). Liver overload in Brazilian triathlets after-ironman completion is related to muscle fatigue. *Annals of Hepatology*, 7(3): 245-248.

Burkill, H. (2000).The Useful Plants of West Tropical Africa. *Royal Botanic Gardens*, 4(2): 686.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International journal of food microbiology*, 94 (3): 223-53.

Byod, C.E. and Tucker, C.S. (1998). Pond Aquaculture Water Quality Management. Kluwer Academic Publishers, Massachusetts, USA, Pp. 92.

Cabello, F.C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, 8(7): 1137-44.

Cagauan, A.G., Galaites, M.C. and Fajardo, L.J. (2004). Evaluation of botanical piscicides on Nile Tilapia *(Oreochromis niloticus* L.*)* and mosquito fish (*Gambusia affinis* Baird and Girard). Proceedings of 6th International Symposium on Tilapia in Aquaculture; 12–16 Sept 2014; Manila, Philippines, Pp. 179-187.

Campbell, T.W (2012). Haematology of fish. In: Thrall, M.A; Weiser, G; Allison, R (eds). Veterinary haematology and clinical chemistry. Ames (IA): John Wiley and Sons.Pp. 293-312.

Campbell, T.W. and Ellis, C.K. (2007). Haematology of birds. In: Campbell, T. W. and Ellis, C.K. (Eds). *Avian and Exotic Animal Haematology and Cytology* (Third edition.). Blackwell publishing professional, Pp. 3-50.

Capkin, E., Altinok, I. and Karahan, S. (2006). Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. *Chemosphere*, 64: 1793-1800.

Cengiz, E.I. and Unlu, E. (2002), Histopathologica changes in the gills of mosquitofish, *Gambusia affinis* exposed to endosulfan. *B. Environ. Contam. Tox.*, 68: 290-296.

Cerqueira, C.C.C. and Fernandes, M.N. (2002). Gill tissue recoveryafter cooper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicology and Environmental Safety*, 52: 83-91.

Chakraborty, S.B. and Hancz, C. (2011). Application of phytochemicals as immunostimulant antipathogenic and antistress agents in finfish culture. *Aquaculture*, 3: 103-119.

Chandra, S.R.J., Neelima, P. and Govinda, R.K. (2017).A review on the toxicity and other effects of Dichlorvos, an organophosphate pesticide to the freshwater fish. *Bioscience Discovery*, 8(3): 402-415.

Chang, P.H. and Plumb, J.A. (1996). Histopathology of experimental *Streptococcus* sp. infection in tilapia, *Oreochromis niloticus* (L.) and channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases*, 19(3): 235-241.

Chiquieri, A., Di Maio, F.R. and Peixoto, A.L. (2004). A distribuição geográfica da família Rubiaceae Juss. na Flora Brasiliensis de Martius. *Rodriguésia*, 55: 47-57.

Chopra, R.N. (1956). Glossary of Indian Med Plants. *CSIR*, Pp. 39.

Chung, G., O'Neil, N.J. and Rose, A.M. (2011). CHL-1 provides an essential function affecting cell proliferation and chromosome stability in Caenorhabditis elegans. *DNA Repair (Amst)*, 10(11): 1174-82

Cinar, K. and Senol, N. (2006). Histological and histochemical characterization of the mucosa of the digestive tract in flower fish (*Pseudophoxınus antalyae*). *Anatomy Histology and Embryology*, 35: 141-151.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18: 403-414.

Clay, D, (1979). Population biology, growth and feeding of African cafish (*Clarias gariepinus*) with special reference to juveniles and their importance in fish culture. *Arch. Hydrobiol.*, 87(4): 453-482.

Conserva, L.M. and Ferreira, J.C. (2012). Borreria and Spermacoce species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. *Pharmacognosy Revision*, 6(11): 46-55.

Coppo, J.A., Mussart, N.B. and Fioranelli, S.A. (2002). Physiological variations of enzymatic activities in blood of Bullfrog, *Rana catesbeina* (Shaw, 1802).*Revision Veterinariam,* 12(13): 22-27.

Cormier, S.M., Neiheisel, T.W., Wersing, P., Racine, R.N. and Reimschuessel, R. (1995). New nephron development in fish from polluted waters: a possible biomarker. *Ecotoxicology and Environmental Safety,* 4: 157-168.

Coutinho, C. and Gokhale. K.S. (2000).Selected oxidative enzymes and histopathological changes the gills of *Cyprinus carpio* and *Oreochromis mossambicus* cultured in secondary sewage effluent. *Water Research*, 34: 2997-3004.

Cox, T., Griffiths, A. and Rial-Gonzalez, E. (2000). Research on Work-Related Stress, Office for Official Publications of the European Communities: Luxembourg.

Cunjak, R.A. (1988a). Behaviour and microhabitat of young Atlantic salmon (Salmo salar) during winter. *Canadian Journal of Fish and Aquatic Science*, 45: 2156-2160.

Dabas, A., Nagpure, N.S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S. (2012). Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel (*Channa punctatus*). *Fish Physiology and Biochemistry*, 38: 469-482.

Dalziel, J.M. (1956).Useful Plants of West Tropical Africa, Crown Agents for Overseas Governments, London. Pp. 179-183.

Damas, J., Remacle-Volon, G., Deflandre, E. (1986). Further studies of the mechanism of counter irritation by turpentine. Naunyn Schmiedebergs Arch. *Pharmacology,* 332: 196-200.

Dang, Z. (2000). Adaptive stress responses in fish gills. PhD. Njimegen: Katholeke Universiteit Nijmegen.

Danjuma, B.Y., Abdulsalam, S. and sulaiman, A. D. I. (2012).Kinetic investigation of Escravos crude oil contaminated soil using natural stimulants of plant sources. *International Journal Emerging Trends in Engineering and Development,* 2(5): 478-486.

Daoust P.Y., Wobeser G., Newstead J.D. (1984): Acute pathological effects of inorganic mercury and copper in gills of rainbow trout.*Veterinary Pathology*, 21: 93-101.

Das, B.K. and Mukherjee, S.C. (2000).Sub lethal effect of quinalphos on selected blood parameters of *Labeo rohita* (Ham.) fingerlings. *Asian Fisheries Science*, 13: 225-233.

Das, B.K. and Mukherjee, S.C. (2003). Toxicity of *cypermethrin* in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. Comparative *Biochemistry and Physiology,* 134: 109-121.

Datta, D. and Sinha, G.M. (1989). Responses induced by long term toxic effects of heavy metals on fish tissues concerned with digestion absorption and fish tissues concerned *with digestion absorption and excretion. Gegerbaurs Morphol. Jahrb,* 135: 627-657.

Datta-Munshi, J.S. and Dutta, H.M. (1996). Fish morphology: *Horizon of New Research*.

Davis, K.B., Griffin, B.R. and Gray, W.L. (2002). Effect of handling stress on susceptibility of channel catfish Ictalurus punctatus to *Ichthyophthirius multifiliis* and channel catfish virus infection. *Aquaculture*, 214: 55-66.

Davies, K.J. (2000). Oxidative stress antioxidant defenses and damage removal, repair and replacement systems. *IUBMB Life*, 50: 279-289.

De Graaf, G. and Janssen, H. (1996). Artificial reproduction and pond rearing of the African catfish Clarias gariepinus in sub-Saharan Africa. Fisheries Technical Paper 362. FAO, Rome, Italy.

De Graaf, G.J., Galemoni, F. and Banzoussi, B. (1995). The artificial reproduction and fingerlinging production of the African Catfish, Clarias gariepinus (Burchell, 1822) in protected and unprotected ponds. *Aquaculture Research,* 26: 233-242.

De la Fuente, J., Guillen, I.,Martinez, R. and Estrada, M. (1999).Growth regulation and enhancement in tilapia: basic research findings and their applications. *Genetic Analysis: Biomolecular Engineering,* 15: 85-90.

De La Torre, F.R., Ferrari, L. and Salibia´N.A. (2005). Biomarkers of a native fish species (*Cnesterodon decemmaculatus*) application to the water toxicity assessment of a peri-urban polluted river of Argentina, *Chemosphere*, 59: 577-583.

Defoirdt, T. (2007). Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. *Trends in biotechnology,* 25(10): 472-9.

Defoirdt, T., Sorgeloos, P. and Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current opinion in microbiology,* 14(3): 251-8.

Delashoud, M., Pousty, I.B. and Khojasteh, S.M.(2010). Histology of bighead carp (*Hypophthalmichthys nobilis*) intestine. *Global Veterinary,* 5: 302-306.

Dessein, S., Robbrecht, E. and Smets, E. (2006). A new heterophyllous Spermacoce species (*Rubiaceae*) from the Marungu highlands (D. R. Congo) *Novon,* 16: 231-4.

Denev, S.A. (2008). Ecological alternatives of antibiotic growth promoters in the animal husbandry and Aquaculture. DSc. Thesis, Department of Biochemistry Microbiology, Trakia University, Stara Zagora, Bulgaria,Pp. 294.

Devadoss, S., Murugaiyan, I., Rajan, M. and Thangaraj, P. (2013). Evaluation of phytochemical, antioxidant and antimicrobial properties of ethnomedicinal plant *Psychotria nilgiriensis* Den and Gang. *International Journal* o*f Pharmacy and pharmacological Sciences*, 5: 417-422.

Devi, R. and Banerjee, T.K. (2007). Haematological disturbances due to ammonia toxicity in the air-breathing fish *Channa stiata*. *Biochemistry and Cell Arch*ives, **7**: 217-223.

Diaz, A.O., Garcia, A.M., Figueroa, D.E. and Goldemberg, A.L. (2008). The mucosa of the digestive tract in *Micropogonias furmieri*. A light and electron microscope approach. *Anatomy Histology Embryology*, 37: 251-256.

Dienye, H.E. and Olumuji, O.K. (2014). Growth performance and haematological responses of African mud catfish *Clarias gariepinus* fed dietary levels of *Moringa oleifera* leaf meal. *Net Journal of Agricultural Science*, 2(2): 79-88.

Dixon, J, Gulliver, A. and Gibbon, D. (2001). Farming Systems and Poverty: Improving farmers' livelihoods in a chaging world.Food and Agriculture Organization & World Bank, Rome & Washington. www.fao.org/farmingsystems/

Dorin, D., Sire, M.F. and Vernier, J. (1993). Endocytosis and intracellular degradation of heterologous protein by eosinophilic granulocytes isolated from rainbow trout (*Oncorhynchus mykiss*) posterior intestine.*Biology of Cell*, 79: 219-224.

Draper, G.J., Stiller, C.A., Cartwright, R.A., Craft, A.W. and Vincent, T.J. (1993). Cancer in Cumbria and in the vicinity of the Sellafield nuclear installation. *British Medical Journal*, 306(6870): 89-94.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews,* 82: 47-95.

Dubale, M.S. and Shah, P. (1979). Toxic effect of cadmium nitrate on the liver of*Channa punctatus*, *Experientia*, 35(5): 643-644.

Dugenci, S.K., Arda, N. and Candan, A. (2003). Some medicinal plants as immunostimulant for fish. *Journal of Ethnopharmacology, 88*: 99-106.

Durmaz, H., Sevgiler, Y. and Uner, N. (2006). Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus*. *Pesticide Biochemistry and Physiology,* 84: 215-226.

Duthie, G.G. and Tort, L. (1985). Effect of dorsal aortic cannulation on respiration and haematology of the Mediterranean dogfish, *Scyliorhinus canicula. Comparative Biochemistry and Physiology*, 81: 879-883.

Edsall, L.S. and Spiegel, S. (1999). Enzymatic Measurement of Sphingosine 1-Phosphate, Analytical biochemistry, 272(1): 80-86.

El-Kashief, M.A., Shalloof, K.A.S. and Authman, M.M.N. (2013). Studies on some reproductive characters of tilapia species in Damietta branch of the river Nile. Egypt *Journal of Fisheries and Aquatic Sciences*, Pp.1-17.

El-Sayed, A.F.M., El-Gobashy, A., Al-Amoudi, M. (1996). Effects of pond depth and water temperature on the growth, mortality and body composition of Nile tilapia, Oreochromis niloticus (L.). *Aquaculture Research*, 27: 681-687.

Engelhardt, F.R., Wong, M.P. and Duey, M.E. (1981). Hydromineral balance and gill morphology in rainbow trout Salmo gairdneri, acclimated to fresh and sea water as affected by petroleum exposure.*Aquatic Toxicology*, 1: 175-186.

Ennajar, M., Afloulous, S., Romdhane, M. and Bouajila, J. (2011). Influence of the Process, Season, and Origin on Volatile Composition and Antioxidant Activity of Juniperus phoenicea L. Leaves Essential Oils. *Journal of Foood Science*, 76(2): C224-30.

Ercal, N., Gurer-Orhan, H. and Aykin-Burns, N. (2001). Toxic metals and oxidative stress Part I: Mechanisms involved in metal-induced oxidative damage.*Current Topics in Medicinal Chemistry*, 1: 529-539.

Erkmen B., Caliskan M. and Yerli S.V. (2000). Histopathological effects of cyphenothrin on the gills of the *Lepistes reticulates*. *Veterinary and Human Toxicology*, 42: 71-78.

Evans, W. (2009). *16th edition. Trease and Evans Pharmacognosy.* Saunders Elsevier.

Evans, W.C. (2005). Trease and Evans Pharmacognosy, Fifteen edition, Sanders: An imprint of Elsevier, India, Pp. 306-309.

Ezeasor, D.N. and Stokoe, W.M. (1981).The light and electron microscopic studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, *Salmo gairdneri* Rich. *Journal of Fish Biology*, 18: 527-544.

Ezeri, G.N.O. (2001). Haematological response of *Clarius gariepinus* to bacterial infection and prophylactic treatment with antibiotics. *Journal Aquatic Science*, 16: 22-24.

Ezzat, A.A., Abdel, A.S.H., El Nady, F.E. and Abdel-Barr, M. (1998).Impacts of sub lethal concentrations of lead on hematology of Siganus rivulatus. Proceeding of the 8th international. Conference on environment.

Fafioye, O. (2001). Lehthal and sub-lethal effect of extracts of Parkia biglobosa and Raphia vinifera on some fresh water fauna. *Unpublished PhD dissertation*, Pp. 216.

Fafioye, O. (2005). Plants with piscicidal activities in south Western Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences,* 5: 91-97.

Fafioye, O.O. (2012). Acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus* (Trewavas). *Irjas*, 2(13): 525-530.

Fafioye, O.O., Adebisi, A.A. and Fagade, S.O. (2004).Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *African Journal of Biotechnology,* 3: 627-30.

Fagbenro, O.A. (1992). Utilization of cocoa pod husk in low-cost diets by the clariid catfish, C. isheriensis Syndeham. *Aquacture Fish Management,* 23: 175-182.

Fanta, E., Rios, F.S., Romao, S., Vianna, A.C.C. and Freiberger, S. (2003). Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety,* 54: 119-130.

FAO, WFP and IFAD. (2012). The State of Food Insecurity in the World 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. Rome, FAO. ww.fao.org/docrep/016/i3027e/i3027e.pdf.

FAO. (1977). The State of food and agriculture. http://www.fao.org/docrep/017/ap657e/.

FAO. (2011). FAOSTAT. Food and Agriculture Organization of the United Nations. https://www.feedipedia.org/node/535.1.

FAO. (2011a).The state of the world's land and water resources for food and agriculture (SOLAW) – Managing systems at risk. Rome: Food and Agriculture Organisation of the United Nations and London: *Earthscan*.

FAO. (2016). The State of World Fisheries and Aquaculture: Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome.

Feldmann,B.V., Zinki,J.G. and Jain,N.C.(2000).Schalm's Veterinary Haematology,5th ed. Philadelphia,USA:Lea and Fibiger.

Fernandes, M.N. and Mazon, A.F. (2003).Environmental pollution and fish gill morphology. In: Val, A. L. & B. G. Kapoor (Eds.). Fish adaptations.Enfield, Science Publishers, Pp. 203-231.

Ferreira, S.H., B.B. Lorenzetti, M.S.A. Castro and F.M.A. Correa, 1978. Antialgic Effect of Aspirin-like Drugs and the Inhibition of Prostaglandin Synthesis. In: The

Recognition of Anti-rheumatic Drugs, Dumonde, D.C. and M.K. Jasani (Eds.). MTP Press Ltd., St. Leonard House, Lancaster,Pp.25-37.

Figueirodo-Fernandes, A., Ferreira-cardso, J. and Santos, S.G. (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Veterinária Brasileira*, 27(3):103-109.

Filho, D.W. (1996). Fish antioxidant defences - a comparative approach. *Brazilian Journal of Medical and Biological Research*, 29: 1735-1742.

Fisher, B., Turner, K.R., Morling, P., 2009. Defining and classifying ecosystem services for decision making.*Ecological Economics*, 68(3): 643-653.

Fisheries Section of NSW department of primary Industries. (2006). Carp (*Cyprinus carpio*).

Fliesher, G.A., ELckelberg, E.S. and Elveback, L.R. (1977). Alkaline phosphatase activity in the plasma of children and adolescents.*Clinical Chemistry*, 23(3): 469-472.

Fontana, L., Moreira, E., Torres, M., Fernandez, I., Rios, A., Sanchez, D.F. and Gil, A. (1998). Dietary nucleotides correct plasma and liver microsomal fatty acids alterations in rats with liver cirrhosis induced by oral intake of thioacetamide, *Journal Hepatology*, 28: 662-669.

Forwood, J.M., Harris, J.O. and Deveney, M.R. (2013). Efficacy of bath and orally administered praziquantel and fenbendazole against *Lepidotrema bidyana* Murray, a monogenean parasite of silver perch, Bidyanus bidyanus (Mitchell). *Journal of Fish Diseases*, 36: 939-947.

Fouda, F.M. (2004). Haematological and biochemical studies on the effects of a biological and chemical pesticides on the Nile catfish, *Clarias gariepinus*. *Journal of Egyptian-German Society of Zoology*, 43: 77-97.

Gad, N.S. (2005). Impact of environmental pollution in the southern region of Lake Manzalah Egypt on some biochemical parameters of Tilapia. *Journal of Egyptian german society of Zoology*, 48(A): 279.

Galina, J., Yin, G., Ardó, L. and Jeney, Z. (2009). The use of immunostimulating herbs in fish. An overview of research. *Fish Physiology and Biochemistry*, 35: 669-676.

Gardner, G.R. and Yevich, P.P. (1970). Histological and haematological responses of an estuarine teleost to cadmium. *Journal of Fishery Research*, 27: 2185-2196.

Geidam, M.A., Dauda, E. and Hamza, H.G. (2007). Effects of aqueous stem-bark extract of *Momordica balsamina* Linn on some serum enzymes in normal and ethanol fed rats.*Journal of Biological Sciences*, 7(2): 397-400.

Gernhofer, M., Pawet, M., Schramm, M., Muller, E. and Triebskorn, R. (2001). Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *J. Aqua. Ecosys. Stress Recovery,* 8: 241-260.

GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), (2002. *Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships.* Rep. Stud. GESAMP No. 64, Pp.126.

Gill, T.S. and Epple, A. (1993). Stress-related changes in the haematological profile of the American eel. *Anguilla rostrata*. *Ecotoxicology and Environmental Safety*, 25: 227-235.

Glusczak, L., Miron, D.S., Moraes, B.S., Simo˜es, R.R., Schetinger, M.R.C., Morsch, V.M., Loro, V.L., 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen). *Comparative Biochemistry and Physiology*, 146C: 519-524.

Gornal, A.G., Bardwil, G.S. and David, M.M. (1949). Determination of serum proteins by mean of Biuret reactions. *Biochemistry*, 177: 751-766.

Groenewald, A.A.V.J. (1964). Observations on the food habits of Clarias gariepinus Burchell, the South African freshwater barbel (Pisces: Clariidae) in Transvaal. *Hydrobiologia*, 23: 287-291.

Gutteridge, J.M.C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage.*Clinical Chemistry*, 41(12): 1819-1828.

Halappa, R. and David, M. (2009). Behavioural responses of the fresh water fish, *Cyprinus carpio* (Limmaeus) following sublethal exposure to chlorpyrifos.*Turkey Journal of Fish Aquatic Science*, 9: 233-238.

Handy, R.D. and Penrice, W.S. (1993).The influence of high oral doses of mercuric chloride on organ toxicant concentrations and histopathology in rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology (C),* 106: 717-724.

Harborne, J.B. (1980). Phytochemical Methods. Chapman and Hall Limited, London, Pp.49-189.

Harikrishnan, R., Balasundaram, C. and Heo, M.S. (2011a). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture,* 317: 1-15.

Harikrishnan, R., Balasundaram, C., Kim, M..C., Kim, J.S., Han, Y.J. and Heo, M.S. (2009b). Innate immune response and disease resistance in Carassius auratus by triherbal solvent extracts. *Fish and Shellfish Immunology*, 27: 508-515.

Harikrishnan, R.., Balasundaram, C. and Heo, M.S. (2012a).Effect of Inonotus obliquus enriched diet on hematology, immune response, and disease protection in kelp grouper,Epinephelus bruneus against *Vibrio harveyi*. *Aquaculture*, Pp. 344-349.

Harikrishnan, R., Kim, J.S., Kim, M.C., Balasundaram, C. and Heo, M.S. (2011b). *Hebricium erinaceum* enriched diets enhance the immune response in Paralichthys olivaceus and protect from Philasterides dicentrarchi infection. *Aquaculture,* 318: 48-53.

Haux, C., Sjobeck, M.L. and Larsson, A. (1985). Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. *Marine Environmenttal Research,* 15: 77-95.

Hawkey, C.M. and Dennet, T.B. (1989). *A Colour Atlas of Comparative Veterinary Haematology*. Wolfe Publishing, London, UK, Pp. 192.

Helander, I.M., Alakomı, H.L., Latva-Kala, K., Mattıla-Sandholm, T., Pol I., Smıd, E.J., Gorrıs, L.G.M.and Von Wrıght, A. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria.*Journal of Agricultural and Food Chemistry*, 46: 3590-3595.

Hemalatha, S. and Banerjee, T.K. (1997a). Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the air-breathing catfish *Heteropneustes fossilis* (Bloch).*Biological Research*, 30: 11-21.

Herfindal, D.R. and Gourley, E.T. (2002). Textbook of Therapeutic Drug and Disease Management. Seventh edition. Lippincott Williams and Wilkins, Philadelphia, USA, Pp. 83.

Hesser, E.F. (1960). Methods for routine fish hematology.*Program. Fish Cult*ure, 22: 164-1.

Hinton D.E., Baumann P.C., Gardner G.R., Hawkins W.E., Hendricks J.D., Murchelano R.A. and Okihiro M.S. (1992). Histopathologic biomarkers, In: Hugget R., Kimerle R., Merles P. and Bergman H. (Eds), Biomarkers Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publ., Boca Raton, Pp. 155-195.

Hinton, D.E. and Lauren, D.J. (1990).Liver structural alterations accompanying chronic toxicity in fishes potential biomarkers of exposure. In: McCarthy, J.F. Shugart, L. R. (eds.) Biomarkers of environmental contamination. Lewis Publishers. Boca Raton, Pp.17-57.

Hodge, D.M., DeLong, J.M., Forney, C.F. and Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in

plant tissues containing anthocyanin and other interfering compounds. *Planta,* 207(4): 604-611.

Hoffman, J.J. and Cole, J.R. (1977). Phytochemical investigation of Adenium obesum Forskal (Apocynaceae): Isolation and Identification of cytotoxic agents. *Journal of Pharmaceutical Science,* 66(9): 1336-1338.

Hogendoorn, H. (1979). Controlled propagation of Africa catfish, clarias lazera (C&V). Reproductive biology and field experiment. *Aquaculture*, 17(4): 323-333.

Hori, T.S.F., Avilez, I.M., Iwama, G.K., Jhonson, G. and Afonso, L.O.B. (2008). Impairment of the stress response in matrinxa juveniles (*Brycon amazonicus*) exposed to low concentrations of phenol .*Comparative Toxicology and Pharmacology,* 147: 416-423.

Hrubec, T.C., Smith, S.A. and Robertson, J.L. (2001). Age related differences in haematologic and plasma chemistry analytes of hybrid striped basis *Morore chrysons*x *Morore saxatilis. Veterinary Clinical Pathology*, 30: 8-15.

Hued, A.C., Oberhofer, S. and Bistoni, M.D. (2012). Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of Jenynsia multidentata (Anablepidae, Cyprinodontiformes). *Archive of Environmental Contamination and Toxicology,* 62(1): 107-117.

Ibrahim, A.T.A. (2011). Antioxidative potential of selenium and vitamin E against mercury induced toxicity in *Clarias gariepinus* (Burchell, 1822) from Assiut-Egypt. Ph.D. Thesis, Zoology Department, Faculty of Science, Assiut University, Egypt.

Iguchi, K., Ogawa, K., Nagae, M. and Ito, F. (2003). The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture,* 220: 515-523.

IIRR, IDRC, FAO, NACA and ICLARM, (2001). Utilizing Different Aquatic Resources for Livelihoods in Asia: a resource book. International Institute of Rural Reconstruction, International Development Research Centre, Food and Agriculture Organization of the United Nations, Network of Aquaculture Centers in Asia-Pacifi c and International Center for Living Aquatic Resources Management

Innocent, X., Aly-Fathima, M.S. and Dhanalakshmi,. (2011). Studies on the immunostimulant activity of Coriandrum sativum and resistance to Aeromonas hydrophila in Catla catla. *Journal of Applied Pharmacology Science,* 1:132-135.

Jakoby, W.B. and Habig, W.H. (1980) in Enzymatic Basis of Detoxification (Jakoby, W. B., ed.), Academic Press, New York, Pp. 63-94.

James, P., Thorpe, I.J., Kokkinos, N., Morkot, R. and Frankish, J. (1991). Centuries of Darkness: context, methodology and implications. *Cambridge Journal Archive*, 1: 228-35.

Janseen, J. (1987). Hatchery management of African Clariid catfish Claria gariepinus (Burchell, 1822). In: Coche, A. and Edwards, D. (Eds).Selected Aspects of *Warm Water Fish Culture*. FAO/UNDP, Rome, Pp.181.

Jhosi, P.H.P and Bose, M. (2003).Effect of lindane and malathion exposures to certain blood parameters in a fresh water fish Clarias batrachus. *Pollution Research*, 21: 55-57.

Ji, S.C., Jeong, G.S., Gwang-Soon, I., Lee, S.W., Yoo, J.H. and Takii, K. (2007a). Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. *Fish. Science*, 73: 70-76.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S. and Pokethitiyook, P. (2003). Biochemical and histopathological effects of glyphosate herbicides on Nile tilapia (*Oreochromis niloticus*). *Environmental and Toxicology*, 18(4): 260-268.

John. J., Rensland, H., Schlichting, I., Vetter, G., Borasio, D., Goody, R.S. and Wittinghofer, A. (1993). Kinetic and structural analysis of the Mg(2+)-binding site of the guanine nucleotide-binding protein p21H-ras. *Journal biological chemistry*, 268: 923-929.

Jothivel, N. and Paul, V.I. (2008). Evaluation of the acute toxicity of the seed of *Anamirta cocculus* (Linn.) and its piscicidal effect on three species of freshwater fish. *The internet Journal of Toxicology*, 5: 1.

Judd, R.A. (1964). Freshwater fishes and drainage basins in southern Mrica. 1. The Orange and south coastal drainage basins. *South Africa Journal of Science*, 60: 17-21.

Kadry, S.H.M., Yacoub, A.M., Siliem, T.A and Mabrouk, D.B. (2003). Injuries in kidney tissue of the Girls, Ain Shams University, Egypt, pp: 293. Mugil *Liza ramada* as a biomarker for environmental pollution in the lake Manzalah. *Egyptian Journal of Aquatic Biology and Fisheries*, 7(3): 1-24.

Kaiser, J. (2001).Bioindicators and Biomakers of Environmental pollution and Risk Assessment.Science Publishers, Enfield, USA. Pp 10-20.

Kajita, Y., Sakai, M., Atsuta, S. and Kobayashi, M. (1990). The immunomodulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. *Fish Pathology*, 25: 93-98.

Kakuta, I. and Murachi, S. (1997). Physiological response of carp, *Cyprinus carpio*, exposed to raw sewage containing fish processing wastewater.*Environmental Toxicology and Water Quality*, 12(1): 1-9.

Kaoud, H.A., Zaki, M.M., El-Dahshan, A.R., Saeid, S. and El-Zorba, H.Y. (2011). Amelioration the Toxic Effects of Cadmium-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*. *Life Science Journal*, 8(1).

Karakaya, A.F. (2011). Collaborative environments to enhance creativity of design students: Use of collaborative creativity support tools. Unpublished dissertation, Bilkent University, Ankara, Turkey.

Kavitha, C., Ramesh, M., Kumaran, S.S., and Larshmi, S.A. (2012). Toxicity of *Moringa oleifera* seed extract on some haematological and biochemical profiles in a freshwater fish *Cypririns carpio Experimental Toxicological Pathol*ogical, 64: 681-687.

Kawarazuka, N. and Bene, C. (2011). The potential role of small fish species in improving micronutrient deficiencies in developing countries: *building evidence*, 14: 1927-1938.

Keith, F.P. and Weber, J.L. (1979). The effect of carbon tetrachloride on the total plasma protein concentration of rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology*, 63: 37-42.

Kela, S.L., Ogunsusi, A.A., Ogbogu, V.C. and Nwude, N. (1989). Susceptibility of two – week old *L. natalenses* to some plants extracts. Revue Elev. *Medicine Veteren pays. Trop.,* 42(2): 189-192.

Kelly, M.L., Berry, J.R., Dwyer, D.A., Grinari, J.M., Chouinard, P.Y., Van-Amburgh, M.E. and Bauman, D.E. (1998). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *Journal of Nutrition,* 128(5): 881-5.

Kemal, J. (2014). Laboratory Manual and Review on Clinical Pathology. OMICS Group eBooks, Foster City. CA 94404, USA, Pp. 5-18.

Kerr, M.G. (2005). Diagnostic procedures for the private practice laboratory: Clinical biochemistry and heamatology. In: Kahn, C.M. (Ed). The Merck Veterinary Manual. Ninth edition. Merck & Co., Inv., Whitehouse station, New Jersey, USA, Pp. 1339-1368.

Kiemer, M.C.B. and Black, K.D. (1997). The effects of hydrogen peroxide on the gill tissues of Atlantic salmon, *Salmo salar L*. *Aquaculture,* 153: 181-189.

Kiyohara, M., Nakatomi, T., Kurihara, S., Fushinobu, S., Suzuki, H., Tanaka, T. (2012). alpha-N-acetylgalactosaminidase from infant-associated bifidobacteria belonging

to novel glycoside hydrolase family 129 is implicated in alternative mucin degradation pathway. *Journal of Biology and Chemistry*, 287: 693-700.

Knight, A.P. and Walter, R.G. (1983). In: A Guide to Plant Poisoning of Animals in North America, A.P. Knight and R.G. Walter (Eds.)

Knox, W.E. and Greengard, O. (1965). In: *An introduction to enzyme physiology*, *Advan. Enzyme Regul.* (Ed. Weber, G.) Pergamon Press, New York, London, 3: 247-248.

Konar, R.N. (1975). In vitro studies on Pinus II. The growth and morphogenesis of cell culture from Pinus gerardiana.*Phytomorphology Silver Jubilee*, 55-59.

Konar, S.K. (1970). Nicotine as a fish poison.*Te Progressive Fish Culturist,* 32: 103-104.

Kori-siakpere, O. and Ubogu, E.O. (2008). Sub-lethal haematological effects of zinc on the fresh water fish,Heteroclarias sp. (Osteichthyes:Claridae). *African journal of Biotechnology*,7(12): 2068-2073.

Kori-Siakpere, O., Ake, J.E.G. and Idoge, E. (2005). Haemological characteristics of the African snakehead, *Parachanna obscura*. *African Journal of Biotechnology,* 4(6): 527-530.

Kori-Siakpere, P. (1985). Haematological characteristics of *Clarias isheriensis. Journal of Fish Biology,* 27: 259-263.

Kruatrachue, M., Rangsayatorn, N., Pokethitiyook, P., Singhakaew, S. (2003). Histopathological Changes in the Gastrointestinal Tract of Fish, Puntius gonionotus, Fed on Dietary Cadmium, *Bulletin of Environmental Contamination and Toxicology*, 71(3); 561-9.

Kumar, V., Singh, B.P. and Rani, S. (2004). The bird clock: A complex multi-oscillatory 658 and highly diversified system. *Biology and Rhythm Research*, 35: 121-144.

Kumaraguru, A.K., Beamish, F.W.H. and Ferguson, P. (1982). Direct and circulatory paths of Permethrin causing histopathological changes in gills of S. gairdneri. *Journal of Fish Biology,* 20: 87-91.

Lambert, R.J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology,* 91(3): 453-62.

Lee, S., Najiah, M., Wendy, W. and Nadirah, M. (2009). Chemical composition and antimicrobial activity of the essential oil of Syzygium aromaticum flower bud (clove) against fish systemic bacteria isolated from aquaculture sites. *Frontier Agriculture China,* 3: 332-336.

Legendre, M., Teugels, G.G., Cauty, C. and Jalabert, B. (1992).A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell,

1822), *Heteropneustes longifilis* Valencinnes, 1840 and their reciprocal hybrids (Pisces, Clariidae). *Journal of Fish Biology*, 40: 59-79.

Li, J., Menzel, W. P., Yang, Z., Frey, R. A., and Ackerman, S. A. (2003). Highspatial-resolution surface and cloud-type classification from MODIS multispectral band measurements. *Journal of Applied Meteorology*, 42: 204-226.

Limdi, J.K. and Hyde, G.M. (2003). Evaluation of abnormal liver function tests. *Post Graduate Medicine*, 79(932): 307-312.

Lind, O. (1979). *A handbook of limnological methods.* St. Louis, USA: C.V. Mosby Co, Pp. 199.

Liogier, H. (1990). Description Flora of Puerto Rico and Adjacent Island. *editorial Dela Universidad de Puerto, Rio piedras,* 5: 436.

Little, E.E. and Brewer, S.K. (2001). Neurobehavioral toxicity in fish D. Schlenk, W.H. Benson (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts New Perspectives: *Toxicity and the Environment*, vol. 2, Taylor and Francis, London and New York, Pp. 139-174.

Little, E.E. and Finger, S.E. (1990). Swimming behaviour as an indicator of sublethal toxicity in fish.*Environmental Toxicology and Chemistry*, 9: 13-19.

Livingstone, D.R., Lemaire, P., Matthews, A., Peters, L., Bucke, D. and Law, R.J. (1993). Prooxidant, antioxidant and 7-Ethoxyresorufin O-Deethylase (EROD) activity responses in liver of Dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Marine Pollution Bulletin,* 26: 602-606.

Lockhart, W.L. and Metner, D.A. (1984). Fish serum chemistry as a pathology tool. In: Cairns, V. W., Hodson, P. V., Nriagu, J.O. (eds.) Contaminant effects on fisheries. Willy, Pp.73-85.

Logambal, S.M., Venkatalakshmi, S. and Michael, R.D. (2000). Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Hydrobiologia,* 430: 113-120.

Loomis, T.A. and Hayes, A.W. (1996). Loomis's Essentials of Toxicology, fourth edition. Academic Press, California, U.S.A. Pp. 282.

Lorenzi, H. and Matos, F.J. (2002). Plantas medicinais do Brasil. São Paulo. *Nova Odessa*, Plantarum. Pp. 512.

Lovsin, J. and Drucker, D.J. (2000). New frontiers in the biology of GLP-2. *Regulatory peptides*, 90(2000): 27-32.

Lucia, M.C. and Jesu, C.F.J. (2012). *Borreria* and *Spermacoce* species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. *Pharmacognosy Revision*, 6(11): 46-55.

Luskova, V., Svoboda, M. and Kolarova, J. (2002). The effect of diazinon on blood plasma biochemistry in Carp (*Cprinus carpio* L.). Acta Vet. Brno. 71: 117-123.

Maheswaran, R., Devapaul, A., Velmurugan, B. and Ignacimuthu, S. (2008). Haematological studies of freshwater fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrated Biology*, 2(1): 49-54.

Majak, W. (2001). Review of toxic glycosides in rangeland and pasture forages. *Journal of Range Management,* 54: 494-498.

Makkar, H.P.S., Francis, G. and Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal,* 1 (9): 1371-1391

Mallat, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fish and Aquatic Science,* 42: 630-648

Mandal, P.K. and Kulshrestha, A.K. (1980). Histopathological changes induced by the sublethal surnithjon in *Clarius batrachus* (Linn). *Indian Journal Exploratory biology*, 18: 547-552.

Marnett, L.J. (1999). Lipid peroxidation-DNA damage by malondialdehyde, *Mutation Research,* 424: 83-95.

Marnett, L.J. (2000). Oxyradical and DNA damage, *Carcinogenesis*, 21(3): 361-370.

Martinez, C.B.R., Nagae, M.Y., Zaia, C.T.B.V. and Zaia, D.A.M. (2004).Morphological and physiological acute effects of lead in the neotropical fish, *Prochilodus lineatus*. *Brazilian Journal of Biology*, 64(4):797-807.

Martinez-Espinosa, M. (1996). Report of the expert consultation on small-scale rural aquaculture. Rome, Italy, 28-31 May 1996. *FAO Fisheries Report* No. 548. Rome, FAO, Pp. 182.

Marzoug, H.N.B., Romdhane, M., Lebrihi, A., Mathieu, F., Couderc, F., Abderraba, M., Khouja, M.L., Bouajila, J. (2011). *Eucalyptus oleosa* Essential Oils: Chemical Composition and Antimicrobial and Antioxidant Activities of the Oils from Different Plant Parts (Stems, Leaves, Flowers and Fruits). *Molecules*, 16: 1695-1709.

Masotti, V., Juteau, F., Bessiere, J.M. and Viano, J. (2003). Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. *Journal for Agriculture and Food Chemistry,* 51(24): 7115- 7121.

Matouke, M.M. and Abui, O.A. (2015). Haematological and histological evaluation of African catfish *Clarias gariepinus* following acute exposure to methanolic extract of *Khaya senegalensis*. *Research Agriculture Livestock and Fisheries,* 2(3): 475-482.

Mayer-Gostan, N., Wendelaar, B.S.E. and Balm, P.H.M. (1987). Mechanisms of hormone actions on gill transport. In: Vertebrate endocrinology: fundamentals and biomedical implications. Volume 2. Edited by Pang, P.K.T and Schreibman, P. Boston: Academic press.

Maynart, G., Pousset, J. L., Mboup, S. and Denis, F. (1980). Antibacterial activity of borreverine, an alkaloid isolated from Borreria verticillata (Rubiaceae) C R Seances Soc. *Biol Fil,* 174: 925-8.

Mazon, A.F., Pinheiro, G.H.D. and Fernandes, M.N. (2002). Haematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*.

McCarthy, M.S., Hafs, H.D. and Convey, E.M. (1979). Serum hormone patterns associated with growth and sexual development in bulls. *Journal of Animal Science*, 49: 1012-1020.

McLaughlin, J. and Garofalo, J. (2002). The desert rose (*Adenium obesum*). Miami-Dade: Miami-Dade County/University of Florida Cooperative Extension Service, Pp. 66.

Mekkawy, I.A.A., Hussein, S.Y., Abd-El-Nasser, M. and Ahmed, S.M. (1996). Comparative studies on the effects of herbicide atrazine on someblood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. *Journal of Egyptian-German Society of Zoology,* 19: 283-319.

Mekkawy, I.A.A., Mahmoud, U.M., Wassif, E.T. and Naguib, M. (2011). Effects of Cadmium on Some Haematological and Biochemical Characteristics of *Oreochromis niloticus* (linnaeus, 1758) Dietary Supplemented with Tomato paste and vitamin E. *Journal of Fish Physiology and Biochemistry,* 37(1): 71-84.

Mettam, R. (1941). Plant poisoning in the domestic animals. *Farm Forest,* 1: 58-60.

Meyers, S., Schauer, W., Balzi, E., Wagner, M., Goffeau, A. and Golin, J. (1992). Interaction of the yeast pleiotropic drug resistance genes PDR1 and PDR5. *Current Genetics,* 21(6): 431-6.

Mgbojikwe, L.O. (2000). Acaricidal properties of the aqueous stem bark extract of Adenium obesum. PhD Dissertation. University of Jos, Jos, Nigeria.

Middleton, E.J. (1998). Effect of plant flavonoids on immune and inflammatory cell function. *Advance Experimental Medical Biology,* 439: 175-82.

Mirand, C.D. and Zemelman, R. (2002). Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms,*Sciemce Total Environment,* 293: 207-218.

Misra, H.P. and Fridovich, I. (1972).The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biology and Chemistry,* 247(10): 3170-3175.

Modesto, K.A. and Martinez, C.B.R. (2010). Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere,* 81: 781-787.

Mohamed, F.A.E.S. (2009). Histopathological studies on some organs of *Oreochromis niloticus*, *Tilapia zillii* and *Synodontis schall* from El-Salam canal, Egypt. *Egyptian Journal of Aquatic* Biology *and Fisheries,* 7(3): 99-138.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G. and Mohanta, K.N. (2013). Aquaculture and stress management: a review of probiotic intervention: stress management by probiotics administration. *Journal of Animal and Physiology and Animal Nutrition (Berl),* 97: 405-430.

Moller, M., Herzer, K., Wenger, T., Herr, I. and Wink, M. (2007). The alkaloid emetine as promising agent for the induction and enhancement of drug induced apoptosis in Leukaemia cells. *Oncology Reports,* 18: 737-744.

Monteiro, D.A., Rantin, F.T. and Kalinin, A.L. (2009). The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxã, *Brycon cephalus* (Günther, 1869) exposed to organophosphate insecticide Folisuper 600 BR® (methyl parathion).*Comparative Biochemistry and Physiology C-Toxicology and Pharmacology,* 149: 40-49.

Moore, R.T. (1980). Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts.*Botanica Marina,* 23(6): 361-373.

Moreira, V.F., Oliveira, R.R., Mathias, L.,Braz-Filho, R. and Vieira, I.J. (2010). New chemical constituents from Borreria verticillata (Rubiaceae) Helv. *Chemical Acta,* 93: 1751-1777.

Moss, D.W., Eaton, R.H., Smith, J.K. and Whitby, L.G. (1967). Association of inorganic-pyrophosphatase activity with human alkaline-phosphatase preparations. *Biochemical journal,* 102: 53.

Mousa, M.A. and Khattab, Y.A. (2003). The counteracting effect of Vitamin C (ascorbic acid) on the physiological perturbations induced by ochratoxin intoxication in African-Sharp-Tooth catfish (*Clarias gariepinus*). *Journal of Egypt Academy and Environmental Development,* 4: 117-128.

Moussa, T.A. (1956). Morphology of the accessory air-breathing organs of the teleost *Clarias lazera* (C&V). *Journal of Morphology,* 98:125-160.

Murray, H.M., Wright, G.M. and Goff, P. (1996). A Comparative histological and histochemical study of the post-gastric alimentary canal from three species of *pleuronectid*, the Atlantic halibut, the yellow tail flounder and the winter flounder. *Journal of Fish Biology*, 48: 187-206.

Murtala, Y., Babandi, A., Sunusi, M.M., Shehu, D., Babagana, K. and Alhassan, A. J. (2005). Effect of Aqueous Leaf Extract of *Borreria verticillata* Species of Sudano-Sahelian Savanna on CCl4 Induced Hepatotoxicity. *Journal of National Sciences Research*, 5(24): 2224-3186.

Musa, S.O and Omoregie, E. (1999). Haematological changes in the mudfish, *clarias gariepinus* (Burchell) exposed to malachite green. *Journal of Aquatic Sciences,* 14: 37-42.

Nagegowda, D.A. (2010). Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. *FEBS letters. Federation of European Biochemical Societies* , 584(14): 2965-73.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024.

Neelima, P., Srinivasa, R.G., Govinda, R.K. and Chandra, S.R.J. (2015). Cypermethrin (A Synthetic Pyrethroid) Induced Histopathological Alterations In Gill, Liver And Kidney Of A Non-Target Organism Fish. *International journal of bioassay*, 4(07): 4149-4154.

Nellis, D.W. (1997). *Poisonous Plants and Animals of Florida and the Caribbean*.Pineaple press Inc., USA, Pp.14-15.

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.

Nigro, M., Falleni, A., Del Barga, I., Scarcelli, V., Lucchesi, P., Regoli, F. and Frenzilli, G. (2006). Cellular biomarkers for monitoring estuarine environments: transplanted versus native mussels. *Aquatic Toxicology,* 77: 339-347.

Nounou, A.H., Soliman, M.M., Rizkalla, E.H. and Assad, M.N. (1997). Haematological and biochemical changes in *Clarias lazera* as a result of long term exposure to trial combination of lead, mercury and arsenic. *Egypt Journal of Agricultural Research*, 75(1): 247-270.

Novotny, S. (2005). Systemic pharmacotherapeutics of the cardiovascular system. In C. Kahn, *The Merck Veterinary Manual* (9th ed.). Whitehouse Station, New Jersey, USA: Merck & Co., Inc., Pp. 1966-1979.

Nussey, G., Van-Vuren, J.H.J. and du-Preez, H.H. (1995). Effect of copper on the haematology and osmoregulation of the Mozambique tilapia *Oreochromis mossambicus* (Cichlidae). *Compendium Biochemistry Physiology Pharmacology Toxicology and Endocrinology,* 111: 369-380.

Nwafili, S.A. and Gao, T. (2007). Is the Dutch domesticated strain of Clarias gariepinus (Burchell, 1822) a hybrid? *African Journal of Biotechnology,* 6(8): 1072-1076.

Nwani, C.D., Nwadinigwe, A.O., Joshua, P.E., Onyeke, C.C., Ogbonna, S.U., Ukonze, J. A. and Eze, S. O.O. (2016). Hepatic antioxidant status and haematological parameters in African catfish, clarias gariepinus juvenile exposed to sublethal concentration of *Psychotria microphylla*. *The Journal of Animal & Plant Sciences,* 26(1): 275-281.

Nya, E.J. and Austin, B. (2009). Use of garlic, Allium sativum, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases,* 32: 963-970.

Oakes, K.D. and Van der Kraak, G.J. (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology,* 63: 447-463.

Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical studies and Comparative efficacy of the crude extract of some homoestatic plants in Edo and Delta States of Nigeria. *Global formal of pure and and Applied Science,* 86: 203-208.

Obiezue, R.N., Okoye, I.C., Ikele, B.C. and Obi, I.K. (2011). Evaluation of Biochemical Parameters of *Clarias Gariepinus* exposed To Sub lethal Concentration of *Cypermethrin*. *Animal Research International,* 8(3): 1480-1484.

OECD (2001). *Guidelines for the testing of chemicals No.423:Acute Oral Toxicity-Acute Toxic Class Method (adopted 17th December 2001).* Organization for Economic Co-operation and Development, Paris,France.

Ogbulie, J.N. and Okpokwasili, G.C. (1999). Haematological and histological responses of *Clarias gariepinus* and *heterobranchus bidorsalis* to some bacterial disease in rivers state, Nigeria. *Journal of National Science Foundation of Sri Lanka,* 27(1): 1-16.

Okechi, J. K. (2004). Profitability assessment: a case study of African catfish (clarias gariepinus) farming in the Lake Victoria basin, Kenya. Fisheries Training Programme. The United Nations: Iceland.

Okomoda, J., Ayuba, V.O. and Omeji, S. (2010). Haematological changes in *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to acute concentration of formalin, *Production Agriculture and Technology,* 6(1): 92-101.

Okwu, D.E. (2004).Phytochemical, and vitamin contents of two indigenous species of South Eastern Nigeria, *Journal of Sustainable Agriculture and Environment,* 6: 30-34.

Okwu, D.E. (2005).Phytochemicals, Vitamins and Mineral content of two Nigerian medicinal Plants. *International Journal of Molecular Medicine and Advance Sciences,* 1(4): 375-381.

Olagunju, F.I., Adesiyan, I.O. And Ezekiel, A.A. (2007). Economic viability of cat fish production in oyo state, Nigeria. *Journal of Human Ecology*, 21(2): 121-124.

Oliveira Ribeiro, C.A., Pelletier, E., Pfeiffer, W.C. and Rouleau, C. (2000). Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. *Environmental Research*, 83: 286-292.

Olushola, M.A., Kafilat A.B. and Adebayo A.O. (2014). "Accumulation and Anti-oxidant Enzymes as biomarkers of heavy metal Exposure in *Clarias gariepinus* and *Oreochromis niloticus*." *Applied Ecology and Environmental Sciences,* 2(5): 114-122.

Olusola, S.E., Emikpe, B.O. and Olaifa, F.E. (2013). The potentials of medicinal plants extracts as bio-antimicrobial in aquaculture. *International Journal of Medical Aromatic Plants*, 3: 404-412.

Omoniyi, I., Agbon, A.O. and Sodunke, S.A. (2002). Effect of lethal and sublethal concentrations of Tobacco (Nicotiana Tobaccum) leaf Dust Extract on weight and Heamatological changes in *Clarias Gariepinus* (Burchell). *Journal of applied science and environmental management*, 6: 37-41.

Omoregie, E., Eseyin, T.G. and Ofojekwu, P.P.C. (1994). Chronic effects of formalin on erythrocytic coumts and plasma glucose of the Nile Tilapia,*Oreochromis niloticus*. *Asian Fisheries Science*, 7: 1-6.

Omoregie, E. and Oyebanji, (2002). Oxytetracycline-induced blood disorder in Nile tilapia, *Oreochromis niloticus*. *Journal of World Aquaculture Society*, 33(3): 377-382.

Onusiriuka, B.C. and Ufodike, E.B.C. (2000). Effects of sublethal concentrations of Akee apple, *blighia sapida* and sausage plant, *Kigelia Africana* on tissue chemistry of African catfish,*Clarias gariepinus*. *Journal of Aquatic Science*, 15: 47-49.

Orji, O.U., Ibiam, U.A. and Aja, P.M. (2014). Acute toxicity studies of the lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* juveniles. *International Journal of Biology and Biological Sciences,* 3(4): 038-044.

Orlowski, A., Grygiel, W., Grzebielec, R. and Wyszynski, M. (1997). Polish acoustic survey on pelagic fish distribution in ICES Subdivisions 24, 25 and 26 in the Baltic Sea carried out in October 1996.*Environmental factors*.

Ortiz, J.E.,Torres, T., Valle, M., Lucini, M., Liamas, F.J. (2003). Biomarkers, flora and fauna ofthe bottom of Venta Micena section (Grce, Granada). Palaeoenvironmental information.*GEOGACETA*, 34: 171-174.

Oryan, A., Maham, M., Rezakhani, A. and Maleki, M. (1996). Orphological studies on experimental oleander poisoning in cattle. *Zentralblatt for Veterinarmedizin*, 43: 6235-634.

Ostrander, G.K. (1996).Techniques in Aquatic Toxicology. CRC Press LLC, Boca Raton, FL, Pp. 577-587.

Oyen, L. (2008). Adenium obesum (Forssk) Roem and Schult. In G. G.-F. Schmelzer, *Plant Resources of Tropical Africa:Medicinal Plant 1*. Wageningen, Netherlands: PROTA Foundation/Backhuys Publishers/CTA, 11: 46-49.

Pacheco, M. and Santos, M.A. (2002). Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety*, 53: 331-347.

Palace, V.P., Khaper, N., Qin, Q. and Singal, P.K. (1999). Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease.*Free Radical Biology and Medicine*, 26(5-6): 746-61.

Palozza, P. and Krinsky, N.I. (1992a). Antioxidant effects of carotenoids in vivo and in vitro: an overview. *Methods Enzymology*, 213: 403-420.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B. and Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. and Schn.). *Science of Total Environment*, 309: 105-115.

Panepucci, R.A., Panepucci, L., Fernandes, M.N., Sanchez, J.R. and Rantin, F. T. (2001). The effect of hypoxia and recuperation on carbohydrate metabolism in pacu (Piaractus mesopotamicus). *Brazilian Journal of Biology*, 61: 547-554.

Panigrahi, A. and Azad, I.S. (2007). Microbial intervention for better fish health in aquaculture:the Indian scenario. *Fish Physiology and Biochemistry*, 33: 429-440.

Parent, L.M., DeLorenzo, M.E. and Fulton, M.H. (2011). Effects of the synthetic pyrethroid insecticide, permethrin, on two estuarine fish species.*Journal of Environmental Sciences and Health*, 46: 615-622.

Park, J.Y. and Kim, I.S. (2001). Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. *Journal of Fish Biology*, 58: 861-872.

Parke, D.V., Piotrowsk, I.J.K. (1996). Glutathione: its role in detoxication of reactive oxygen species and environmental chemicals. *Acta Pol Toxicol*, 4: 1-14.

Pasnik, D.J., Evans, J.J., Panangala, V.S., Klesius, P.H., Shelby, R.A. and Shoemaker, C.A. (2005). Antigenicity of Streptococcus agalactiae extracellular products and vaccine efficacy.*Journal of Fish Diseases,* 28: 205-212.

Pavaraj, M., Balasubram, V., Baskaran, S. and Ramasamy, P. (2011). Development of immunity by extract of medicinal plant Ocimum sanctum on common carp Cyprinus carpio (L.). *Research Journal of Immunology*, 4: 12–18. *(PDF) Use of Medicinal*
*Plants in Aquaculture.*Available from:https://www.researchgate.net/publication/315850361_Use_of_Medicinal_Plants_in_Aquaculture [accessed Aug 01 2018].

Payne, S.E. (2005). Aneamia. In: Kahn, C.M. (Ed.). The Merck Veterinary Manual.Ninth edition.Merck & Co., Inc., Whitehouse Station, New Jersey, USA, Pp. 8-15.

Pearson, D. (1979). Chemical Analysis of food. Seventh edition. Churchill livingstone, Edinburgh, Pp. 575.

Pelgrom, S., Lamers, L., Lock, R., Balm, P. and Wendelaar, B.S. (1995). Integrated physiological response of tilapia, *Orechromis mossambicus*, to sublethal copper exposure. *Aquatic Toxicology*, 32: 303-32.

Perry, S.F. and Laurent, P. (1993). Environmental effects on fish gill structure and function, In: Rankin J.C. and Jensen F.B. (ed.), Fish Ecophysiology. Chapman and Hall, London. Pp. 231-264.

Peteri, A., Nandi, S. and Chowdhury, S.N. (1992.). Manual on seed production of African catfish (*Clarias gariepinus*). FAO document repository, BGD/87/045/92/22. Rome, Italy: FAO.

Pichersky, E., Noel, J. and Dudareva, N. (2006). Biosynthesis of plant volatiles: *Nature's Diversity and ingenuity,* 10: 808-811.

Pilo, B. Asnani, M.V. and Shah, R.V. (1972). Studies on wound healing and repair in pigeon liver II. Histochemical studies on acid and alkaline phosphatase during the process, *Journal of Animal Morphology and Physiology*, 19: 205-212.

Piner, P. and Uner, N. (2013). Oxidative stress and apoptosis was induced by bio-insecticide spinosad in liver of *Oreochromis niloticu*s. *Environmental Toxicology and Pharmacology,* 36: 956-963.

Poleksic, V. and Mitrovic-Tutundzic, V. (1994). Fish gills as a monitor of sublethal and chronic effects of pollution. In: Mulle, R., Llyod, R. (Eds.), Sublethal and Chronic Effects of Pollutants on Freshwater Fish. Fishing News Books, Oxford, London, Pp. 339-352.

Poleksic, V., Pekic, Q.S., Cupina, B., Petric, D. and Drochner, W. (2006). Curricular reform at Serbian Agricultural Faculties: achievements of the TEMPUS JEP towards meeting the needs for rural development. Proceedings of the 8th

European Conference on Higher Agricultural Education (ECHAE): The Public and the Agriculture and Forest Industry,Pp.194-197.*(PDF) TEMPUS CASA PROJECT – A SUSTAINABLE TOOL*....Available from: https://www.researchgate.net/publication/318912950_TEMPUS_CASA_PROJECT__A_SUSTAINABLE_TOOL_FOR_KNOWLEDGE_AND_INNOVATION_TRANSFER_IN_ANIMAL_SCIENCES [accessed Jul 30 2018].

Powell, M.D., Briand, H.A., Wright, G.M. and Burka, J.F. (1993). Rainbow trout (*Oncorhynchus mykiss* Walbaum) intestinal eosinophilic granule cell (EGC) response to *Aeromonas salmonicida* and *Vibrio anguillarum* extracellular products. *Fish Shellfish Immunology,* 3: 279-289.

Pratt, D. S. and Kaplan, M. S. (2000).Evaluation of abnormal enzyme results in asymptomatic patients. *New England Journal of Medicine,* 342: 1266-1271.

Prinsloo, J.F. and Schoonbee, H.J. (1987). Investigations into the feasibility of a duck-fish-vegetable integrated agriculture aquaculture system for developing areas in South Africa. Water SA, 13: 109-118.

Puangkaew, J., Kiron, V., Satoh, S. and Watanabe, T. (2005). Antioxidant defense of rainbow trout (Oncorhynchus mykiss) in relation to dietary n-3 HUFA highly unsaturated fatty acids and vitamin E contents. *Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology*, 140(2): 187-96.

Quesada, S.P., Paschoal, J.A.R. and Reyes, F.G.R. (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—a review. *Journal of Food Science,* 78: 1321-1333.

Rad, F., Bozaoglu, S., Ergene, G.S., Karahan, A. and Kurt, G. (2006). Effects of different long day photoperiods on somatic growth and gonadal development in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture,* 255(1-4): 292-300.

Rahman, M.M., Varga, I. and Choudhury, S.N. (1992). *Manual on Africsn Magur (Clarias gariepinus) culture in bangladesh.*

Raj, N.K., Sripal, R.M., Chaluvadi, M.R. and Krishna, D.R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential, Indian Journal of *Pharmacology*, 33: 2-16.

Rali, T., Wossa, S.W. and Leach, D.N. (2007). Comparative chemical analysis of the essential oil constituents in the bark, heartwood and fruits of *Cryptocarya massoy* (Oken) Kosterm. (Lauraceae) from Papua New Guinea. *Molecules Basel Switzerland*, 2(12): 149-154.

Ramamurthy, V., Kothari, R.M. and Bhojan, J. (1987). Application of fungal cellulase in improving the milk yield. *Biotechnology Letters*, 9: 369-372.

Ramírez-Duarte, W.F., Rondón-Barragán, I.S. and Eslava-Mocha, P.R. (2008). Acute toxicity and histopathological alterations of Roundup herbicide on‘‘cachama blanca’’ (*Piaractus brachypomus*). *Pesqi Veterinary Brasilia*, 28: 547-554.

Ravanaiah, G. and Narasimha Murthy, C.V. (2010). Accumulation of heavy metals in selected tissues of fish,Tilapia mossambicus collected from the Pennar estuary.*Life science bulletin,* 7(2): 217-222.

Rec. G.S.C.C. (1972). Determination of Alkaline Phosphatase. *Z. Clin. Chem. Klin. Biochem*, 10: 281-291.

Refiel, C.W. and Travill, A.A. (1979). Structure and carbohydrate histochemistry of the intestine of ten Teleostean species. *Journal of Morphology,* 162(3): 343-359.

Rehman, H., Abbas, S. and Lohahet, N. (2003). *Laboratory Manual of Physiology*, Society of Veterinary Physiology, Lahore, Pakistan.

Reimschuessel, R. (2001). A fish model of renal regeneration and development. *Ilar Journal*, 42: 285-291.

Reitman, S. and Frankel, S. (1957). A Colorimeteric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology,* 28(1): 56 -63.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B. and Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current and future perspectives status and future perspectives. *Aquaculture*, 433: 50-61.

Ribeiro, C.A.O., Neto, F.F., Mela, M., Silva, P.H., Randi, M.A.F., Rabitto, I.S., Costa, J.R.M.A. and Pelletier, E. (2006). Haematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. *Environmental Research*, 101: 74-80.

Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A.M., Henriksson, P.J.G., Murray, F.J., Little, D.C., Dalsgaard, A. and Van den Brink, P.J., (2013). Feed additives and probiotics in four major internationally traded farmed in Asia. *Aquaculture*, 412: 231-243.

Risbourg, S.B. and Bastide, J. (1995). Hepatic perturbations induced by a herbicide (atrazine) in juvenile grey mullet *Liza ramada* (Mugilidae Telostei):an ultra structural study. *Aquatic Toxicology,* 31: 217-229.

Roberts, R. (1978). The patho-physiology and systemic pathology of teleost. In R. Roberts, *Fish Pathology*. London: Bailliere Tindall. Pp. 55-91.

Roberts, R.J (2012). Fish pathology. In R.J Roberts. Fish pathology Third edition. WB Saunders, Edinburgh. 359 pp.

Rodrigues, E.L. and Fanta, E. (1998). Liver histopathology of the fish Brachydanio rerio acute exposure to subletal livels of the organophosphate dimetoato 500. *Revista Brasileira de Zoologia*, 15: 441-450.

Roganovic-zafirova, D., Jordanova, M., Panov, S. and Velkova-jordanoska, L. (2003). Hepatic capillariasis in the Mediterranean barbell (Barbus meridionalis petenyi Heck.) from Lake Ohrid. *Folia Veterin*aria, 47(1): 35-37.

Romero Ormazábal, J.M., Feijoó, C.G. and Navarrete Wallace, P.A. (2012). Antibiotics in aquaculture- use, abuse and alternatives. In E.D. Carvalho, *Health and Environment in Aquaculture*, Pp. 159.

Rosety-Rodríguez, M., Ordoñez, F.J., Rosety, M., Rosety, J.M., Ribelles, A. and Carrasco, C. (2002). Morpho-histochemical changes in the gills of turbot, *Scophthalmus maximus* L., induced by sodium dodecyl sulfate. *Ecotoxicology and Environmental Safety*, 51: 223-228.

Ruane, N.M., Nolan, D.T., Rotllant, J., Tort, L., Balm, P.H.M. and Bonga, S.E.W. (1999). Modulation of the response of rainbowtrout (*Oncorhynchus mykiss* Walbaum) to confinement, by an ectoparasitic (Argulus foliaceus L.) infestation and cortisol feeding. *Fish Physiology and Biochemistry*, 20: 43-51.

Sahu, S., Das B. K., Pradhan ,J., Mohapatra ,B.C., Mishra ,B.K. and Sarangi N. (2007). Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings, Fish and Shellfish Immunology, 23:109-118.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172: 63-92.

Sakr, S. and Gabr, (1991). Ultra structural changes induced by diazinon and neopybuthrin in skeletal muscles of Tilapia nilotica. Proceed. *Zoology Soceity*, A.R.E. 21: 1-14.

Salah, W., Miller, N.J., Pagauga, G., Tijibung, B.A.P., Rice, E. and Evans, C. (1995). Prlyphenolic flavonis as scavenger of aqueous phase radicals as chain breaking oxidant. *Archive of Biochemistry and Biology*, 2: 339-346.

Saliu, J.K. and Bawa-Allah, K.A. (2012). Toxicological effects of lead and zinc on the antioxidant enzyme activities of post juvenile *Clarias gariepinus*. *Resource and Environment*, 2: 21-26.

Sallie, R., Tredger, J.M. and William, R. (1991). Drugs and the liver. *Biopharmaceutical Drug Disposit*, 12: 251-259.

Sambasiva Rao, K.R.S. (1999). Pesticide impact on fish metabolism.Discovery Publishimg House, New delhi, India, Pp. 66-70.

Sánchez, R., Margalida, A.,González, L.M. and Oria, J. (2008). Biases in diet sampling methods in the Spanish Imperial Eagle Aquila adalberti. *Ornis Fennica*, 85: 82-89. 2008.

Santos, M.A., Pacheco, M. and Ahmad, I. (2004). *Anguilla anguilla* L. antioxidants responses to in situ bleached kraft pulp mill effluent outlet exposure. *Environment International*, 30: 301-308.

Saravanan, M., Vasantha Kumar, D., Malarvizhi, A. and Ramesh, M. (2010). Biosafety of Azardirachta indica (A. Juss) leaves extracts on certain biochemical parameters in Labeo rohita. *Journal of Biopesticides*,3: 227-231.

Sastry, K.V. and Gupta, P.K. (1978). Effect of mercuric chloride on the digestive system of a teleost fish, Channa punctatus. *Bulletin of Environmental Contamination and Toxicology*, 20(3): 353-60.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, **S.** (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus Bloch*. *Ecotoxicology and Environment Safety*, 56: 295-301.

Schmidt, H., Bernet, D., Wahli, T., Meier, W. and Burkhardt-Holm, P. (1999). Active biomonitoring with brown trout and rainbow trout in diluted sewage plant effluents. *Journal of Fish Biology*, 54(3): 585-596.

Schwaigner, J., Ferling, H., Mallow, U., Wintermayr, H., Negele, R.D. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofane. Part I. Histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology*, 68: 141-150.

Sellappan, S., Akoh, C.C. and Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. Journal of Agricultural and *Food Chemistry*, 50: 2432-2438.

Senturk, H., Ozdesa, D., Gundogdu, A., Duran, C. anf Soylak, M. (2009). Removal of phenol from aqueous solutions by adsorption onto organomodified Tirebolu bentonite: equilibrium, kinetic and thermodynamic study. *J. Hazard. Mater.*, 172: 353-362.

Sevigler, Y., Oruc, O.E. and Uner, N. (2004). Evaluation of etoxazole toxicity in the liver of Oreochromis niloticus. *Pesticide Biochemistry and Physiology*, 78: 1-8.

Seyfried, E.E., Newton, R.J., Rubert, K.F., Pedersen, J.A. and McMahon, K.D. (2010). Occurrence of tetracycline resistance genes in aquaculture. Facilities with varying use of oxytetracycline. *Microbiology and Ecology*, 59: 799-807.

Shah, S.L. and Altindag, A. (2005). Alterations in the immunological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal treatments

with mercury, cadmium and lead. *Turkey Journal of veterinary and animal science,* 29: 1163-1168.

Shahi, J. and Singh, A. (2011).Effect of bioactive compounds extracted from euphorbious plants on haematological and biochemical parameters of *Channa punctatus*. *Institute of Medicine in the tropics, Sao Paulo,* 53(5): 259-263.

Shajiselvin, C.D., Kottai Muthu, A. and Suresh, K. (2010). Evaluation of in vivo antioxidant and lipid peroxidation effect of various extracts of the whole plant of *Borreria hispida* (Linn) on rat fed with high fat diet. *International Journal of Pharmaceutical Sciences Review and Research,* 3(1): 66-69.

Shalaby, A.M., Khattab, Y.A. and Abdel Rahman, A.M. (2006). Effects of garlic (Allium sativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *Journal of Venomous Animals Toxins,* 12: 172-201.

Shalaby, A.M.E. (2007). Effect of EDTA on reduction of cadmium toxicity on growth, some haematological and biochemical profiles of Nile tilapia (*Oreochromis niloticus*). *Journal of fisheries and aquatic science,* 2: 100-109.

Shalloof, K.A.S. and Salama, H.M.M. (2008). Investigations on Some Aspects of Reproductive Biology in *Oreochromis niloticus* (Linnaeus, 1757) Inhabited Abu-zabal Lake, Egypt. *Global Veterinaria,* 2(6): 351-359.

Sharma, S., Barrie, L.A., Plummer, D., McConnell, J.C., Brickell, P.C., Levasseur, M., Gosselin, M. and Bates, T.S. (1999). Flux estimation of oceanic dimethyl sulfide around North America.*Journal of Geophysical Research*, 104(D17).

Sherman, K.E. (1999). Aminotransferase in clinical practice. *Archives of Internal Medicine,* 151: 260-265.

Siddiqui, A.Q. and Al-harbi, A.H. (1995). Evaluation of three species of tilapia, red tilapia and a hybrid tilapia as culture species in Saudi Arabia.*Aquaculture*, 138: 145-157.

Sies, H. and Stahl, W. (1995). Vitamins E and C, β- carotene, and other carotenoids as antioxidants. *American Journal of Clinical Nutrition, Bethesda,* 62(6): 1315-1321.

Silva, R.H.M., Lima, Nd.F.M., Lopes, A.J.O., Vasconcelos, C., Mesquita, J.W.Cd., Msquita, L.S.Sd., Lima, F.C.V.M., Ribeiro, M.Nd.S., Ramos, R.M., Cartágenes, Md.Sd.S. and Garcia, J.B.S. (2017). Antinociceptive Activity of *Borreria verticillata*: In vivo and In silico Studies. *Frontiers Pharmacology,* 8: 283.

Singh, A.P., Singh, S., Bhartiya, P. and Yadav, K. (2010). Toxic effect of phorate on the serum biochemical parameters of Snake headed fish *Channa punctatus* (Bloch). *Advances in Bioresearch*, 1(1): 177-181.

Singh, D. and Singh, A. (2002). Biochemical alterations in freshwater fish *Channa punctatus* due to lattices of *Euphorbia royleana* and *Jatropha gossypifolia*. *Environmental Toxicology and Pharmacology,* 12: 129-136.

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American journal of Enol Viticult,* 16: 144-153.

Sofowora, A. (2008). Medicinal Plants and Traditional Medicines in Africa . *Third edition Spectrum Books Limited,* 197(204): 306-310.

Sogbesan, O. (2006).Effects of different organic substrates on growth and survival of long winged termite (*Macrotermes subhyabrius*) under laboratory conditions. *African Journal of General Agriculture,* 2(2): 3744.

Sogbesan, O.A. and Ugwumba, A.A.A. (2008). Nutrition evaluation of termite (Macrotermes subhyalinus). Meal as animal protein supplement in the diets of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. *Turkish journal of fisheries and aquatic sciences,* 8: 149-157.

Solomon, R.J. And Taruwa. S.M. (2011). The growth comparison of two catfishes (*C. gariepinus* and *Heteroclarias*).*Nature and Science,* 9(8): 138-148.

Soufy, H., Soliman, M., El-Manakhy, E. and Gaafa, A. (2007). Some biochemical and pathological investigations on monosex tilapia following chronic exposure to carbofuran pesticides.*Global Veterinaria,* 1: 45-52.

Stara, A., Machova, J. and Velisek, J. (2012). Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp**,** *Cyprinus carpio L. Environmental Toxicology and Pharmacology,* 33(2): 334-343.

Stockham, S.L. and Scott, M.A. (2002). *Fundamentals of veterinary clinical pathology.* Iowa, USA: Blackwell Publishing Company. Pp. 908.

Storey, K.B. (1996). Oxidative stress: animal adaptation in nature. *Brazilian Journal of Medical and Biological Research,* 29: 1715-1733.

Suchismita, D. and Abhik, G. (2013). A Study on Acute Toxicity, Behaviour and Growth in Indian Flying Barb, Esomus Danricus (Hamilton-Buchanan) on Exposure to Organochlorine Pesticide, Endosulfan (Ec 35). *International Journal Of Environmental Sciences,* 3(6): 2218-2223.

Suvetha, L., Ramesh, M. and Saravanan, M. (2010). Influence of cypermethrin toxicity on ionic regulation and gill Na^+/K^+-ATPase activity of a freshwater teleost fish *Cyprinus carpio.Environmental Toxicology and Pharmacology,* 29: 44-49.

Svecevieus, G. (2001). Avoidance response of rainbow trout, Oncorhyncus mykiss to heavy metal model mixtures. A comparision with acute toxicity tests. *Bull Environ Contam Toxicol,* 67: 680-687.

Svoboda, M. (2001). Stress in fishes (a review*), Bulletin VÚRH Vodnany,* 4: 169-191.

Svoboda, M., Luskova, V. and Drastichova, Z.U. (2001). The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L). *Acta veterinary Brno,* 70: 457-465.

Tacon, A.G.J. (2001). Increasing the contribution of aquaculture for food security and poverty alleviation.Tagboto, S. and Townson, S. (2001). Antiparasitic properties of medicinal plants and other naturally occurring products.*Advances in Parasitology*, 50: 199-295

Takashima, F. and Hibya, T. (1995). An atlas of fish histology normal and pathological features, 2nd ed. Tokyo, Kodansha Limited. Pp.147.

Talas, Z.S. and Gulhan, M.F. (2009). Effects of various propolis concentrationson biochemical and haematological parameters of rainbow trout (*Oncorhynchus mykiss).Ecotoxicology and Environmental Safety*, 72: 1994-1998.

Talpur, A.D. and Ikhwanuddin, M. (2013). Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish and Shellfish Immunology,* 34: 254-264.

Tasneem, M., Siddique, F. and Ahmad, A.(2014). Stabilizers: Indispensable substances in dairy products of high rheology.*Critical Review in Food science and Nutrition,* 54: 869-879.

Temmink, J.H.M., Field, J.A., Van Haastrecht, J.C. and Merkelbach, R.C.M. (1989). Acute and subacute toxicity of bark tannings in carp (*Cyprinus carpio*).*Water Research*, 23(3): 341-344.

Tennyson, A.G., Dhar, S. and Lippard, S.J. (2008). Synthesis and characterization of {Ni(NO)}10 and {Co(NO)2} 10 complexes supported by thiolate ligands. *Journal of America Chemistry Society,* 130: 15087-15098.

Teugels, G.G. (1986). Systemic revision of African species of the Genus Claria (Pisces: *Claridae*).Annals musee Royal de l' Afrique Central, *Sciences Zoologiques*, 247: 1-199.

Thadani, S.T. and Salunke, S.P. (2014). Effect of Carica papaya seed powder on liver and muscle of commonly cultured catfish *Clarias batrachus*. *CIBTech Journal of Zoology,* 3: 86-92.

The, S.J., Adams, S.M. and Hinton, D.E. (1997). Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquatic Toxicology*, 37: 51-70.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S. and Jarikhuan, S. (2003). Histopathological alterations of white sea bass, *Lates calcarifer* in acute and sub-chronic cadmium exposure. *Evironmental Pollution,* 121: 307-321.

Tijjani, A., Ndukwe, I.G. and Ayo, R.G. (2011b). Studies on antibacterial activity of *Adenium obesum* (Apocynaceae) stem-bark. *Continental Journal of Microbiology,* 5(1): 12-17.

Tijjani, A., Ndukwe, I.G. and Ayo, R.G. (2012). Isolation and characterization of Lup-20(29)-ene-3,28-diol (Betulin) from the stem bark of *Adenium obesum* (Apocynaceae). *Tropical Journal of Pharmaceutical Research,*11(2): 259-262.

Tijjani, A., Sallau, M.S. and Sunusi, I. (2011a). Synergistic activivty of methanol extract of Adenium obesum (Apocynaceae) stem bark and oxytetracycline against some clinical bacterial isolates. *Bayero Journal of Pure and Applied Sciences,* 4(1): 79-82.

Tilak, K.S., Satyavardhan, K. and Thathaji, P.B. (2003). Biochemical changes induced by fenvalerate in the freshwater fish *Channa punctata*. *Journal of Ecotoxicology Environment Monitoring*, 13(4): 261-270.

Tilak, K.S. and Yaeobu, K. (2002). Toxicity and effect of fenvalerate on fish, *Ctenopharyngodon idellus*. *Journal of Ecotoxicology and Envrionmental Monitoring,* 12(1): 09-15.

Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, A.K., Nautiyal C.S., Mittal, S., Tripathi, A.K. and Tohri, B.N. (2005). Diversity of plant growth and soil health supporting bacteria.*Current Science*, 89: 136-150.

Tiwari, S. and Singh, A. (2004). Piscicidal activity of alcoholic extracts of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. *African Journal of Traditional Complementary and Alternative Medicine* (CAM), 1(1): 15-29.

Trease, G.E. and Evans, W.C. (1983). Pharmacology, 11th Edition.Braillier Tiridel and Macmillan Publishers.

Triebskorn, R., Casper, H., Scheil, V. and Schwaiger, J. (2007). Ultrastructural effects of pharmaceuticals (carbamazepine, clofibric acid, metoprolol, diclofenac) in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). *Annal of Bioanal Chem,* 387: 1405-1416.

Turina, A.V., Nolan, M.V., Zygadlo, J.A. and Perillo, M.A. (2006). Natural terpenes: self-assembly and membrane partitioning.*Biophysical Chemistry*, 122(2): 101-113.

Ultee, A., Bennik, M. H.J. and Moezelaar, R. (2002). The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen Bacillus cereus. *Society* , 68(4): 1561-1568.

Umeda, N., Nibe, H., Hara, T. and Hirazawa, N. (2006). Effects of various treatments on hatching of eggs and viability of oncomiracidia of the monogenean *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini*. *Aquaculture*, 253: 148-153.

Uner, N., Oruç, E.O., Canli, M. and Sevgiler, Y. (2001). Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). *Bulletin of Environmental Contamination and Toxicology*, 67: 657-664.

Uys, W., Hecht, T. and Walter, M. (1987). Changes in digestive enzyme activities of *Clarias gariepinus* (Pisces: Clariidae) after feeding. *Aquaculture*, Pp. 243-250.

van der Oost, R., Beyer, J. and Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmenta Toxicology and Pharmacology*, 13: 57-149.

Van der Putte, I. and Pärt, P. (1982). Oxygen and chromium transfer in perfused gills of rainbow trout (*Salmo gairdneri*) exposed to hexavalent chromium at two different pH levels. *Aquatic Toxicology*, 2: 31-45.

Van Heerden, D., Vosloo, A. and Nikinmaa, M. (2004). Effects of short term copper exposure on gill structure, methallothionein and hypoxia-inducible factor-1a´ (HIF-1a) levels in rainbow trout (*Oncorhyncus mykiss*). *Aquatic Toxicology*, 69: 271-280.

Van Vuren, J.H.J., Van Fer Merwe, M. and Du Preez, H.H. (1994). The effect of copper on the blood chemistry of *Clarias gariepinus* (Clariidae). *Ecotoxicology and Environment Safety*, 29: 187-199.

Varshney R. and Kale R.K. (1990). Effects of Calmodulin Antagonists on Radiation-induced Lipid Peroxidation in Microsomes. *International Journal of Radiation Biology*, 58: 733-743.

Vaseeharan, B. and Thaya, R. (2013). *Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture*. Retrieved from *Aquaclture International*.

Veiga, M.M., Silva, A.R.B. and Hinton, J.J. (2002). Technical, Social and Environmental Aspects of Artisanal Gold Mining in the Amazon Region. Chapter 11, p.277-306.

In: Extração de Ouro - Princípios, Tecnologia e Meio Ambiente. R.B.E.Trindade & O.Barbosa Filho. Published by CETEM/CNPq, Rio de Janeiro, Brazil ISBN 85:7227-1503 (in Portuguese).

Velíšek L, Shang E, Velíšková J, Chachua T, Macchiarulo S, Maglakelidze G, Wolgemuth DJ, Greenberg DA.(2011).GABAergic neuron deficit as an idiopathic generalized epilepsy mechanism: the role of BRD2 haploinsufficiency in juvenile myoclonic epilepsy. *PLuS One*. 6(2): 36-56.

Velkova-Jordanoska, L. (2002). Histopatological and molecular-diagnostical study of the hepatic capillarity in black barbel (Barbus meridionalis petenyi Heck.) in Lake Ohrid. MSc Thesis, Univ. St.Kiril and Methodius, Skopje, R. Macedonia (Mc).

Venkatesan, S., Pugazhendy, K., Meenambal, M., Sangeetha, D., Vasantharaja, C., Jayachandren, K. and Prabakaran, S. (2012). Protective Role of Spirulina on the Variation of Haematological Parameter Induced by Herbicide Atrazine in the Fresh water Fish *Cyprinus carpio* (Linn). *International Journal of Pharmaceutical & Biological Archives,* 3(1): 249-254.

Vicentini, N.M., Dupuy, N., Leitzelman, M., Cereda, M.P. and Sobral, P.J.A. (2005). Prediction of cassava starch edible film properties by chemometric analysis of infrared spectra. *Spectroscopy Letters,* 38(6): 749-767.

Vieira, I.J., Mathias, L., Braz-Filho, R. and Schripsema, J. (1999). Iridoids from *Borreria verticillata. Org Lett,* 1: 1169-71.

Viveen, W.J., Richter, C.J., Van Oodot, P.G., Janssen, J.A. and Huisman, E.A. (1985). Practical Manual for the Culture of the African Catfish, Clarias gariepinus. Section for Research and Technology, Ministry for Development Co-operation. The Hague, Natherlands, Pp. 128.

Wagh, V.D., Wagh, K.V., Tandale, Y.N. and Salve, S.A. (2009). Phytochemical, pharmacological and phytopharmaceutics aspects of Sesbania grandiflora (Hadga): A review.*Journal of Pharmacy Research,* (5): 889-892.

Wagner, T and Congleton, J.L (2004). Blood chemistry correlates of nutritional condition, tissue damage and stress in migrating juvenile Chinook Salmon (*Onchorynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences,*61: 1066-1074.

Wang, N. and Daun, J. K. (2006). Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (*Lens culinaris*). *Food Chemistry,* 95 (3): 493-502.

Wedemeyer, G.A. and McLeay, D.J. (1981). Methods for determining the tolerance of fishes to environmental stressors.In A. D. Pickering (ed.).Stress and fish. *Academic Press,* New York. Pp. 247-275.

Wedmeyer, C.A. and Yasutake, W.K. (1977). Clinical methods for the assessment of the effect of environmental stress on fish health. *United States Technical States Papers and United States Fish Wildlife Services,* 89: 1-8.

Walencik, E.F and Witeska, K.P (2007). Studies on the haematology of certain freshwater teleosts. *Anatomy Anzeiger,* 123: 520-533.

Welling, S.R. and McCain, B.B. (1976). In: Progress in environmental tumor research. Hamburger F. Ed. Karger. Basel, 20: 55-74.

Wells, D., Beck, N., Delikaraoglou, D. and Vanicek, P. (1986). Guide to GPS positioning. *Canadian GPS Associates, and University of New Brunswick.*

West, J.D. and Marnett, L.J. (2006). Endogenous reactive intermediates as modulators of cell signaling and cell death. *Chemical Research in Toxicology,* 19(2): 173-194.

Wester P.W., Canton J.H. (1991). Comparative Biochemistry and Physiology (C), 100, 115-117.

Wilhelm Filho, D., Torres, M.A., Marcon, J.L., Fraga, C.G. and Boveris, A. (2000). Comparative antioxidant defenses in vertebrates-emphasis on fish and mammals. *Trends Comparative Biochemistry and Physiology,* 7: 33-45.

Winzer, K., Becker, W., Van Noorden, C.J.F. and Kohler, A. (2000). Shorttime induction of oxidative stress in hepatocytes of the European flounder (*Platichthys flesus*). *Marine Environmental Research,* 50: 495-501.

Woo, P.T.K. and Bruno, D.W. (1998): Fish diseases and disorders: viral, bacterial and fungal infections, CABI 3, Pp. 479–511.

World Health Organization (2007). Guidelines for safe recreational water environments Volume 1: *Coastal and Fresh Waters*, Geneva, 1-33.

Wroblewski, B.M. (1985). 15-21-year results of the Charnley lowfriction arthroplasty. *Clin Orthop.*, 211: 30-5.

Wu, C.C., Liu, C.H., Chang, Y.P. and Hsieh, S.L. (2010). Effects of hot-water extract of Toonsinensis on immune response and resistance to Aeromonas hydrophila in Oreochromisa mossambicus. *Fish Shellfish Immunology,* 29: 258-263.

Yamauchi, T. and Abe, E. (1990). Cardiac glycosides and pregnanes from *Adenium obesum* (Studies on the constituents of *Adenium obesum*). *Chemistry and Pharmacology Bulletin,* 64(2): 669-672.

Yamawaki, K., Hashimoto, W., Fujii, K., Koyama, J., Ikeda, Y. and Ozaki, H. (1986). Hemochemical changes in carp (*Cyprinus carpio*) exposed to low cadmium concentrations.*Nippon Suisan Gakkaishi Bulletin,* 52: 459-466.

Yang, P., Liou, K.N., Mishchenko, M.I. and Gao, B.C. (2000). Efficient finite-difference time-domain scheme for light scattering by dielectric particles: Application to aerosols. *Applied Optics*, 39: 3727-3737.

Yaro, I. (1967). Common names of local fish in English,Hausa,Nupe,Ijaw and Kanuri. In W. Reed, *Fish and Fisheries of Northern Nigeria*. Zaria, Nigeria: Gaskiya corporation. Pp. 203-212. Yonar,M.E./

Yekeen, T.A. and Fawole, O.O. (2011). Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus. African Journal of Biotechnology*, 10(64): 14090-14096.

Yildirim, Y.B. and Turan, F. (2010). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, Clarias gariepinus. *J. Anim. Vet. Adv.*, 9: 327-331.

Yonar, S.M., Pala, A. and Saglam, N. (2015). Trichlorfon-induced haematological and biochemical changes in Cyprinus carpio: Ameliorative effect of propolis. *Diseases of Aquatic Organisms*, 114(3)

Yuan, C., Li, D., Chen, W., Sun, F., Wu, G., Gong, Y., Tang, J., Shen, M. and Han, X. (2007). Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry*, 33: 93-101.

Zaid, H., Talior-Volodarsky, I., Antonescu, C., Liu, Z. and Klip, A. (2009). GAPDH binds GLUT4 reciprocally to hexokinase-II and regulates glucose transport activity. *Biochemistry Journal*, 419: 475-484.

Zheng, K., Padman, R. and Johnson, M.P. (2009). An interface-driven analysis of user interactions with an electronic health records system. *Journal of American Medical Information Association*, 16(2): 228-237.

Zhang, S., Bi, H. and Liu, C. (2007). Extraction of bio-active components from *Rhodiola sachalinensis* under ultra high hydrostatitic pressure separation and purification Technology, 57: 275-280.

Zheng, Z.W., Ho, C.R. and Kuo, N.J. (2007). Mechanism of weakening of west Luzon eddy during La Nin˜a years.*Geophysical Research Letters*, 34: 11604.

Zielinski, S. and Portner, H. (2000). Oxidative stress and antioxidative defense in cephalopods: a function of metabolic rate or age?, *Comparative biochemistry and physiology*, Pp.147-160.

APPENDICES

APPENDIX I



Plate XXIX: *Adenium obesum* plant



Plate XXX: Borreria verticillata plant



Plate XXXI: Clarias gariepinus (Burchell, 1822)



Plate XXXII: Concrete Tank for Acclimatization of Fish



Plate XXXIII: Acute Toxicity Bioassay AOE



Plate XXXIV: Glass Aquirium Tank Sub Lethal Treatment Set-Up before Introductionof Extracts and Stocking



Plate XXXV: Fish in sublethal treatment tanks after stocking before the introduction of extracts



Plate XXXVI: Aspirating blood from the caudal vein lateral approach using a wet towel to calm the fish

APPENDICES

APPENDIX I

Table 4.22: The pH profile acute bioassay aqueous extract *Adenium Obesum* stem bark 96-hr exposure

	pH 96-HOUR EXPOSURE			
GROUP	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL	7.30±0.00	7.30±0.00	7.30±0.00	7.30±0.00
AOE1	7.37±0.01	7.38±0.00	7.36±0.02	7.35±0.01
AOE2	7.46±0.01	7.46±0.03	7.47±0.03	7.48±0.03
AOE3	7.57±0.03	7.58±0.02	7.56±0.01	7.57±0.01
AOE4	7.70±0.02	7.71±0.01	7.74±.06	7.70±.06
AOE5	7.74±0.33	7.75±0.33	7.76±0.33	7.77±0.33

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE (7.8mg/l), AOE (8.5mg/l), AOE (9.5mg/l), AOE (11.5mg/l)

APPENDIX II

Table 4.23: Dissolved oxygen profile Acute Bioassay Aqueous Extract *Adenium Obesum* Stem Bark 96-hr exposure

	DISSOLVED OXYGEN			
GROUP	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03
AOE1	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03
AOE2	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03
AOE3	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03
AOE4	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03
AOE5	4.87±0.03	4.87±0.03	4.87±0.03	4.87±0.03

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX III

Table 4.24: Total dissolved solid profile bioassay aqueous extract *Adenium obesum* stem bark 96-hr exposure

	TOTAL DISSOLVED SOLID			
GROUP	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL	378.00±0.01	378.33±0.88	378.67±0.67	379.00±0.58
AOE1	379.00±0.58	394.00±0.58	397.00±0.58	401.00±0.58
AOE2	401.00±0.578	415.00±2.51	426.67±0.33	438.33±0.33
AOE3	438.33±0.58	441.00±0.58	444.33±0.33	448.67±0.33
AOE4	448.67±0.33	454.00±0.58	466.33±0.88	468.67±0.33
AOE5	468.67±0.33	473.33±0.33	477.00±0.58	483.00±0.58

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX IV

Table 4.25:Electrical conductivity profile acute bioassay aqueous extract *Adenium Obesum* stem bark 96-hr exposure

	ELECTRICAL CONDUCTIVITY(μS/cm)			
GROUP	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL	477.33±0.33	478.33±0.33	478.67±0.33	479±0.33
AOE1	479±0.58	481±0.58	482.67±0.33	485.67±0.33
AOE2	485.67±0.33	486.67±0.33	487.33±0.33	488.67±0.33
AOE3	488.67±0.33	490.67±0.33	491.33±0.67	493.67±0.33
AOE4	493.67±0.33	495.67±0.33	497.33±0.33	497.67±0.33
AOE5	497.67±0.33	498.67±0.33	499.33±0.33	501±0.33

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX V

Table 4.26: Temperature profile acute bioassay aqueous extract *Adenium Obesum* stem bark 96-hr exposure

	Temperature (˚C)			
GROUP	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL	27.60±.00	27.27±.33	27.60±.01	27.61±.01
AOE1	27.60±.00	27.27±.33	27.60±.01	27.61±.01
AOE2	27.60±.00	27.27±.33	27.60±.01	27.61±.01
AOE3	27.60±.00	27.27±.33	27.60±.01	27.61±.01
AOE4	27.60±.00	27.27±.33	27.60±.01	27.61±.01
AOE5	27.60±.00	27.27±.33	27.60±.01	27.61±.01

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX VI

Table 4.27: Probit analysis aqueous extract of *Adenium Obesum* stem bark

Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log (Concentration)[a]		
	Estimate	Decrease Bound	Upper Bound	Estimate	Decrease Bound	Upper Bound
LC1	5.355	1.183	7.035	.729	.073	.847
LC2	5.644	1.411	7.262	.752	.149	.861
LC3	5.836	1.577	7.411	.766	.198	.870
LC4	5.984	1.714	7.526	.777	.234	.877
LC5	6.108	1.835	7.620	.786	.264	.882
LC6	6.215	1.945	7.702	.793	.289	.887
LC7	6.310	2.046	7.775	.800	.311	.891
LC8	6.397	2.141	7.841	.806	.331	.894
LC9	6.477	2.231	7.901	.811	.349	.898
LC10	6.551	2.317	7.957	.816	.365	.901
LC15	6.868	2.711	8.196	.837	.433	.914
LC20	7.131	3.071	8.394	.853	.487	.924
LC25	7.365	3.415	8.371	.867	.533	.933
LC30	7.581	3.757	8.735	.880	.575	.941
LC35	7.788	4.102	8.893	.891	.613	.949
LC40	7.988	4.457	9.050	.902	.649	.957
LC45	8.188	4.827	9.209	.913	.684	.964
LC50	8.388	5.218	9.374	.924	.718	.972
LC55	8.394	5.636	9.50	.934	.751	.980
LC60	8.809	6.088	9.743	.945	.784	.989
LC65	**9.036**	**6.581**	**9.966**	**.956**	**.818**	**.999**

APPENDIX VII

Table 4.28: Clinical sign exhibiteb in acute bioassay of *Adenium Obesum* stem bark (24Hr-48Hr)

	Extract	Exposure period	
Clinical signs	(mg/l)	24-h	48-h
Repeated attempts to jump out of water	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	++
Erratic movements	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	++
Aggression	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	++
Excessive mucous secretion	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	+++
Frequent opercula movements	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	++
Air gulping	CONTROL	-	-
	AOE1	+	+
	AOE2	+	+
	AOE3	+	+
	AOE4	+	+
	AOE5	++	++

AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX VIII

Table 4.29: Clinical signs exhibited in the contact phase (72-hr to 96-hr) of the toxicity of aqueous extract of *Adenium Obes*um stem bark

	Extract	Exposure period			
Clinical signs	(mg/l)	24-h	48-h	72-h	96-hr
Exposed snout	CONTROL	-	-	-	-
	AOE1	-	+	±	+
	AOE2	-	+	±	+
	AOE3	-	+	±	+
	AOE4	-	+	-	+
	AOE5	-	+	-	+
Motionless	CONTROL	-	-	-	-
	AOE1	-	+	+	+
	AOE2	-	+	+	+
	AOE3	-	+	+	+++
	AOE4	-	+	+	+++
	AOE5	-	+	++	+++
Different postures (vertical,angualr,flat)	CONTROL	-	-	-	-
	AOE1	-	+	+	+
	AOE2	-	+	+	++
	AOE3	-	+	++	+++
	AOE4	-	+	++	+++
	AOE5	-	++	++	+++
Sudden dart	CONTROL	-	-	-	-
	AOE1	-	+	+	+
	AOE2	-	+	+	++
	AOE3	-	+	++	+++
	AOE4	-	+	++	+++
	AOE5	-	+++	++	+++
Swirling and/or sluggish movements	CONTROL	-	-	-	-
	AOE1	-	+	+	+
	AOE2	-	+	+	++
	AOE3	-	+	++	+++
	AOE4	-	+	++	+++
	AOE5	-	++	++	+++
Loss of balance	CONTROL	-	-	-	-
	AOE1	+	+	+	+
	AOE2	+	+	+	++
	AOE3	+	+	++	+++
	AOE4	+	+	++	+++
	AOE5	++	++	++	+++

Data are presented as means ± SEM. KEYS: AOE1 (6.5mgl), AOE2 (7.8mg/l), AOE3 (8.5mg/l), AOE4 (9.5mg/l), AOE5 (11.5mg/l)

APPENDIX IX

Table 4.30: Acute bioassay of aqeuous extract of adenium obesum stem bark mortality profile 96-hour

Concentration (mg/l)	Number of survivors at 96 hours			TOTAL FISH	ALIVE	Mortality	Mortality (%)
	REPLICATE 1	REPLICATE 2	REPLICATE 3				
GRP1	10	10	10	30	30	0	0
GRP2	10	10	10	30	20	10	33.33
GRP3	10	10	10	30	16	14	46.67
GRP4	10	10	10	30	8	22	73.33
GRP5	10	10	10	30	7	23	76.67
GRP6	10	10	10	30	1	29	96.67