

**EVALUATION OF THE ANTIOXIDANT, ANTI-INFLAMMATORY,
HEPATOPROTECTIVE AND CYTOTOXIC PROPERTIES OF
MORINDA LUCIDA Benth**

By

ADEJO, GODWIN OJOCHOGU

**DEPARTMENT OF BIOCHEMISTRY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

NOVEMBER, 2012

**EVALUATION OF THE ANTIOXIDANT, ANTI-INFLAMMATORY,
HEPATOPROTECTIVE AND CYTOTOXIC PROPERTIES OF
MORINDA LUCIDA Benth**

By

ADEJO, GODWIN OJOCHOGU

(B.Sc Biochemistry, ABU 1999; M.Sc Biochemistry, UNIBADAN 2003)

[Ph.D/SCIE/46080/2004-05]

**A RESEARCH DISSERTATION SUBMITTED TO
THE POST GRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY ZARIA,
NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIRMENT FOR THE
AWARD OF DOCTORATE DEGREE IN BIOCHEMISTRY
DEPARTMENT OF BIOCHEMISTRY, AHMADU BELLO
UNIVERSITY ZARIA**

NOVEMBER, 2012

DECLARATION

I hereby declare that the work in this dissertation titled: “**Evaluation of the Antioxidant, Anti-inflammatory, Hepatoprotective and Cytotoxic Properties of *Morinda lucida* Benth**” was performed by me in the Department of Biochemistry, Ahmadu Bello University, under the supervision of Professor S. E. Atawodi, Professor D. A. Ameh and Dr. S. Ibrahim. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Adejo, Godwin Ojochogu

.....

.....

[Ph.D/SCIE/46080/2004-05]

(Signature)

(Date)

CERTIFICATION

This dissertation titled: “**Evaluation of the Antioxidant, Anti-inflammatory, Hepatoprotective and Cytotoxic Properties of *Morinda lucida* Benth**” meets the regulations governing the award of Doctorate Degree in Biochemistry (Ph.D) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

.....		
Chairman, Supervisory Committee	(Signature)	(Date)
Professor S. E Atawodi		

.....		
Member, Supervisory Committee	(Signature)	(Date)
Professor D. A. Ameh		

.....		
Member, Supervisory Committee	(Signature)	(Date)
Dr. S. Ibrahim		

.....		
Head of Department	(Signature)	(Date)
Dr. H. M. Inuwa		

.....		
Dean, School of Postgraduate Studies	(Signature)	(Date)
Professor Adebayo Abiodun Joshua		

DEDICATION

I dedicate this work to the two most cardinal personalities in my life:

God-Almighty - my maker and my all-in-all.

Daniel Adu'Ojo Edeh – the director of Ministry of Mercy Orphanages,
and the only one who really understand me and have exemplified himself
what a true father should be.

ACKNOWLEDGEMENTS

Most important thanks to God the Almighty for charting my course through this phase of life that I would never wish to pass through again, lest there is anything like reincarnation.

My unquantifiable regards go first to Professor S. E. Atawodi, Professor D. A. Ameh and Dr. S. Ibrahim for their supervision, high level understanding, advice and forthrightness in making sure that this work was not just completed, but properly executed.

I also appreciate the Head of Department of Biochemistry for her understanding and support towards the completion of this work. I wish to thank all the lecturers and staff of the Department of Biochemistry, A.B.U. Zaria for their advice, technical assistance, and provision of needed research materials, particularly Mr. Yakubu Apeh, Mr. Stephen Jok, and Mr. John Shaffa. Thanks to Mr. Uche Ndidi and Dr. Emmanuel Balogun for helping me greatly with the statistics.

Plenty of appreciation goes to the Vice Chancellor (VC) of Bingham University, Professor Felix Anjorin for his great encouragement. Also to Professor Olusola Ladeji, the Head of Biochemistry Department, Bingham University for all his advice, help, and the permission to use the departmental laboratory for my research while ensuring that basic research materials are available for my use. Same goes to Professor S. O. Adeyemo, the Dean of Science and Technology, Bingham University.

Many thanks to Professor F. S. Idachaba (Fmr. VC of Kogi State University, Anyigba), Professor E. M. Abdulrahman (VC of Kaduna State University, Kaduna), Professor Sonnie Oniye (ABU Zaria), Associate Professor (Mrs.) Anthonia Eniaijeju (Late), Professor L. Lajide (FUTA), Dr. M.O. Aremu (Chemistry, NSUK), Mr. Isaac Enemaku (Bursary, Bingham University) and Dr. Peter Ogedebe (Head, Computer Science Department, Bingham University) for their advice and encouragement towards the completion of this programme.

I must thank Professor H.O.A. Oluma (Dean of Science, Uni-Agric Makurdi) for his useful advice, Professor Jonathan Okafor for sending me the first list of medicinal plants of my research interest, Dr. A.S. Agbaji (NARICT, Zaria) and Mr. P.J.I. Idachaba for their fatherly supports, concern and interventions.

Lots of thanks go to Mr. Bamidele Okoli, Mr. Johnmark G. Audam, Mr. Raymond Jonathan, Miss. Scholastica Ifeonu, Mrs. Beatrice Akawu, Miss Blessing Suleiman and the entire technical staff of Biochemistry and Chemistry Departments of Bingham University for all their logistic supports. Mr. Kingsley Okoyomoh must be thanked for being helpful in many ways.

Thanks to all the staff of 'Innovative Biotech', Keffi for being so involved and helpful with my molecular biology experiment, especially Dr. Simon Agwale, Mr. Akyala Ishaku, Mr. Zungwe Timothy, Miss Iperepolu Odundayo, Oyindo Marcellinus, Paulinus Obinna and Lilian Duruemeruo.

Many thanks also to Hajia Aisha Abubakar of NIPRD, Abuja for being resolute on using the state-of-the-arts equipment for my anti-oxidant experiments. Special thanks to Miss Samuel Joyce Yakubu for all my printings.

Thanks to Mr. Ajibola Meraiyebu for providing his powerful camera (JVC Super LoLux) during my ethnobotanical surveys/plant collection and histopathology photographs, to Mr. Paul Amade (Physics Department, Bingham University) for helping me with instrument for my anti-inflammatory experiment, and Mr. Friday Ojeamiren for providing me with space in the animal house and general care for my experimental animals.

Thanks to Mr. E. O. Umoru (NEPA, Abuja), Charity Ojone Omogohi (my sister), Mr. and Mrs. S. S. Adejo (my parents), whose financial support which they thought was so little and their prayers meant so much to me. My parents were particularly understanding despite my inability to help with their health needs during the many grueling years of my pursuit.

Thanks to Diamond Bank Plc, Keffi Branch and staff especially Mr. Joel Haruna and Miss Mariette Obodozie, to Bingham University Cooperative and Multipurpose Society especially Mr. Richard Kyarem and to Ministry of Mercy Orphanages for their supports.

Thanks to my wife – Unekwu Evelyn John and two daughters - Sharon and Faith for understanding and enduring with all the deprivations to which I had subjected them.

Thanks to Uju Iliemene for being very helpful with my experiments and to Mr. Christopher Akintayo, without whom this work would have still been 'many miles' behind.

Lots of appreciations go to Mr. Funsho Olowoniyi for standing by me through out the turbulent period of the pursuit of a Ph.D in Biochemistry.

Thanks to Iye-Kaka (Late) for all the prayers she laid up for me before she departed mother earth.

ABSTRACT

The anti-oxidant, anti-inflammatory, hepatoprotective and cytotoxic effects of different plant parts of *Morinda lucida* were studied. Methanolic extracts of the plant parts, viz: the whole fruit, leaf, stem bark, and root were prepared and the yields varied in the order: stem bark > fruit > leaf > root. LD₅₀ test in rats indicated non-toxicity of all the plant parts at doses of 5000mg extract/kg body weight. Anti-oxidation assays presented a higher activity of superoxide dismutase (SOD) among the group post-treated with bark extract. In the pre-treatment (hepatoprotective) experiment, there was a significantly higher SOD activity ($p<0.05$) in the leaf and bark groups than the 'CCl₄ only' group, indicating antioxidative effects. However, the vitamins (α -Tocopherol and ascorbic acid) groups showed significantly higher values ($p<0.05$) than the entire experimental groups. Glutathione peroxidase (GPx) activity was significantly higher in the bark and leaf extracts post-treatment group than the 'CCl₄ only' group ($p<0.05$). The extract pre-treatment group also showed the bark and leaf presenting significantly higher activity than the 'CCl₄ only' group. Catalase enzyme activity was significantly higher ($p<0.05$) in the leaf extract pre-treatment group. Obtained value was comparable to the normal control (73.1 ± 10.6 Umol/min/mg protein). Anti-inflammatory properties at doses of 100, 300 and 500mg extract/kg body weight exhibited inhibitory effect on carrageenan-induced rat paw oedema in a dose-dependent manner. Respectively, *Morinda lucida* leaf extracts showed percentage (%) inflammatory inhibition of 83.53 ± 10.50 , 91.67 ± 8.33 , and 100.00 ± 0.00 . Stem bark extract showed 58.33 ± 15.37 , 91.67 ± 8.33 and 91.67 ± 8.33 . Fruit extract showed 58.33 ± 15.37 , 75.00 ± 17.08 and 100.00 ± 0.00 ; and root extract showed 100.00 ± 0.00 at all the doses. In the serobiochemical assays, liver function tests showed lowest AST activity in the root extract among the extract post-treatment group while the fruit extract showed the lowest among the pre-treatment group ($p<0.05$). The ALT activity of all the different extracts demonstrated significant reductions ($p<0.05$) in the post-treatment (hepatocurative) group as compared to the positive control. A similar pattern of lowered ALT activity was also observed in the pre-treatment group, with the bark extract showing the lowest value. Total bilirubin concentration was significantly lower ($p<0.05$) in all the extracts post-treatment group, with values obtained for leaf and fruit being comparable to the normal control group. In the extract pre-treatment group, all the extracts showed significantly lower total bilirubin concentration than the 'CCl₄ only' group ($p<0.05$) and comparable to the values obtained for the vitamin groups. Lipid profile studies showed that cholesterol-lowering effect of the extracts was most effective in the post-treatment group administered with bark extract even though all the different extracts exhibited similar effects. But in the pre-treatment group, the leaf extract was most active. HDL concentration was significantly higher ($p<0.05$) among the extracts pre-treated groups except in the leaf extracts where there was no significant difference, compared with the 'CCl₄ only' group. Also, in the extract post-treatment group, all the groups showed significantly higher ($p<0.05$) HDL concentration than the 'CCl₄ only' group. The LDL concentration was only significantly lower among the bark extract post-treatment group, while in the extract pre-treatment group, the leaf and bark presented significantly lower ($p<0.05$) LDL concentrations, comparable to the normal group. Triglyceride concentrations were significantly lower in all the extract post-treatment groups when compared to 'CCl₄ only' group ($p<0.05$). A similar trend was also observed in the extracts pre-treatment groups, presenting significantly lower ($p>0.05$) triglyceride concentrations than the 'CCl₄ only' group. Kidney function assays viz: total creatinine and total urea concentration were also carried out. Although, all the parts showed a reduction in creatinine concentration ($p<0.05$) in both the extract pre-treated and post-treated groups, creatinine levels showed that the most significant nephroprotective effects ($p<0.05$) was exhibited by the leaf extract while the extracts of the other plant parts did not. Total urea

concentrations were lowest in the root extract pre-treated group. All the pre-treatment groups were however lower in total urea than the 'CCl₄ only' group. In the post-treatment groups all the extracts demonstrated lowered total urea concentration than the 'CCl₄ only' group. Total protein concentration was higher in all the post-treatment groups than 'CCl₄ only' group. In the extract pre-treatment group, only the leaf and root extracts group showed higher total protein concentration than the 'CCl₄ only' group. Cytotoxic properties using the brine shrimp assay which included the hatchability assay and lethality assay revealed that while that of root, leaf, and stem bark showed half-inhibitory concentration (IC₅₀) of 190mg/L, 210mg/L and 407mg/L respectively, the fruit extract showed IC₅₀ of 170mg/L and exerted total hatchability inhibition on the artemia cysts at 600mg/L concentration. The lethality assay revealed that the leaves possessed the highest cytotoxicity properties among all the plant parts, at 316.2mg/L followed by the root extract at 398.1mg/L while the least was the fruit extract at 631mg/L. Plasmid nicking assay showed the genotoxic properties of the extracts at lower concentration of 100µg/10µL. Only the bark extracts demonstrated genoprotective effect. The genotoxic properties of the extracts increased with increased concentration. Histological slides of liver and kidney tissues showed that all the fruit, leaf and root extracts possessed only slight to non-hepatoprotective or hepatocurative effects except the bark extract, and whose effects on hepatoprotection was most pronounced. These results suggest that different parts of *Morinda lucida* possess significant anti-oxidant, anti-inflammatory, hepatoprotective/ hepatocurative, cytotoxic and genotoxic properties (at lower concentrations however), to justify their various uses in folklore medicine.

TABLE OF CONTENTS

Cover page	i
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgements	vi
Abstract	ix
Table of Contents	xi
List of Figures	xxii
List of Tables	xxiii
List of Plates	xxvi

CHAPTER ONE

INTRODUCTION	1
1.1 Statement of the problem	2
1.2 Justification	4
1.3 Aim	4
1.4 Specific objectives	5

CHAPTER TWO

LITERATURE REVIEW	6
2.1 Botanical description of <i>Morinda lucida</i>	6
2.2.1 Description of physical features of <i>Morinda lucida</i>	6
2.2.2 Traditional medicinal uses of <i>Morinda lucida</i>	7
2.2.3 Properties of <i>Morinda lucida</i>	7
2.2.4 <i>Morinda lucida</i> in Ethnomedicine	11

2.2.5	Phytochemistry of <i>Morinda lucida</i>	13
2.2.5.1	Polyphenols and Flavonoids	13
2.2.5.2	Polyphenols and Flavonoids in plants	13
2.2.5.3	Beneficial effects of polyphenols	14
2.2.5.4	Flavonoids	15
2.3	Oxidative stress and anti-oxidation	16
2.3.1	Oxidative stress	16
2.3.2	Anti-oxidation	17
2.3.3	Natural sources of antioxidants	18
2.4	Inflammation and anti-inflammation	18
2.4.1	Inflammation	18
2.4.2	Types of inflammation.....	19
2.4.3	Acute Inflammation	19
2.4.4	Chronic Inflammation	20
2.4.5	Miscellaneous kinds of inflammation	21
2.4.6	Inflammogenic factors	21
2.4.6.1	Hydrolytic enzymes	21
2.4.6.2	Prostaglandins (PGS)	22
2.4.6.3	Complement System	23
2.4.6.4	Protein Breakdown Process	24
2.4.6.5	Calcium	25
2.4.6.6	Cyclic Nucleotides	25
2.4.7	Anti-inflammation	26
2.5	Hepatotoxicity and Hepatoprotection	27
2.5.1	Liver diseases and Medicinal Plants use	31
2.6	Cytotoxicity and cytotoxic compounds	38

2.6.1	Genotoxicity	48
-------	--------------------	----

CHAPTER THREE

3.0	MATERIALS AND METHODS	53
3.1	Materials	53
3.1.1	Materials used for anti-oxidation experiments.....	53
3.1.2	Materials used for anti-inflammation experiments	53
3.1.3	Materials used for hepatoprotection experiments	54
3.1.4	Materials used for cytotoxicity experiments	54
3.2	Methodology	55
3.2.1	Collection and authentication of plant sample.....	55
3.2.2	Plant extracts preparation and determination of percentage yield	55
3.3.1	Care for experimental rats	56
3.3.2	Choice of carbon tetrachloride for antioxidant and hepatotoxicity experiments.....	56
3.3.3	Animal experimental groupings for anti-oxidation experiment.....	56
3.3.4	Extract pre-treatment (hepatoprotective effect) experiment.....	57
3.3.5	Extract post-treatment (hepatocurative effect) experiment.....	58
3.3.6	Collection of animal samples	58
3.4.1	Preparation of Serum	59
3.4.2	Preparation of tissue homogenate	59
3.4.3	Animal experiment groupings for anti-inflammation experiment	60
3.4.4	Carrageenan-induced rat paw oedema anti-inflammation groups	60
3.4.5	LD ₅₀ determination.....	61
3.4.6	Anti-oxidation experiments (<i>In vivo</i>).....	61
3.4.6.1	Determination of lipid peroxidation.....	62
3.4.6.2	Determination of Catalase (Cat) activity.....	62
3.4.6.3	Determination of Glutathione peroxidase (GPx) activity.....	63

3.4.6.4	Determination of Superoxide dismutase (SOD) activity.....	64
3.4.7	Lipid profile studies	65
3.4.7.1	Total serum triglyceride (TG).....	65
3.4.7.2	Total serum Cholesterol (TC).....	66
3.4.7.3	High density lipoprotein (HDL)	67
3.4.7.4	Low density lipoprotein (LDL)	68
3.4.8	Anti-inflammatory test	69
3.4.8.1	Induced paw oedema assay.....	69
3.4.8.2	Nitric oxide assay	70
3.4.9	Hepatoprotective/hepatocurative tests.....	71
3.4.9.1	Induction of hepatotoxicity	71
3.4.9.2	Determination of Aspartate aminotransferase (AST) activity	71
3.4.9.3	Determination of Alanine aminotransferase (ALT) activity.....	72
3.4.9.4	Determination of Bilirubin concentrations	72
3.4.9.5	Conjugated (Direct) bilirubin.....	73
3.4.9.6	Unconjugated (Indirect) bilirubin	73
3.4.9.7	Total bilirubin	73
3.5.1	Determination of nephrological effects.....	74
3.5.1.1	Creatinine concentration	74
3.5.1.2	Determination of blood Urea	74
3.5.1.3	Total protein	75
3.6	Cytotoxicity tests	76
3.6.1	Brine shrimp hatchability assay	76
3.6.2	Brine shrimp lethality assay.....	77
3.6.3	Preparation of artificial sea water.....	78

3.6.4	Preparation of egg yolk suspension	78
3.6.5	Genotoxicity test: Plasmid nicking assay	78
3.6.6	Quantification of flavonoids and polyphenols:.....	79
3.6.7	Total flavonoid content determination	79
3.6.8	Total polyphenolic content determination.....	80
3.7	Statistics	80

CHAPTER FOUR

4.0	RESULT	81
4.1	Percentage yield of extracts	81
4.2	Results of LD ₅₀ dose determination	83
4.3	Body and organ weight changes in rats treated with methanolic extracts of parts of <i>M. lucida</i>	85
4.3.1	Relative body weight changes of rats pre-treated with extracts before CCl ₄ administration.....	85
4.3.2	Relative liver/body weight ratio of rats pre-treated with extracts before CCl ₄ administration.....	85
4.3.3	Relative kidney/body weight ratio of rats pre-treated with extracts before CCl ₄ administration.....	86
4.3.4	Relative body weight changes of rats post-treated with extracts after CCl ₄ administration.....	89
4.3.5	Relative liver/body weight ratio of rats post-treated with extracts after CCl ₄ administration.....	89
4.3.6	Relative kidney/body weight ratio of rats post-treated with extracts after CCl ₄ administration.....	90
4.4	Results of biochemical assays	92
4.4.1	Anti-Oxidation Results: Lipid peroxidation	92
4.4.2	Concentration of thiobarbituric acid reactive substances in rats	

pre-treated with extracts before CCl ₄ administration:.....	92
4.4.3 Superoxide dismutase (SOD) concentration in rats pre-treated with extracts before CCl ₄ administration:.....	93
4.4.4 Glutathione peroxidase (GPx) concentration in rats pre-treated with extracts before CCl ₄ administration:.....	93
4.4.5 Catalase (CAT) concentration in rats pre-treated with extracts before CCl ₄ administration	94
4.4.6 Concentration of thiobarbituric acid reactive substances in rats post-treated with extracts after CCl ₄ administration	95
4.4.7 Superoxide dismutase (SOD) Concentration in rats post-treated with extracts after CCl ₄ administration	95
4.4.8 Glutathione peroxidase (GPx) concentration in rats post-treated with extracts after CCl ₄ administration	97
4.4.9 Catalase (CAT) concentration in rats post-treated with extracts after CCl ₄ administration	97
4.5 Results of anti-inflammatory experiments	100
4.5.1 Carrageenan-induced rat paw oedema	100
4.5.2 Inflammation inhibition by methanolic fruit extract	100
4.5.3 Inflammation inhibition by methanolic leaf extract	100
4.5.4 Inflammation inhibition by methanolic bark extract	101
4.5.5 Inflammation inhibition by methanolic root extract	101
4.5.6 Anti-inflammation result: Nitric oxide assay	102
4.6 Results of liver function experiments	108
4.6.1 Aspartate aminotransferase (AST) concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts	108
4.6.2 Alanine aminotransferase (ALT) concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	108
4.6.3 Direct bilirubin concentration in rats tested for hepatoprotective	

	effect of <i>M. lucida</i> extracts against CCl ₄ damage.....	109
4.6.4	Indirect bilirubin concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	110
4.6.5	Total bilirubin concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	110
4.6.6	Aspartate aminotransferase (AST) concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	113
4.6.7	Alanine aminotransferase (ALT) concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	113
4.6.8	Direct bilirubin concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage.....	114
4.6.9	Inirect bilirubin concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage.....	114
4.6.10	Total bilirubin concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage.....	115
4.7	Results of lipid profile experiments	118
4.7.1	Total triglyceride concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	118
4.7.2	Total cholesterol concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	118
4.7.3	High density lipoprotein (HDL) concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	119

4.7.4	Low density lipoprotein (LDL) concentration in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	120
4.7.5	Total triglyceride concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	123
4.7.6	Total cholesterol concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	123
4.7.7	High density lipoprotein (HDL) concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	124
4.7.8	Low density lipoprotein (LDL) concentration in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	125
4.8	Results of kidney function experiments	126
4.8.1	Creatinine Concentration in rats pre-treated with extracts before CCl ₄ administration	127
4.8.2	Total urea concentration in rats pre-treated with extracts before CCl ₄ administration	127
4.8.3	Total protein concentration in rats pre-treated with extracts before CCl ₄ administration	128
4.8.4	Creatinine Concentration in rats post-treated with extracts after CCl ₄ administration	130
4.8.5	Total urea concentration in rats post-treated with extracts after CCl ₄ administration	130
4.8.6	Total protein concentration in rats post-treated with extracts after CCl ₄ administration	131
4.9	Results of cytotoxicity experiments	132
4.9.1	Inhibitory effect of extracts on <i>Artemia</i> cyst.....	133
4.9.2	Lethal effect of extracts on <i>Artemia</i> nauplii	133

4.9.3	Results of genotoxic effects of <i>Morinda lucida</i> extracts on pUC18 plasmid DNA	136
4.9.3.1	Genotoxic effects of <i>M. lucida</i> fruit extract	136
4.9.3.2	Genotoxic effects of <i>M. lucida</i> leaf extract	138
4.9.3.3	Genotoxic effects of <i>M. lucida</i> bark extract	140
4.9.3.4	Genotoxic effects of <i>M. lucida</i> root extract	142
4.10	Histopathology of liver and kidney tissues of rats treated with <i>M. lucida</i> extracts	143
4.10.1	Liver histopathology in CCl ₄ induced rats	144
4.10.2	Kidney histopathology in CCl ₄ induced rats	151
4.11	Results of haematological studies	158
4.11.1	Red Blood Cell count	158
4.11.2	Packed Cell Volume	158
4.11.3	Haemoglobin count	158
4.11.4	White Blood Cell count	159
4.11.5	Lymphocytes count	159
4.11.6	Neutrophil count	160
4.11.7	Platelet count	161
4.11.8	Mean Corpuscular Volume	161
4.12	Relative total flavonoid and total polyphenol content	165

CHAPTER FIVE

DISCUSSION	168
5.1 Percentage of extracts yield	168
5.2 LD ₅₀ determination	168
5.3 Effect of extracts against CCl ₄ on rats body and organ weights	169

5.3.1	Body weight changes	169
5.3.2	Relative liver/body weight changes	170
5.3.3	Relative kidney/body weight changes	170
5.4	Antioxidant effect of extracts against CCl ₄ in rats	171
5.4.1	Lipid peroxidation	171
5.4.2	Superoxide dismutase (SOD) activity.....	173
5.4.3	Glutathione peroxidase (GPx) activity.....	174
5.4.4	Catalase (CAT) activity	175
5.5	Anti-inflammatory effects of extracts	177
5.5.1	<i>M. lucida</i> methanolic fruit extract	177
5.5.2	<i>M. lucida</i> methanolic leaf extract	178
5.5.3	<i>M. lucida</i> methanolic bark extract	179
5.5.4	<i>M. lucida</i> methanolic root extract	180
5.5.5	Anti-inflammatory test (Nitric oxide assay)	181
5.6	Effect of extracts against CCl ₄ on liver function	181
5.6.1	AST and ALT activities, and Bilirubin concentration	181
5.7	Effect of extracts against CCl ₄ on lipid profile	184
5.7.1	Total cholesterol concentration	184
5.7.2	Total triglyceride concentration	185
5.7.3	High density lipoprotein (HDL) concentration	185
5.7.4	Low density lipoprotein (LDL) concentration	186
5.8	Effect of extracts against CCl ₄ on kidney function	188
5.8.1	Creatinine and urea concentration	188
5.8.2	Total protein concentration	189
5.9	Cytotoxic effects of extracts	189

5.9.1	Hatchability effect of extracts on <i>Artemia</i> cysts (brine shrimp eggs).....	189
5.9.2	Lethality effect of extracts on <i>Artemia</i> nauplii (brine shrimps).....	190
5.10	Genotoxic effect of extracts on pUC18 plasmid	191
5.11	Effects of extracts against CCl ₄ on histopathology of liver and kidney tissues of rats	194
5.12	Effects of extracts against CCl ₄ on haematological parameters of rats.....	195
5.13	Total flavonoid and total polyphenol contents of different parts of <i>M. lucida</i>	198
6.0	Summary, conclusions and recommendations	200
6.1	Summary	200
6.2	Conclusions	203
6.3	Recommendations	204
	REFERENCES	205
	APPENDIX I.....	227
	APPENDIX II	257
	APPENDIX III	260

LIST OF FIGURES

Figure 2.1: Plant-derived anticancer agents in clinical use	40
Figure 2.2: Plant-derived anticancer agents for future development.....	41
Figure 4.6: Percentage nitric oxide inhibition of different parts of <i>M. lucida</i> plant	107

LIST OF TABLES

Table 2.1: List of some plants evaluated for hepatoprotective effects.	30
Table 2.2: List of some plant-based formulations marketed for hepatic disorders.	33
Table 2.5: Plant derived anticancer agents in clinical use	42
Table 2.5: Plant derived anticancer agents for future development	44
Table 4.1: The percentage yields of methanol extract of different plant parts of <i>M. lucida</i> , following 12hrs of defatting with Petroleum ether	82
Table 4.2: LD ₅₀ dose of methanol extract of different parts of <i>M. lucida</i> in rats following oral administration.....	84
Table 4.3: Relative change in body weight and target organ/body weight ratios of extract pre-treated rats before CCl ₄ administration	88
Table 4.4: Relative change in body weight and target organ/body weight ratios of extract post-treated rats after CCl ₄ administration.	91
Table 4.5: TBARS concentration, and activities of SOD, GPx and CAT enzymes in liver of rats pre-treated with methanolic extracts of different parts of <i>M. Lucida</i> before CCl ₄ administration.....	96
Table 4.6: TBARS concentration, and activities of SOD, GPx and CAT enzymes in liver of rats post-treated with methanolic extracts of different parts of <i>M. Lucida</i> after CCl ₄ administration	99
Table 4.7: Effect of methanolic fruit extract of <i>M. lucida</i> on carrageenan induced rat paw oedema.....	103
Table 4.8: Effect of methanolic leaf extract of <i>M. lucida</i> on carrageenan induced rat paw oedema.....	104
Table 4.9: Effect of methanolic bark extract of <i>Morinda lucida</i> on carrageenan induced rat paw oedema.....	105

Table 4.10: Effect of methanolic root extract of <i>Morinda lucida</i> on carrageenan induced rat paw oedema.....	106
Table 4.11: Activities of AST and ALT, and Bilirubin (Direct, Indirect and Total) concentration in rat serum tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	112
Table 4.12: Activities of AST and ALT, and Bilirubin (Direct, Indirect and Total) concentration in serum of rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage:.....	117
Table 4.13: Concentration of cholesterol, triglyceride, HDL and LDL in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	122
Table 4.14: Concentration of cholesterol, triglyceride, HDL and LDL in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄ damage	126
Table 4.15: Concentration of creatinine, urea and total protein in rats pre-treated with methanol extracts of <i>M. lucida</i> before CCl ₄ administration	129
Table 4.16: Concentration of creatinine, urea and total protein in rats post-treated with methanol extracts of <i>M. lucida</i> after CCl ₄ administration	132
Table 4.17: The IC ₅₀ dose values of methanolic extracts different parts of <i>M. lucida</i> obtained from the hatchability assay on <i>Artemia</i> cysts.....	134
Table 4.18: The LC ₅₀ dose values of methanolic extracts different parts of <i>M. lucida</i> obtained from the lethality assay on <i>Artemia</i> nauplii	135
Table 4.19: Haematological changes in rats tested for hepatoprotective effect of <i>M. lucida</i> extracts against CCl ₄ damage	163
Table 4.20: Haematological changes in rats tested for hepatocurative effect of <i>M. lucida</i> extracts against CCl ₄	164
Table 4.21: Total flavonoid contents of methanolic extracts of parts of <i>M. lucida</i>	166
Table 4.22: Total polyphenol contents of methanolic extracts of parts of <i>M. lucida</i>	167

LIST OF PLATES

Plate 1: <i>M. lucida</i> plant (growing from a stump)	8
Plate 2: <i>M. lucida</i> stems, branches and leaves.....	9

Plate 3: <i>M. lucida</i> flower (in bud), fruits, placentation, and dry fruit.....	10
Plate 4: Effect of <i>M. lucida</i> methanolic fruit extract on the integrity of supercoiled pUC18 plasmid DNA in the presence of Fenton's reagent.....	138
Plate 5: Effect of <i>M. lucida</i> methanolic leaf extract on the integrity of supercoiled pUC18 plasmid DNA in the presence of Fenton's reagent.....	140
Plate 6: Effect of <i>M. lucida</i> methanolic bark extract on the integrity of supercoiled pUC18 plasmid DNA in the presence of Fenton's reagent.....	142
Plate 7: Effect of <i>M. lucida</i> methanolic root extract on the integrity of supercoiled pUC18 plasmid DNA in the presence of Fenton's reagent.....	144
Plate 8: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of <i>M. lucida</i> fruit extract	146
Plate 9: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of <i>M. lucida</i> leaf extract	147
Plate 10: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of <i>M. lucida</i> bark extract	148
Plate 11: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of <i>M. lucida</i> root extract	149
Plate 12: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-administered with α -tocopherol (Vitamin E)	150
Plate 13: Liver sections of CCl ₄ - induced hepatotoxicity in rats pre-administered with ascorbic acid (Vitamin C)	151
Plate 14: Kidney tissue sections of rats treated with 100mg/kg body weight of <i>M. lucida</i> fruit extract before and after CCl ₄ injection.....	153
Plate 15: Kidney tissue sections of rats treated with 100mg/kg body weight of <i>M. lucida</i> leaf extract before and after CCl ₄ injection	154
Plate 16: Kidney tissue sections of rats treated with 100mg/kg body weight of <i>M. lucida</i> bark extract before and after CCl ₄ injection	155
Plate 17: Kidney tissue sections of rats treated with 100mg/kg body weight of <i>M. lucida</i> root extract before and after CCl ₄ injection	156

Plate 18: Kidney tissue sections of CCl ₄ - injected rats pre-administered with α -tocopherol (Vitamin E).....	157
Plate 19: Kidney tissue sections of CCl ₄ - injected rats pre-administered with ascorbic acid (Vitamin C).....	158

CHAPTER ONE

INTRODUCTION

Traditional medicine has been used for thousands of years throughout the world. These uses, particularly for the treatment of diseases, date back to prehistoric times. The Egyptian ‘‘Ebers Papyrus’’ was one of the earliest written records detailing the use of herbs in the treatment of illness. Herbal remedies are currently used to treat a plethora of acute and chronic disorders ranging from common acute cold to various chronic types of cancers (Gonzalez-Avila, 2003; Mohammad, 2006; Mbarek *et al.*, 2007; Konate *et al.*, 2010; Francois *et al.*, 2010 and Santanu *et al.*, 2010). It was estimated that 50% of Western drugs are plant based materials or are parts of the drug models (Trager and Jensen, 1976). Many drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity.

Today, the search for natural compounds and other preparations of plant origin that can be used to prevent or mitigate maladies have been intensified. For instance, some medicinal and food plants such as *Moringa oleifera* (Atawodi *et al.*, 2010), *Khaya senegalensis* (Atawodi *et al.*, 2009), *Syzygium aromaticum* (Atawodi, 2011), *Hibiscus esculentus* (Atawodi *et al.*, 2009), *Canarium schweinfurthii* (Atawodi, 2010) have been demonstrated not only to contain phytochemicals with strong antioxidant capacity. But there still lies a very vast unexplored field in Nigeria for the discovery of such plants. This project is therefore designed to evaluate an indigenous medicinal plant of Northern Nigeria (*Morinda lucida*) for anti-oxidant, anti-inflammatory hepatoprotective, cytotoxic (and genotoxic) effects of its different parts.

1.1 Statement of problem

The sheer magnitude and mortality of epidemics during the era of plagues, is quite difficult to imagine (Kenrad and Williams, 2012). Over the past centuries, communicable diseases were the major causes of death as a result of illness across the globe. At that time, life expectancy was determined by whether people were exposed to epidemics that were mostly uncontrollable. But after World War II, there was a huge advancement in medical research that came up with antibiotics, vaccinations and various pharmaceuticals and there was much life improvement with good health. But almost simultaneously non-communicable diseases became the bane of the world, largely because of the new life style brought about by industrialization. As such heart diseases, cancer, diabetes, genetic disorders like premature ageing, inflammatory diseases and many more became a real burden for health systems in developed countries and were thus associated with economic development and so-called ‘diseases of the rich, (Boutayeb and Boutayeb, 2005; Wan-Ibrahim *et al.*, 2010 and Muslim *et al.*, 2010).

At present, non communicable diseases caused by western diet and lifestyle, environmental pollution, lack of early diagnostic tools, smoking of tobacco, fake and inefficacious drugs, have become more and more prevalent in developing countries where they greatly increase the burden of infectious diseases. Worse is that the health systems in low and middle income countries are unable to support the burden of diseases (Atawodi, 2005; Boutayeb and Boutayeb, 2005; and Bo *et al.*, 2007; Minhua *et al.*, 2009; Kumar *et al.*, 2010).

A number of non communicable diseases seem to be both interrelated and to share the same etiology. For instance, evidences are replete that reactive oxygen species (ROS) from exogenous sources are involved in the pathogenesis of a large number of diseases including cancer (Park and Pezzuto, 2002; Gonzalez-Avila, 2003), diabetes, inflammation (Atawodi,

2005; Singh *et al.*, 2009), Alzheimer and Parkinson disease (Atawodi, 2005) and nucleic acid damage (Usuh *et al.*, 2005; Singh *et al.*, 2009). A potent scavenger of these ROS could therefore serve as possible preventive intervention for free radical-mediated diseases (Nanasombat and Teckchuen, 2009).

A substantial body of evidence links the production of reactive oxygen species, and subsequently, oxidative stress and damage to the pathogenesis of age-related and chronic diseases (Vuillaume, 1987; Witz, 1991 and Trush and Kensler, 1991). Oxidative stress can occur through overproduction of reactive oxygen and nitrogen species through either endogenous or exogenous insults. For instance, in genotoxicity which may have commenced with acute inflammation may result in carcinogenesis, and the unregulated or prolonged production of cellular oxidants has been linked to mutation (oxidant-induced DNA damage), as well as modification of gene expression. Under normal physiological conditions, cells are capable of counterbalancing the production of reactive oxygen species with antioxidants. Endogenous cellular antioxidant defenses are mainly enzymatic and include superoxide dismutase, glutathione peroxidase, and catalase. Superoxide dismutases are localized to the cytosol and mitochondria and function to reduce superoxide anion to hydrogen peroxide and water. Glutathione peroxidases, localized in the cytosol and mitochondria, remove the majority of hydrogen peroxide, whereas catalase, located in peroxisomes, is responsible for the removal of high levels of hydrogen peroxide (Abuja and Albertini, 2001). Non-enzymatic antioxidants such as vitamin E, vitamin C, β -carotene, glutathione, and coenzyme-Q function to quench reactive oxygen species (Clarkson and Thompson, 2000). When the redox balance is shifted in favor of cellular oxidants, oxidative damage to nucleic acids, lipids, or proteins can result and produce modification to cell function and cell viability.

1.2 Justification

At the dawn of the third millennium, non-communicable diseases are sweeping across the entire globe. There is an increasing trend in developing countries, where the demographic and socio-economic transition imposes more constraints on dealing with double burden of infectious and non- infectious diseases in a poor environment, characterized by inadequate health systems. It is predicted that by 2020, non-communicable diseases will cause seven out of every ten deaths in developing countries. Many of the non communicable diseases can be prevented by tackling associated risk factors (Boutayeb and Boutayeb, 2005) for instance, dealing with the issue of oxidative stress.

Available data has for instance, shown that liver cancer at present is the second most common cancer in Nigerian men after prostate cancer while it still remains the most common cancer in men from various parts of sub-Saharan Africa (Koulibaly *et al.*, 1997). Survival rate for cancer of the liver is between 30 – 40% for early localized cancer while in all stages combined, it is only 5%. But sadly also, detection at early stages is mostly non-existent in Africa (Okobia, 2003).

1.3 Aim

The aim of this research is to build on the existing body of knowledge on the antioxidant, anti-inflammatory, hepatoprotective and cytotoxic properties of Nigerian foodstuffs and medicinal plants.

1.4 Specific objectives

The objectives of this study are:

- i. To evaluate the antioxidant effect of the different parts of *Morinda lucida*.
- ii. To establish the anti-inflammatory properties of the different parts of *Morinda lucida*.
- iii. To determine the hepatoprotective/hepatocurative effects of the extract of the different parts of *Morinda lucida* on the integrity of the liver and associate disorders like hyperlipidemia and hyperbilirubinaemia.
- iv. To assess the cytotoxic (and genotoxic) properties of the different parts of *Morinda lucida*.
- v. To relate the total flavonoid content with the total polyphenol composition.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical description of *Morinda lucida*

Morinda lucida Benth, a Rubiaceae, is known commonly as ‘brimstone tree’ or ‘Kuhia’ (English), ‘Arbre à soufre’ (French), and ‘Moindo’ (Poland). In Nigeria, it is called ‘oruwo’ (Yoruba), ‘nfia’ or ‘Ezeogu’ (Igbo), ‘ogele’ (Igala), among other many different indigenous tribal names, while in other African countries it is called ‘Uhon’ (Ivory Coast) and ‘hojologbo’ (Sierra Leone). It is a medium sized evergreen shrub or small to medium-sized tree up to (18 –25) meters tall, with the branches often crooked or gnarled. The tree is gifted with a crooked hole and rather short twisted branches. The bark is rough, grey in colour, forming irregular patches but at times the bark may be smooth, scaly, grey to brown coloured, often with some distinct purple layers. The leaves are about 7 – 15 cm long by 3.5 – 7.5cm and broad, while the flowers are white with a narrow glabrous corolla-tube about 2.5cm. (Adomi and Umukoro, 2010; Nwakaego, 2012).

2.2.1 Description of physical features of *Morinda lucida*

The leaves are opposite, simple and entire, with stipules that are ovate or triangular ranging in length from 1–7mm. It usually possesses an inflorescence with a stalked head of between 4–7mm in diameter. The peduncle is up to 8cm long bearing a stalked cup-shaped gland at the base. The flowers are bisexual, regular, in fives, heterostylous, and faintly fragrant (Burkill, 1997). The calyxes are cup-shaped, about 2mm long, and persistent. The corollas are salver-shaped, about 1.5cm long, white or greenish yellow, with ovate-lanceolate lobes of up to 5mm × 2.5mm. The ovary is inferior, bi-cellular with style of about 8–11mm long. It possesses two stigma lobes of 4–7mm long, five stamens, piercing through the corolla throat,

with short filaments (Zimudzi and Cardon, 2005). The fruit is a drupe, with several arranged together into an almost globose succulent syncarp of about 1–2.5 cm in diameter. The fruits are soft and black when mature. The seeds are ellipsoid, with dimensions: 3.5mm × 2mm × 0.5mm (Zimudzi and Cardon, 2005).

2.2.2 Traditional medicinal uses of *Morinda lucida*

In West Africa, *Morinda lucida* is an important plant in traditional medicine. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis, feverish conditions during child birth and even cancer (Adomi and Umukoro 2010; Soladoye *et al.*, 2010; Ebiloma *et al.*, 2011). The plant is also employed in cases of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhea (Adesida, and Adesogan, 1972). In Nigeria *Morinda lucida* is one of the 4 most used traditional medicines against fever. In Côte d'Ivoire a bark or leaf decoction is applied against jaundice and in DR Congo it is combined with a dressing of powdered root bark against itch and ringworm (Abbiw, 1990).

2.2.3 Properties of *Morinda lucida*

Up to 18 (eighteen) anthraquinones have been isolated from the wood and bark of *Morinda lucida*, including the red colorants 1-methylether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin (Zimudzi and Cardon, 2005). Two anthraquinols known as oruwal and oruwalol, which give a yellow colour have also been found, which seem to be the intermediates in the formation of anthraquinones. In addition to anthraquinones, tannins, flavonoids and saponosides have also been isolated (Adesida, and Adesogan, 1972).



Plate 1: *Morinda lucida* plant (growing from a stump)



Plate 2: *Morinda lucida* stems, branches and leaves

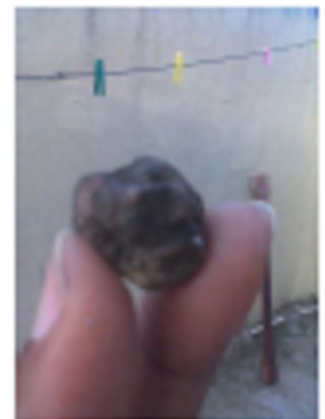
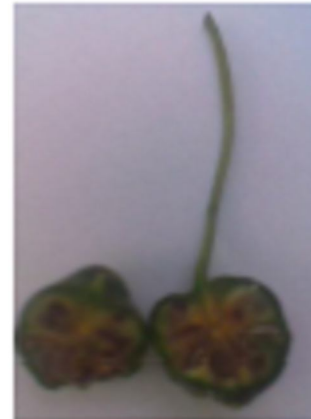


Plate 3: *Morinda lucida* (a). flowers (in bud), (b.) fruits, (c). placentation, (d). dry fruit

2.2.4 *Morinda lucida* in Ethnomedicine

Ethnomedicine, from native's point of view, is the study of the indigenous way of treating and managing certain diseases that affect people living in a given environment. This approach is particularly useful for the study of indigenous therapeutic agents since it allows the researcher to understand treatment patterns according to native explanatory models, instead of seeing only through the lens of biomedicine (Neuwinger, 2000). *Morinda lucida* is a medicinal plant that grows in many African countries and it is widely used as a medicine in West Africa. The plant is generally used as ingredients of fever teas, which are usually taken, for the traditional treatment of malaria (Awe and Makinde, 1998).

In West Africa, *Morinda lucida* is an important plant in traditional medicine. In Nigeria, *Morinda lucida* is one of the four most used plants in the preparation of traditional medicines against fever. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. In some cases, the plant is employed in the treatment of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhea (Adesida *et al.*, 1972).

In Côte d'Ivoire, a bark or leaf decoction is applied against jaundice and in DR Congo, the decoction of the stem bark or leaf is combined with a dressing of powdered root bark against itch and ringworm (Abbiw, 1990). Adewunmi *et al.* (1984) reported in their work that the bark, root and leaf are bitter. These workers stated that the infusion or decoction of these parts is used for the treatment of yellow fever and other forms of fever. They also reported that the decoction of the leaf is applied to the breast of women at weaning of their infants to prevent infections. However, *Morinda lucida* is used generally for febrifuge, analgesic and

laxative (Bever, 1996) while the decoction of the stem bark is used for the treatment of severe jaundice. As reported by Adjanohoun *et al.* (1991) *Morinda lucida* is used locally in the treatment of irregular menstruation, insomnia and jaundice though did not state the parts that are useful in this purpose.

Burkill, (1991) also stated that locally, *Morinda lucida* is used in the treatment of wound infections, abscesses and chancre. The primary syphilitic ulcer associated with swelling of local lymph glands and is painless, indurated, solitary and highly infectious. Also amongst the Igede People in Benue State, Nigeria, it was reported by Igoli *et al.* (2005) and Ogaji *et al.* (2006) that the decoction of the *Morinda lucida* is used twice or thrice daily as anti – diarrhea, while the leaves are used for treatment of infertility in women.

Morinda lucida leaves are used as an ingredient of “fever teas”, which are usually taken for the traditional treatment of malaria. The plant is also used as a general febrifuge, analgesic and laxative. A weak decoction of the stem bark is used for the treatment of severe jaundice (Oliver-Bever, 1986). The major constituents of *Morinda lucida* extract are various types of alkaloids-anthraquinones and anthraquinols. Adewunmi and Adesogan (1984) isolated and characterized two compounds, oruwalol and oruwal, and ten anthraquinones from the stem of the plant. Anthraquinones isolated from *Morinda lucida* were shown to possess potent *in vitro* activity against *Plasmodium falciparum* (Koumaglo *et al.*, 1992). Trypanocidal activity of the methanol extract of the leaf of *M. lucida* was reported by Asuzu and Chineme (1990) while Obih *et al.* (1985) reported the antimalarial activity of *Morinda lucida* against *Plasmodium berghei berghei* in mice.

2.2.5 Phytochemistry of *Morinda lucida*

The major constituents of *M. lucida* extracts are various types of alkaloids - anthraquinones and anthraquinols (Adewunmi *et al.*, 1984). From the wood and bark of *Morinda lucida*, 18 anthraquinones have been isolated, including the red colorants 1- methylether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin. Two compounds (Oruwalol and Oruwal) as well as ten anthraquinones) were isolated and characterized from the stem (Adesogan *et al.*, 1984). In addition to anthraquinones, tannins, flavonoids and saponosides have been isolated. Adesogan *et al.* (1983), Rath *et al.* (1995) and Koumagho *et al.* (1992) isolated anthraquinones and oruwacin from the roots of *M. lucida*. Trease and Evans (2007) also confirmed the presence of the above constituents in their report. Two known triterpenic acids, ursolic and oleanolic acids were isolated from the leaves (Lawal *et al.*, 2012). Three compounds (digitolutein, rubiadin 1-methyl ether and damnacanthal) (Koumaglo *et al.*, 1992) and stigmatserol (Fleischer *et al.*, 2011) were extracted from the stem bark and roots of *M. lucida*.

2.2.5.1 Polyphenols and flavonoids

2.2.5.2 Polyphenols and flavonoids in plants

Polyphenols are secondary compounds widely distributed in the plant kingdom. They are divided into several classes, namely, phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins) stilbenes and lignans, which are distributed in plants and food of plant origin (Manach *et al.*, 2004; 2005).

Phenolics are an important aspect of fruit quality because of their contribution to the taste, colour and nutritional properties of fruit (Cheynier, 2005). There are evidences showing that phenolic substances act as antioxidants by preventing the oxidation of LDL-lipoprotein, platelet aggregation, and damage of red blood cells (Cheynier, 2005). Additionally, phenolics act as: (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents and, (iv) clarifying agents (Proestos *et al.*, 2005). They are responsible for red wine colour, astringency and bitterness and contribute to its sensory profile (Lesschaeve & Noble, 2005). They are derived from the fruit and vine stems, or by the yeast metabolism. In addition, phenolics serve as important oxygen reservoirs and substrates for browning reactions.

Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to produce multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improving the quality and nutritional value of food (Kahkonen *et al.*, 1999). These phenolic compounds are classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another.

2.2.5.3 Beneficial effects of polyphenols

The beneficial effects of polyphenols are mainly attributed to their antioxidant properties, since they can act as chain breakers or radical scavengers depending on their chemical structures (Rice-Evans, 2001). Polyphenols may also trigger changes in the signalling pathways and subsequent gene expression (Chen *et al.*, 2002; Pfeilschifter *et al.*, 2003). It has been reported that a red grape juice concentrate inhibited lipid peroxidation of LDL by

prolonging the lag phase by 2.7 times relative to a control, when evaluated at a total phenolic concentration of 10µM gallic acid equivalents (GAE) (Landbo & Meyer, 2001). It was also confirmed that the red grape juices tested blocked lipid peroxidation of LDL at 20µM GAE, while white grape juice exerted pro-oxidant activity at $5 \pm 20\mu\text{M}$ GAE (Landbo & Meyer, 2001).

2.2.5.4 Flavonoids and Types

Flavonoids are molecules with a phenolic benzopyran structure and they occur only in plants where they are present predominantly as glycosides. The flavonoids may themselves be divided into six subclasses as a function of the type of heterocycle involved; namely flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins and lignans are especially common in leaves, flowering tissues, and woody parts such as stems and barks (Kahkonen *et al.*, 1999). They are important in the plant for normal growth development and defence against infection and injury. Flavonoids also partly provide plant colouration present in flowers, fruits, and leaves as pigments. Plant polyphenols comprise a great diversity of compounds, among which flavonoids and several classes of non-flavonoids are usually distinguished (Harborne, 1989). The latter are mostly rather simple molecules, such as phenolic acids and stilbenes, but they also include complex molecules derived from them such as stilbene oligomers, gallotannins, ellagitannins, and lignins, while the former share a common nucleus consisting of two phenolic rings and an oxygenated heterocycle. More than 4000 flavonoids have been identified in plants, and the list is constantly expanding (Harborne & Williams, 2000).

2.3 OXIDATIVE STRESS AND ANTI-OXIDATION

2.3.1 Oxidative Stress

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems (Chanda and Dave, 2009). ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders (Halliwell, 1994; Rackova *et al.*, 2007) such as cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas *et al.*, 2007), Alzheimer's disease (Smith *et al.*, 2000) mild cognitive impairment (Guidi *et al.*, 2006), Parkinsons disease (Bolton *et al.*, 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna *et al.*, 1997), ageing (Hyun *et al.*, 2006), atherosclerosis (Upston *et al.*, 2003). Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries (Jainu and Shyamala, 2005). Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system (Wiseman and Halliwell, 1996).

Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize

reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($\cdot\text{O}_2^-$) and hydroxyl radicals ($\text{OH}\cdot$), as well as non-free radicals (H_2O_2) and singlet oxygen ($^1\text{O}_2$) (Halliwell, 1995).

In the body, free radicals are derived from two sources: endogenous sources, (e.g. nutrient metabolism, ageing process) and exogenous sources (e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents and pesticides). When oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko *et al.*, 2006). ROS can attack various substrates in the body and contribute to the development of chronic diseases. For example, oxidatively modified LDL has been hypothesized to be a causative agent in the development of cardiovascular diseases (Touyz, 2004). Exogenous chemicals and endogenous metabolic processes in human body produce free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death. Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states. During normal metabolic processes, human body generates more than 2kg of $\cdot\text{O}_2^-$ - per year (Evans and Halliwell, 1999).

2.3.2 Anti-Oxidation:

Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide

dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury (Bergendi *et al.*, 1999). Molecules such as vitamin C and E inhibit lipid peroxidation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage.

2.3.3 Natural sources of antioxidants

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006). There are many synthetic antioxidants in use. It is reported, however, the compounds have several side effects (Ito *et al.*, 1983), such as risk of liver damage and carcinogenesis in laboratory animals (Gao *et al.*, 1999; Williams *et al.*, 1999; Osawa and Namiki, 1981). There is therefore a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

2.4 INFLAMMATION AND ANTI-INFLAMMATION

2.4.1 Inflammation

Although there are various definitions of inflammation, its occurrence has been described as a vital response of tissue to injury (Naik *et al.*, 1976). Jalil *et al.* (2003) described inflammation

as the reaction of living tissues to injury, infection or irritation, while Mantri and Witiak (1994) defines inflammation as a disease involving localized increases in number of leukocytes and a variety of complex mediator molecules including prostaglandins. Considering this definition, it becomes tempting to describe inflammation as a protective and normal response to any kind of noxious stimulus that may alter the normal physiological process of the host, varying from acute transient and highly localized response to simple-mechanical injury or to the complex persistent response involving the whole organism (Naik *et al.*, 1976).

These initial responses may initiate further a series of biochemical, immunological and cellular events, which may range in time from recognition of the noxious stimulus through mobilization of natural defense mechanisms, ending with physical repair and restoration of function of the injured tissue. Thus inflammation can be defined simply by summing up all these processes, as a complex, vascular lymphatic and local tissue reaction elicited in animals by the presence of viable and non-viable irritants (Naik *et al.*, 1976).

2.4.2 Types of Inflammation

Inflammation may be broadly classified into three categories, namely, acute inflammation, chronic inflammation, and mixed types of inflammation. The mixed type may include allergic and dermatological disorders.

2.4.3 Acute Inflammation

When a tissue injury is caused by a single event such as mechanical trauma, a thermal or chemical burn or a single exposure to non-replicating antigen the protective phenomena results in inflammation and repairative process proceeds smoothly from injury to recovery.

Consequently, whole inflammatory process at least in acute inflammation exemplifies a beneficial homeostatic mechanism trying to restore the affected tissue to its normal healthy state.

2.4.4 Chronic Inflammation

There are many diseases which are distinguished by signs and symptoms characteristic of response to chronic inflammatory process of unidentified origin. Some of these rheumatic disorders are characterized by a lack of detectable anti-globulin (rheumatoid factor) and antinuclear antibodies in the serum. These are often loosely known as collagen disease which suggests the involvement of structure and/or metabolism of collagen in the diseased process. Several classifications have been suggested (Kulonen, 1971). The main diseases include rheumatic fever, rheumatoid arthritis, ankylosing spondylitis and osteoarthritis, but many other disorders exhibiting chronic inflammatory changes, such as periarteritis nodosa, scleroderma and systemic lupus erythematosus are usually included in the general classification (Goodman and Gillman 1970).

There are certain chronic inflammations caused by self replicating parasite like bacterium, virus or neoplasm. These inflammations may become much more complex because of persisting injurious agents or their degraded products. When noxious agents cannot be destroyed or removed early, the inflammatory responses try to isolate them from the rest of the organism by forming granuloma (e.g. in pulmonary tuberculosis and silicosis or gumma as in syphilis). The ultimate response of this type is classified as Gohn complexes which contain viable but imprisoned tubercule bacilli functionally isolated from the host (Naik *et al.*, 1976).

Gout is characterized by acute and chronic inflammatory response to the deposition of microcrystals of sodium urate in the joints and tissues. Presently, the etiology of gouty arthritis is totally well understood.

2.4.5 Miscellaneous kinds of inflammation

This group of disorders may not be essentially considered as inflammatory but whose components are of inflammatory origin. Many of the dermatological conditions consist of acute, sub-acute and chronic inflammatory reactions to various known and unknown prime causes. All these disorders mainly involve skin and can readily be assessed. Some examples of these skin diseases are pemphigus, pemphigoid and discoid lupus. However, these apparently seem to be immunologic but they hardly respond to immunosuppressive therapy (Ebringer and Mackay, 1969). Contact dermatitis on the other hand is a manifestation of delayed hyper-sensitivity, *Encapheli mylites* involving spinal cord and motor incoordination. Rejection of the transplanted organ is clearly initiated by normally protective immunological reactions but it is mediated by the familiar sequence of inflammatory stimulus leading to cardinal signs of inflammation and, above all the loss of function.

2.4.6 Inflammogenic factors

2.4.6.1 Hydrolytic enzymes

Inflammogenic factors have been considered to include lysosomal components such as hydrolytic enzymes or cationic proteins which play important roles in the initiation of inflammation, tissue injury and connective tissue breakdown (Weissman *et al.*, 1969). Another researcher found higher levels of catalytic enzymes in inflamed tissue or serum of arthritic rats as compared to normal animals. In rat adjuvant arthritis, it has been stressed that

the potential destructive capacity of connective tissue is acid hydrolases and it is liberated within the endogenous cellular elements of connective tissue or derived from migrating leukocytes (Anderson, 1970). However, Paulus and Whitehouse (1973) have stressed that the presence of potentially destructive enzymes in serum is not the sole factor in injury, because it was discovered that there was no correlation between maximum enzyme activity and presence of tissue damage, a study that was further supported by the fact that, in rheumatoid arthritis the catabolic activity is not the sole problem, but there is also the articular damage which may be due to adopted leukocytes in synovial tissue.

2.4.6.2 Prostaglandins (PGs)

Prostaglandin 'E's are involved in cellular injury and inflammation. Most of the non-steroidal anti-inflammatory agents are active inhibitors of its production from its precursor, arachidonic acid. Prostaglandins E₁ and E₂ often induce the increase of vascular permeability in animals and flare response in human beings (Horton, 1963; Crunkhorn and Wills, 1971; Kaley and Weiner, 1971, 1971a). PGE type has been identified in the inflammatory exudate of carrageenan-induced inflammation in rats (Wills, 1969). Prostaglandins occur relatively late in inflammatory process and are often associated with migration of leukocytes into inflamed site (Willoughby, 1971). In rabbits, it has been shown that during phagocytosis, prostaglandins are released from leukocyte lysosome (Higgs and Youtten, 1972) and also during endocytosis (Anderson, 1970).

Aspirin, indomethacin and salicylates have been shown by a number of workers to inhibit synthesis or release of PGE₂ and PGF₂- α from arachidonic acid from variety of tissue (Vane, 1971; Smith and Wills, 1971; Ferreira *et al.*, 1971; Smith and Lands, 1971). The inhibition of

prostaglandin synthetase by antiinflammatory agents depends on its order of potency in carrageenin induced inflammation (Tomlinson *et al.*, 1972). Though non-steroidal anti-inflammatory drugs suppress prostaglandin synthesis, recently it has been shown that PGE₁ and PGE₂ at high doses suppress local inflammation of arthritis and carrageenin induced inflammation (Aspinall *et al.*, 1969; Glenn *et al.*, 1972) with some side and toxic effects like hyperplasia, prostatic hyperplasia and diarrhoea which may contribute to its anti-inflammatory effect (Naik *et al.*, 1976). The involvement of prostaglandins in urate crystal inflammation was demonstrated by Denko (1974). He explained that the prolonged inflammation by urate crystals is due to the constant formation or release of prostaglandins from antecedent phospholipids in the membrane. These released prostaglandins may occur with membranolysis. He concluded that urate crystal inflammation may be a membrane disease.

Mechanisms by which prostaglandins elicit 'some of their actions are thought to involve cyclic-AMP (Kahn and Lands, 1973). Also, PGE₁, PGE₂ and PGF₂- α were reported to stimulate adenylate cyclase activity in human mixed leukocytes (Poigar *et al.*, 1973). Endogenous cyclic AMP and cyclic GMP might mediate the opposing effects of prostaglandins on lysosomal enzyme secretion from neutrophils. The prostaglandins, especially the biphasic actions of PGF₂ on neutrophil function support a modulatory function for these tissue hormones in the inflammatory process. Glucocorticoids reduce the rapid accumulation of cyclic GMP provoked by immune reactants but have no effect on levels of cyclic AMP.

2.4.6.3 Complement System

These are reactive proteins which are continually available to all body tissues and play a vital role in the protective mechanism of the organism against exogenous or endogenous injury agents. These complement constituents when set into action against injurious agents often

cause damage to the host. Complement activation further stimulates many other reactions by which many more pathogenic and inflammatory factors are formed for example anaphylatoxin which causes smooth muscle contractions, increases capillary permeability, accumulation of migrated leukocytes, releasing histamine from tissues, formation of high molecular kinins, lysis of platelets and thereby releasing vasoactive amines and other catalytic enzymes (Muller-Eberhard, 1969).

Trypsin inhibitors are irreversible complement inhibitors *in vitro* and some of them are also active *in vivo* but only at sub-toxic dose level (Baker and Hurlbut, 1969; Cory *et al.*, 1972). Cobra venom has long been used as complement inhibitor and is now successfully used as suppressor of immune response in organ transplantation.

2.4.6.4 Protein Breakdown Process

Local protein breakdown process may bring about perpetuation or the reduction of inflammation. Hence it will be advisable to think of the use of some anti-proteolytic agents in inflammatory conditions. If anti-proteolytic drugs can prevent the process like histolysis, kinin, kallikrein formation, fibrin deposition, then it will be a real therapeutic use. However, it has also been shown that exogenous proteases may act as anti-inflammatory agents and increased synthesis of glycoproteins in the liver during inflammatory process. Hence, it is very difficult to say whether the proteolysis may aggravate or inhibit the existing inflammatory process (Naik and Sheth, 1976).

Extracellular proteases from plasma transudate infiltrating leukocytes, chondrocytes and synovial cells may degrade albumin (Barnhart *et al.*, 1968) which perhaps enhance the

protein synthesis and wound repair by delivering amino acids and peptides from mobile amino acid pool. Some preliminary experiments using amino acids, namely, glycine, glutamic acid, phenylalanine and aspartic acid, in treating carrageenan edema and cotton pellet-granuloma showed good anti-inflammatory effect (Naik *et al.*, 1974a).

2.4.6.5 Calcium

The knowledge that extracellular calcium is required for many reactants to provoke enzyme release suggests that calcium entry into the cells is important. Organic substances which provoke lysosomal enzyme secretion from neutrophils do so by first promoting calcium entry into the cells. Increased intracellular calcium could bring about the accumulation of intracellular cyclic GMP, which in turn could signal the secretion of lysosome granule constituents into the extracellular environment. Calcium is required for numerous secretory processes (Rubin, 1970). Woodwin *et al.*, 1973) have reported that calcium is required for rabbit granulocytes to discharge F3-glucuronidase in the presence of leucocidin. Smith and Ignarro (1974) have indicated that calcium influx from the extracellular medium into human neutrophils occurs during cell contact with immune reactants at 37°C. Calcium influx was associated with, but preceded, by lysosomal enzyme secretion from human neutrophils (Ignarro, 1974). Immune reactants such as zymosan treated serum or certain activated complement components operated as calcium ionophores in triggering lysosomal enzyme secretion from human neutrophils (Ignarro, 1974).

2.4.6.6 Cyclic Nucleotides

Cyclic GMP and cyclic AMP serve a second messenger role in the cellular actions of numerous primary hormones or effective substances. Accumulated evidence suggest that

these two naturally occurring cyclic nucleotides play a vital role in expressing the actions of autonomic neuro tumors, prostaglandins, glucocorticosteroids and calcium translation on lysosomal enzyme secretion from human neutrophils (Naik and Sheth, 1976).

Epinephrine, prostaglandins (PG) E₁, norepinephrine, isoproterenol, glucagon or adrenocorticotrophin were reported to stimulate cyclic AMP synthesis in isolated and mixed human leukocytes (Scott, 1970; Bourne and Melmon, 1971). This catecholamine effect was inhibited by beta adrenergic blockers but not by a adrenergic blockers. Ignarro (1974) reported that effect of epinephrine is mediated by intracellular cyclic AMP by virtue of the capacity of this neurohormone to stimulate leukocyte adenylcyclase activity and thereby elevate the levels of cyclic AMP.

Certain vasoactive hormone-mediators of inflammation and cAMP help in protecting host from the dangerous chain of an unregulated immune response. Cyclic AMP is in control of leukocyte functions, mainly histamine release due to antigen-antibody reactions, hypersensitivity etc (Naik *et al.*, 1976).

2.4.7 Anti-Inflammation

Over the recent past, steroidal and non-steroidal anti-inflammatory drugs have been very much in use. But today, due to the links that inflammation has with so many diseases, there is an increased interest in curbing this phenomenon. Although modern drugs are effective in the management of inflammation and associated conditions, but their use is often limited because of side effects (Lipsky, 1999). Recently, there is growing realization that apart from being safer, economical and easily available, herbs, phytochemicals and herbal products can

influence the course of inflammatory diseases and may provide an amalgamation of nutritional substances, which may help in restoring and maintaining wear and tear of tissues. Therefore, it is rational to search, identify and evaluate traditional medicines used for their potential anti-inflammatory properties (Santosh *et al.*, 2010).

Today, the use of medicinal plants has reached a revolutionary proportion. Raji *et al.* (2002) discovered the powerful anti-inflammatory and analgesic agents contained in the extracts of *Zingiber officinale*. *Moussonia deppeana* (Schldl. & Cham) Hanst, is a specie of Mexican medicinal flora used in Veracruz State to treat cases related to stomach pain, renal diseases, cough, tumors and inflammation. Laboratory findings showed that the administration of 100mg/kg , of extracts of hexane and ethanoic acid, offered 39% and 28% inflammatory inhibition respectively, upon the application of doses of 2mg/ear while within the first hour of induction of paw oedema using indomethacin, the ethanoic acid extract presented a 43% inhibition at 100 mg/kg dose (Domínguez-Ortiz *et al.*, 2009). These results support their traditional use in managing and treating inflammatory maladies.

2.5 HEPATOTOXICITY AND HEPATOPROTECTION

Liver is the principal organ in the maintenance of the body's *milieu interior*. It influences the flow of nutrients, controls metabolism of carbohydrates, proteins and fats. It is the major reticulo-endothelial organ in the body as such has important immune function in maintaining the body's normal functionality. It plays key role in drug bio-transformation, disposition of exogenous toxins or therapeutic agents and in so many biochemical reactions. In a life time, the liver is severally damaged due to the overdose of drugs, ingestion of certain chemicals or consumption of alcohol. Damages to the liver that were chemical – driven is known as

hepatotoxicity. Chemical agents such as synthetic agents (CCl₄, galactosamine, lithocholic acid and isoniazid), natural agents (microcystins) and herbal remedies (*Cascara sagrada*) can also contribute to hepatotoxicity. Such damaged liver will lead to many pathological conditions like hepatitis, cholestasis, steatosis, granuloma, etc. Hepatoprotection or anti-hepatotoxicity is the ability of a compound to prevent liver damage (Dhingra *et al.*, 2011).

There are several mechanisms responsible for the induction of hepatic injury. So many chemicals damage the mitochondria, which is the organelle for energy generation in the cell. Thus, its malfunctioning or dysfunctioning as a result of damage releases excessive amount of oxidants which in turn injures hepatic cells. Activation of some enzymes in the cytochrome P-450 system like CYP2E1 also leads to oxidative stress (Jaeschke *et al.*, 2002). Injury to hepatocyte and bile duct cells lead to the accumulation of bile acid in the liver. This endorses further liver damage. Non-parenchyma cells such as Kupffer cells also have role in the mechanism of hepatotoxicity. The exact mechanism of drug induced liver injury remains largely unknown but it appears to involve two pathways, namely, direct hepatotoxicity and adverse immune reactions. The direct hepatotoxicity is drug induced liver injury which originated from the bio activation of drugs to chemically reactive metabolites, which have the ability to interconnect with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress. Furthermore, these reactive metabolites may induce interruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production. This damage of cellular function can ultimately result in cell death and likely liver failure (Dhingra *et al.*, 2011).

Several hepatotoxic agents have been discovered and used to induce hepatotoxicity especially in experimental models. Carbon tetrachloride (CCl_4) has been popularly used to induce liver damages for the study of hepatotoxicity in acute and chronic liver failure. CCl_4 is metabolized by CYP2E1, CYP2B and CYP3A to some extent, to form the trichloromethyl radical ($\cdot\text{CCl}_3$) which can bind to cellular molecules damaging crucial cellular progressions. This radical also react with oxygen to form the trichloromethylperoxy radical ($\text{CCl}_3\text{OO}\cdot$), which is a very reactive specie. The administration of single dose of CCl_4 can cause centrilobular cell necrosis of the liver and changes in lipid profiles within three (3) hours of administration. The level of CCl_4 however, falls such that it is eliminated from the liver within 24 hours (Dawkins 1963).

D-Galactosamine is another hepatotoxic agent which creates a diffuse type of liver injury similar to viral hepatitis. It is assumed to disrupt the synthesis of essential RNA-linked uridine nucleotide pool, altering hepatocellular function and ultimately resulting to cell death. The defected RNA synthesis, as a consequence, leads to a decline in protein synthesis. This mechanism of toxicity brings about an uncontrolled increase in cell membrane permeability, leading to enzyme leakage and resultant cell death. Galactosamine also decreases bile flow and its contents, namely, bile salts, cholic acid and deoxycholic acid. Galactosamine also decreases the number of viable hepatocytes and the rate of oxygen consumption (Saraswat *et al.*, 1996; Dhingra *et al.*, 2011).

Thioacetamide is a selective hepatotoxin that its within a short time period. It interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. Metabolites of thioacetamide are the reason behind such hepatic injury. Thioacetamide is extensively metabolized to acetamide and thioacetamide S-dioxide by the mixed function

oxidase system. The former lacks liver necrotizing properties while the latter is further metabolized by CYP450 monooxygenase system to sulfene and thioacetamide S-dioxide (Jaspreet, 2011; Dhingra *et al.*, 2011). Thioacetamide S-dioxide is extremely reactive which covalently binds to liver macromolecules and initiates liver injury, cirrhosis and ultimate defect in cell permeability that causes escalated Ca^{2+} concentration in the intracellular compartment. Ultimately, there is increased nuclear volume, obstructed mitochondrial activity and cell death ensues.

Hepatotoxicity is also inducible by alcohol. Incidentally, the liver is the organ which is more prone to ethanolic toxic effects. There are documented literatures linking fatty infiltration, hepatitis and cirrhosis to alcohol use. Hepatitis and cirrhosis may occur due to higher lipid peroxidative reaction during the microsomal breakdown of ethanol. It is plausible to infer that alcohol is capable of inducing changes in the composition of membrane lipids *in vivo*. Alcohol leads to altered membrane phospholipid and increased lipid peroxidation, as a result of increased generation of reactive oxygen species (ROS) during ethanol oxidation in the liver. The damaging effect of these free radicals is due to the decrease in antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase. Additional damages may likely result from the direct effect of acetaldehyde formed from the oxidation of ethanol (Kapur *et al.*, 1994, Sandhir and Gill, 1999; Dhingra *et al.*, 2011; Jaspreet, 2011).

The hepatotoxicity of paracetamol is established mostly as a result of overdose or overindulgence, in which case it causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large and excessive lesions. Therapeutic dose of paracetamol is usually metabolized to sulphate and

glucuronide conjugates and further degraded to a reactive midway which is detoxified by conjugation with glutathione. But in overdose, the sulphate and glucuronide conjugation pathways are drenched and drugs are converted to the reactive metabolite. The glutathione is rapidly exhausted and the metabolite accumulated and binds covalently to liver cell proteins, causing necrotic damages to the liver (Dhingra, 2011; Jaspreet, 2011).

Nonsteroidal anti-inflammatory drugs, NSAIDS, have been fingered as a major group of drugs exhibiting hepatotoxicity. Dose-dependent and idiosyncratic reactions are documented. For instance, aspirin and phenylbutazone have been linked with intrinsic hepatotoxicity, while idiosyncratic reactions have been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin (Jaspreet, 2011).

2.5.1 Liver Diseases and Medicinal Plants use

Recently, there has been an increasing demand for ayurvedic and herbal drugs and some medicinal herbs have been proven to possess hepatotprotective potential. Silymarin is a flavonol-lignan mixture extracted from *Silybum marianum*, and noted as a popular remedy for hepatic diseases (PDR, 1998). Presently, herbal formulations touted for curing or managing liver disorders are in the marketplaces but in actual sense, only selected medicinal herbs have been tested for hepatotprotective activity (Bruneton, 1999). Some herbal formulations claiming to be hepatoprotective may actually contain hepatotoxic chemical constituents. Andrographolide (*Andrographis paniculata*), Glycyrrhizin (*Glycyrrhiza glabra*), Picrorrhizin (*Picrorrhiza kurroa*) and Hypo-phyllanthin (*Phyllanthus niruri*) are potential candidates with hepatoprotective activity (Kumar *et. al.*, 2012).

In tables 2.3 and 2.4 are some hepatoprotective herbal treatments and some commercial preparations sourced from medicinal plants for managing liver maladies. These remedies' workings are based on any of the following mechanisms: (1) Anti-hepatotoxicity effects against hepatitis-causing agents, (2) Hepatotrophic effects, i.e., promotion of healing process of the liver, and (3) Hepatoprotective effects, helping to prevent various liver infections.

Table 2.3: List of some plants evaluated for hepatoprotective effects.

S/N	Plant & Family	Part used	Extractant	Animal	Model	Phytochemical	Reference
1	<i>Flacourtia indica</i> (Burm. f.) Merr (Salicaceae)	Leaves	Petroleum ether and ethyl acetate	Albino Wistar rats.	Paracetamol	Tannins, flavonoids,	Mohamed, 2010
2	<i>Annona squamosa</i>	Fruit	Ethanol	Swiss albino mice	1. Isoniazid + rifampicin, 2. Diethylnitrosamine	Alkaloids	Raj <i>et al.</i> , 2009.
3	<i>Silybum marianum</i>	Seeds	Ethanol & Chloroform	Albino Wistar rats.	thioaceta-mide	Flavonoids	Madani <i>et al.</i> , 2008
4	<i>Chamomile recutita</i> capitula		Ethanol	Wistar rats	Paracetamol	Flavonoids	Gupta <i>et al.</i> , 2006
5	<i>Coccinia grandis</i> Linn (Curcubitaceae)	Fruit	Ethanol	Wistar rats	CCl ₄	Phenolic and flavonoid	Vadivu <i>et al.</i> , 2008
6	<i>Wedelia calendulacea</i> L. (Asteraceae)		Ethanol	Wistar rats	CCl ₄	Isoflavonoids	Murugaian <i>et al.</i> , 2008
7	<i>Prosthechea michuacana</i> (Orchidaceae)	Orchid (Aerial parts, seudobulbs)	Methanol	albino rats	CCl ₄ , paracetamol	Apigenina.	Rosa and Rosario, 2009
8	<i>Aegle marmelos</i> (Rutaceae)	Leaves	Water/ethanol	MCF7 and MDA- MB-231 breast cancer cell lines	Ehrlich ascites carcinoma cells	Skimmianine	Pallab <i>et al.</i> , 2009
9	<i>Cassia roxburghii</i> DC	Seed	Methanol	albino rats	ethanol- CCl ₄	Flavonoids	Arulkumaran <i>et al.</i> , 2009
10	<i>Orthosiphon stamineus</i>	Leaves	Methanol	Male <i>Sprague</i> <i>Dawley</i>	Thioacetamide	Rosmarinic acid, flavones, flavonol glycosides	Mohammed <i>et al.</i> , 2011

Table 2.3: List of some plants evaluated for hepatoprotective effects. (Cont'd)

S/N	Plant & Family	Part used	Extractant	Animal	Model	Phytochemical	Reference
11	<i>Ficus carica</i> Linn. (Moraceae)	Leaves, fruit	Methanol	albino rats	CCl ₄ , rifampicin	Flavonoids, coumarin, sterol, anthocyanins	Krishna <i>et al.</i> , 2007), Gond <i>et al.</i> , 2008.
12	<i>Lepidium sativum</i> (L) (Brassicaceae)	Seeds	Methanol	albino rats	CCl ₄	Flavonoids	Afaf <i>et al.</i> , 2008).
13	<i>Sargassum polycystum</i> (Phaeophyceae)	NI	Ethanol	albino rats	D-galactosamine	NI	Meena <i>et al.</i> , 2008).
14	<i>Solanum nigrum</i> (Solanaceae)	Whole plant	Water, methanol	Mice, Rats	Thioacetamide, CCl ₄	Carotenase, lutein, lycopene, vitamin C.	Mohamed <i>et al.</i> , 2010, Elhag <i>et al.</i> , 2011.
15	<i>Eclipta alba</i> (Compositae)	Leave, Root	Methanol, CHCl ₃	Albino Wister rats	CCl ₄	Flavanoids (Coumastone)	Lal <i>et al.</i> , 2010.
16	<i>Foeniculum vulgare</i> Mill. (Umbelliferae)	Seeds (fruit)	NI	Rats	CCl ₄	essential oil	Anil <i>et al.</i> , 2012.
17	<i>Trigonella foenum graecum</i> (Leguminosae)	Seed	Phenol	human Chang liver cells	Ethanol	garcinone E	Anil <i>et al.</i> , 2012.
18	<i>Jatropha curcas</i> Linn (Euphorbiaceae),	Leave	Methanol	Human hepatocellular carcinoma cells	Aflatoxin B1 (AFB1)	Flavonoids	Anil <i>et al.</i> , 2012.
19	<i>Garcinia mangostana</i> Linn. (Guttiferae)	Fruit peel	Methanol	human liver cancer cell lines	CCl ₄	Xanthones (garcinone E)	Anil <i>et al.</i> , 2012.
20	<i>Andrographis paniculata</i> Kalmegh (Acanthaceae)	NI	Methanol	Albino wistar rats	CCl ₄ , Dgalactosamine, ethanol, paracetamol.	Andrographolide	Anil <i>et al.</i> , 2012.

Table 2.3: List of some plants evaluated for hepatoprotective effects. (Cont'd)

S/N	Plant & Family	Part used	Extractant	Animal	Model	Phytochemical	Reference
21	<i>Swertia Chirata</i> (Gentianeaceae)	NI	NI	NI	CCl ₄	NI	Anil <i>et al.</i> , 2012.
22	<i>Phyllanthus amarus</i> (Euphorbiaceae)	NI	NI	NI	Aflatoxin	NI	Anil <i>et al.</i> , 2012.
23	<i>Morinda citrifolia</i> L. (Noni) (Rubiaceae)	NI	NI	NI	CCl ₄	NI	Anil <i>et al.</i> , 2012.
24	<i>Azadirachta indica</i> (Neem) (Meliaceae)	Leave	NI	Albino wistar rats	paracetamol.	NI	Anil <i>et al.</i> , 2012.
25	<i>Fumaria indica</i> (Fumariceae)	NI	Petroleum ether, Water, Methanol	Albino wistar rats	CCl ₄ , paracetamol, rifampicin.	NI	Anil <i>et al.</i> , 2012.
26	<i>Picrorhiza kurroa</i> (Scrophulariaceae)	NI	Ethanol	NI	NI	NI	Anil <i>et al.</i> , 2012.
27	<i>Cassia fistula</i> (Fabaceae)	Leave	<i>n</i> -Heptane	Albino wistar rats	Paracetamol	NI	Anil <i>et al.</i> , 2012.
28	<i>Careya arborea</i> (Myrtaceae)	Bark	Methanol	Mice	Ehrlich ascites carcinoma (EAC) cells	NI	Anil <i>et al.</i> , 2012.
29	<i>Phyllanthus emblica</i> Linn. (Euphorbiaceous)	NI	Ethanol	Albino wistar rats	Ethanol	NI	Anil <i>et al.</i> , 2012.
30	<i>Curcuma longa</i> (Zingiberaceae)	Rhizome	Ethanol	Albino wistar rats	Paracetamol	Curcumin	Anil <i>et al.</i> , 2012.

NI = Not indicated

Table 2.2: List of some plant-based formulations marketed for hepatic disorders.

S/N	Drug	Medicinal plant & family	Plant part	Active constituent	Traditional use	Reference
1	GARLIC8 (ALLIUM)	<i>Allium sativum</i> (Liliaceae)	Bulb	Allicin, propyl disulphide, Allin	Carminative, Expectorant, Anthelmintic	PDR, 1998
2	TURMERIC (CURCUMA)	<i>Curcuma longa</i> (Zingiberaceae)	Fresh Rhizomes	Curcuminoides Volatile oils	Antiinflammatory Condiment	Trease and Evans, 2007; Kokate and Gokhle, 2007.
3	LIQUORICE (MULETHI)	<i>Glycyrrhiza glabra</i> (Leguminosae)	Dried Roots and Stolon	Glycyrrhizin, 18-B- Glycyrrhetic acid	Peptic ulcer, Expectorant.	Handa, 2003
4	PICRORRHIZA (KUTKI)	<i>Picrorrhiza kurroa</i> (Scrophulariaceae)	Dried Rhizomes	Picroside-1, Kutkoside, Amarogentin	Hepatoprotective bitter, bitter tonic	Vadiya <i>et al.</i> , 1996
5	BOLDO	<i>Peumus boldus mol.</i> (Monimiaceae)	Dried leaf	Limonene, Linalol, Rhamnetin, B-pinene	Dyspepsia, Heart burn.	Malhotra, 2001
6	FUMITORY	<i>Fumaria officinalis</i> (Fumariaceae)	Flower	Protopine, Fumaritrine, Fumaricine	Enhance urinary and digestive elimination Function	Malhotra, 2001; Qadry, 2007

Table 2.4: List of some plant-based formulations marketed for hepatic disorders (cont'd)

S/N	Drug	Medicinal plant & family	Plant part	Active constituent	Traditional use	Reference
7	TEA (THEA)	<i>Thea sinensis</i> (Theaceae)	Leaf buds	Caffeine, Theobromine, Theophylline	CNS stimulant, Diuretic	Handa, 2003.
8	GOLDEN SEAL	<i>Hydrastis Canadensis</i> (Ranunculaceae)	Dried rhizome and roots	Hydrastine, Berberine	Conjunctival hyperthermia of allergic or seasonal origin.	Trease and Evans, 2007; Kokate and Gokhle, 2007.
9	KALMEGH	<i>Adrographis paniculata</i> (Acanthaceae)	Leaves or entire aerial	Adrographolide, Kalmeghin, Flavonoids, Phenols	Stomachic, cholagogue, Liver protective, Dyspepsia	PDR, 1998; Handa, 2003.
10	GADUCHI	<i>Tinospora cardifolia</i> (Monispermaceae)	Dried stem	Clerodone, Tinosporal, Tinosporon, Berberine	Antipyretic, Analgesic	Qadry, 2007; Trease and Evans, 2007.
11	PUNARNAVA	<i>Boerhavia diffusa</i> (Nictagenaceae)	Dried whole plant	Punarnavine, Punarnavoside	Hepatoprotective, Diuretic	PDR, 1998.

2.6 CYTOTOXICITY AND CYTOTOXIC COMPOUNDS

Cytotoxicity is a term used to describe how acutely toxic or poisonous to the cell a substance can potentially be. Exposure to cytotoxic substances can result in permanent cellular damage or even death (Karize and Fritsky, 2003).

Substances that can be described as cytotoxic include some chemicals or even other types of cells. In the case of chemical compounds, some naturally produced ones can present in the form of animal venom, such as spiders and snake venoms. The family of vipers, for example, is known to release a type of cytotoxin called haemotoxin, which is able to rupture red blood cells and cause internal bleeding and organ damage. Another dangerous cytotoxin is cardiotoxin, which is often associated with a king cobra's venomous bite. The toxin attaches itself to the muscle cells in the heart, causing the organ to stop pumping blood, which can result to death. As for synthetically produced chemicals, their cytotoxicity does not always bring a negative effect but can actually be used as a treatment. Such is the case for chemotherapy, a common treatment option for cancer patients. What chemotherapy does is either stop these cells from multiplying or ultimately kill them. The good thing about chemotherapy is that many important types of cells in the body – such as those of the heart, brain, and the bones — are not affected, since their normal regeneration rate is not as fast as those of malignant cells. Other types of cells that normally have a rapid turnover, however, may be affected by chemotherapy. These cells can include those in the gut, the hair, and the mouth. This is why cancer patients who are undergoing chemotherapy may experience diarrhoea, hair loss, and soreness in the mouth, among other side effects caused by chemotherapy's cytotoxicity (Karize and Fritsky, 2003).

The human body also produces cytotoxic cells that help fight off infection-causing viruses and foreign bodies. One of these cells is a type of white blood cell called the “cytotoxic T cell,” which has the ability to kill damaged cells due to a virus or a tumor. Another type is the “natural killer cell,” a white blood cell that discharges some proteins and somehow “programs” infected cells to die (apoptosis).

Cytotoxic chemotherapy is one of the chemotherapeutic methods which affect cancerous cells. Cytotoxic agents have been established as one of the most important ways of cancer treatment in the medicine world. Another way to treat cancer is by using cytotoxicity of certain plants. For instance, one of the methods suggested by doctors is the use of specific plants from Malaysia and Thailand, which use cytotoxicity of plants to kill cancerous cells. Plant tissues contain lectins, which kill animal cells; cytotoxic chemotherapeutic agents are developed to help in treatment of cancerous cells (Saetung *et al.*, 2005).

Cytotoxic chemotherapeutic agents interfere with the ability of cancerous cells to divide. This kind of chemotherapy has become one of major therapeutic modalities; the cytotoxic agents divide cells in the body, preventing the dangerous effect of cancerous cells. Cytotoxic agents often include anti-metabolites, antibiotics and alkylating agents. Cytotoxic therapy has proved to be successful in difficult cases, such as in advanced breast cancer. The course of chemotherapy includes several cycles, used in order to minimize possible side effects that the patient might have. Sometimes the use of one chemotherapy regime may be followed by several different regimens to complete the process of medical treatment. For treatment of several types of cancer paclitaxel is often prescribed, which is a chemotherapeutic agent used to treat patients with breast cancer and several other types of cancer, such as lung cancer,

ovarian cancer, head and neck cancers. Paclitaxel is also tested to use for treatment of metastatic melanoma.

Medicinal plants discovered either through systematic screening programs or serendipitously, possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence, despite the advantages of the synthetic and combinatorial chemistry as well as molecular modeling, medicinal plants remain an important source of new drug leads and new chemical entities (Rana and Afifi, 2007). In a study involving 877 small molecules and new chemical entities (NCEs) introduced between 1981 and 2002 nearly half (49%) were natural products, semi-synthetic natural products, semi-synthetic natural products analogues or synthetic compounds based on natural products. Cancer treatments have a prominent position in utilization of medicinal plants as a source of drug discovery; and among the Food and Drug Administration (FDA) approved anticancer and anti-infectious preparations, drugs of natural origin have a share of 60% and 75% respectively (Newman *et al.*, 2003). At present, there is justifiable increased interest in the search of possible anticancer agents from the flora of different countries.

In a recent study, the ethanolic extract of *Salvia dominica*, was found to exhibit nearly five times more cytotoxic effect than its chloroform extract, presenting potential antineoplastic properties. Same plant in an earlier study was demonstrated to possess antiproliferative activity against colorectal adenocarcinoma, choriocarcinoma, prostate adenocarcinoma, B lymphoblast, glioblastoma and endometrium adenocarcinoma cell lines (Fiore *et al.*, 2006). *Salvia dominica* was found to be rich in flavonoids.

The ethanolic extract of *Justicia spicigera* and *Tagetes lucida* as well as aqueous extracts from *Ibervillea sonora*, *Cucurbita ficifolia*, *Tagetes lucida* were investigated for cytotoxic effects using sulforhodamine B assay against T47D (Human Breast cancer) and HeLa (Human cervix cancer) cell lines. All the extracts were shown to be cytotoxic ($ED_{50} < 20\mu\text{g/ml}$) against T47D cell line, meanwhile only the aqueous extract from *Tagetes lucida* and the ethanolic extract from *Justicia spicigera* were cytotoxic to HeLa cell line. But the ethanolic extract from *Justicia spicigera* presented the best cytotoxic effect, correlating with one of its popular uses as a treatment for cancer. Further investigation showed the presence of flavones compounds, viz: Kaempferol-3,7-bisrhamnoside (kaempferitrin) isolated from the ethanol leaf extract, kaempferitrin (II) isolated from the soluble methanol fraction of hexane-ether- methanol (1:1:1) (Vega-Avila *et al.*, 2009).

Saetung *et al.*, (2005) investigated several number of Thai medicinal plants used for cancer treatment and found that ethanolic extracts of *Dioscorea membranacea* and *Curcuma zedoaria* showed specific activity against lung cancer cell lines but were less cytotoxic against normal cells. In yet another research, it was found that methanolic extract of *Solanum nigrum* had significant cytotoxicity effect on HeLa cell line at a low concentration range of between 10 mg/ml to 0.0196 mg/ml by using SRB (Sulphorhodamine B) assay while its inhibitory action on HeLa cell line was in concentration range of between 10 mg/ml to 0.0196 mg/ml by using MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The IC_{50} value and R^2 value of *Solanum nigrum* on HeLa cell and Vero cell were 847.8 and 0.8724, 9088 and 0.1017 respectively by SRB assay while by MTT assay, the IC_{50} value and R^2 value on HeLa cell alone was 265.0 and 0.9496. The IC_{50} value of *Solanum Nigrum* on Vero cell was 6.862 by MTT assay. These values were indicative of the anticancer activity of the methanolic extract of *Solanum nigrum*, by virtue of its significant cytotoxic activity albeit against HeLa cell line than on Vero cell line (Sanjay *et al.*, 2009).

Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world. A number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or preclinical development (Shoeb, 2006). Figures 2.11 and 2.12 show a number of these anticancer agents derived from plants and currently in clinical use and those holding good promise for future development as anticancer agents.

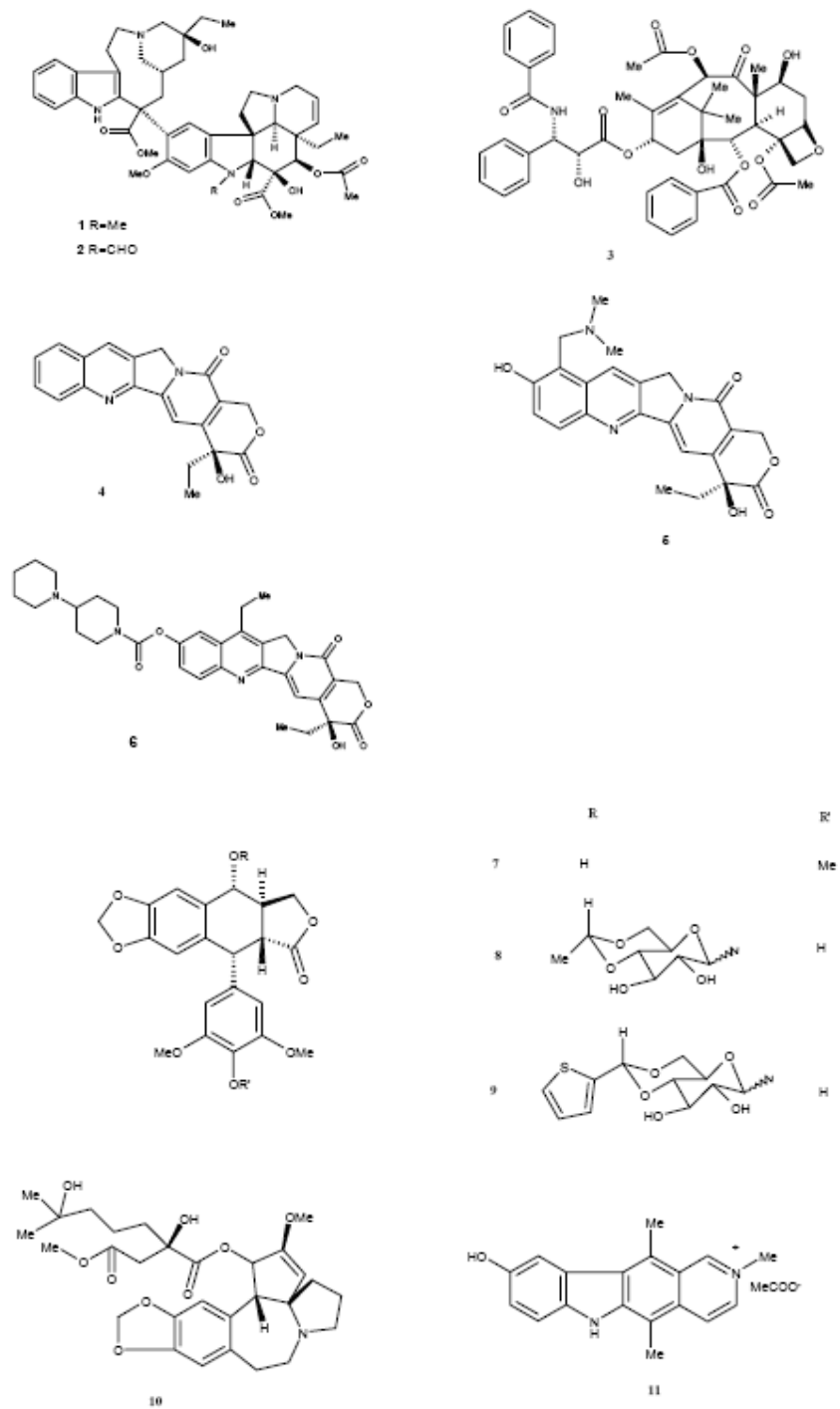


Figure 2.1: Plant-derived anticancer agents in clinical use

Vinblastine (1), and Vincristine (2), Paclitaxel (Taxol®) (3), Camptothecin (4), Topotecan (5), Irinotecan (6), Epipodophyllotoxin (7), Etoposide (8), Teniposide (9), Homoharringtonine, (10), Elliptinium (11).

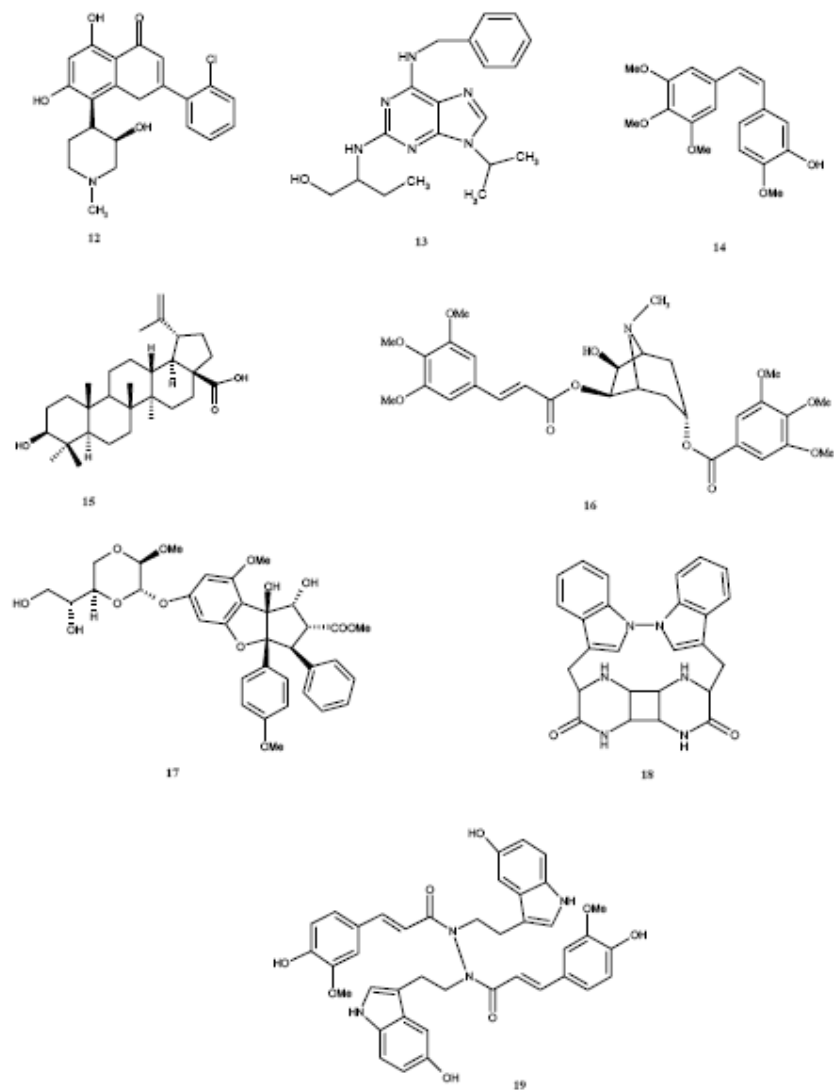


Figure 2.2: Plant-derived anticancer agents for future development.

Flavopiridol (12) Roscovitine (13), Combretastatin A-4 (14) Betulinic acid (15), Pervilleine-A (16) Silvestrol (17) schischkinnin (18) and montamine (19)

Table: 2.1 Plant derived anticancer agents in clinical use

Phytochemical	Name of drug or compound	Plant source	Family	Common name	Cancer type	Reference
Vinca alkaloids	Vinblastine	<i>Catharanthus roseus</i> G. Don	Apocynaceae	Madagascar periwinkle	Leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma.	Cragg and Newman, 2005.
Vinca alkaloids	Vincristine	<i>Catharanthus roseus</i> G. Don	Apocynaceae	Madagascar periwinkle	Leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma.	Cragg and Newman, 2005.
-	Paclitaxel Taxol®	<i>Taxus brevifolia</i> Nutt, <i>Taxus Canadensis</i> Marshall, <i>Taxus baccata</i> L., <i>Taxus baccata</i>	Taxaceae	Bark of the Pacific Yew	Ovarian cancer, advanced breast cancer, small and non-small cell lung cancer and non-cancerous cases.	Rowinsky <i>et al.</i> , 1992; Cragg and Newman, 2005.
-	Camptothecin	<i>Camptotheca acuminata</i> Decne	Nyssaceae	Chinese ornamental tree	Cancer treatment, but dropped due to severe bladder toxicity.	Potmeisel, 1995
-	Topotecan	Semi-synthetic derivative of camptothecin	-	-	Ovarian and small cell lung cancers.	Creemers <i>et al.</i> , 1996; Bertino, 1997.
-	Irinotecan	semi-synthetic derivative of camptothecin	-	-	Colo-rectal cancers.	Creemers <i>et al.</i> , 1996; Bertino, 1997
-	Epipodophyllotoxin	<i>Podophyllum peltatum</i> Linnaeus and <i>Podophyllum emodi</i> Wallich	Berberidaceae	Roots of Podophyllum species	Anti-tumour.	Stahelin, 1973.

Table 2.1: Plant derived anticancer agents in clinical use (cont'd)

Phytochemical	Name of drug or compound	Plant source	Family	Common name	Cancer type	Reference
-	Etoposide	semi-synthetic derivatives of epipodophyllotoxin	-	-	Lymphomas and bronchia cancers.	Cragg and Newman, 2005; Harvey, 1997.
-	Teniposide	semi-synthetic derivatives of epipodophyllotoxin	-	-	Testicular cancers.	Cragg and Newman, 2005; Harvey, 1997.
-	Harringtonine, Homoharringtonine	Cephalotaxus harringtonia var. drupacea (Sieb and Zucc.)	Cephalotaxaceae	Chinese tree	Myelogenous leukemia and chronic myelogenous leukemia.	Cragg and Newman, 2005; Kantarjian <i>et al.</i> , 1996.
-	Elliptinium	<i>Bleekeria vitensis</i> A.C. Sm.	-	-	Breast cancer.	Cragg and Newman, 2005.

Table 2.2: Plant-derived anticancer agents for future development

Phytochemical	Name of drug or compound	Plant source	Family	Plant part	Cancer type	Reference
Alkaloid rohitukine	Flavopiridol	<i>Dysoxylum binectariferum</i> Hook. f.	Meliaceae	-	Broad range of tumors, including leukemia, lymphomas and solid tumors.	Kelland <i>et al.</i> , 2000; Christian <i>et al.</i> , 1997.
Olomucine	Roscovitine	<i>Raphanus sativus</i> L.	Brassicaceae	-	Phase II clinical trials in Europe for colon cancer.	Cragg and Newman, 2005; Meijer <i>et al.</i> , 2003.
-	Combretastatin A-4	<i>Combretum caffrum</i> (Eckl. & Zeyh.) Kuntze	Combretaceae	Bark	Colon, lung and leukemia cancers.	Ohsumi <i>et al.</i> , 1998; Pettit <i>et al.</i> , 1995.
Pentacyclic triterpene	Betulinic acid	<i>Betula</i> species, <i>Zizyphus mauritiana</i> , <i>Zizyphus rugosa</i> , <i>Zizyphus oenoplia</i> .	Betulaceae	-	Human melanoma.	Cichewitz <i>et al.</i> , 2004; Pisha <i>et al.</i> , 1995; Cragg and Newman, 2005.
-	Pervilleine A	<i>Erythroxylum pervillei</i> Baill.	Erythroxylaceae	Root	Against multidrug resistant (MDR) oral epidermoid cancer cell line (KB-V1). In preclinical development.	Silva <i>et al.</i> , 2001; Mi <i>et al.</i> , 2003).
-	Silvestrol	<i>Aglaila sylvestre</i> (M. Roemer) Merrill	Meliaceae	Fruits	Lung and breast cancer cell lines.	Hwang <i>et al.</i> , 2004; Cragg and Newman, 2005.
Alkaloid	Schischkinnin	<i>Centaurea schischkinii</i>	-	Seeds	Human colon cancer.	Shoeb <i>et al.</i> , 2005; Shoeb, 2006.
Alkaloid	Montamine	<i>Centaurea montana</i>	-	Seeds	Human colon cancer.	Shoeb <i>et al.</i> , 2005; Shoeb, 2006.

2.6.1 Genotoxicity

While cytotoxicity refers to the degree to which an agent possesses a specific destructive action on certain cells, genotoxicity describes an agent's damaging ability to the DNA which can cause mutations that can result in cancer. Thus, genotoxicity is a broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced (ICH, 2011). The mechanism for DNA damage, leading to mutation, is due to the attack of hydroxyl radicals on guanine in DNA. Free radicals such as superoxide anion and nitric oxide intervened in the inflammatory process. Nitric oxide can induce cyclo-oxygenase, the rate limiting step enzyme for inflammation process and enhance production of interleukin-1 and tumor necrosis factor (Jang and Murrell, 1998).

The current increasing global wave of interest in the use of medicinal plants in treating/managing diverse ailments has inadvertently increased the chances of introducing compounds that may be genotoxic or genoprotective into the body. According to Passos *et al.* (2010) studies on the genotoxicity and anti-genotoxicity of natural plant extracts can help evaluate the safety and effectiveness of herbal products. Although herbal preparations have been traditionally used in cancer treatment, they could contain bioactivated components that can promote cancer development (Siddique *et al.*, 2008).

For instance, among some 20 local plant extracts tested for genotoxicity to lymphocyte DNA, two of them namely, *Quercus infectoria* Olivier and *Vitex pinnata*

L exerted more than 50% DNA strand breaks, indicating severe damage. These plants have been widely used in traditional treatments and as health supplements in Southeast Asia. Specifically, the Kadazan and Dusun people in Sabah, East Malaysia have long used *Vitex pinnata* to treat cuts and wounds, where the pounded leaves were made into a paste, while the young shoots were consumed for managing hypertension (Wan-Ibrahim *et al.*, 2010). *Quercus infectoria* gall extract had similarly been reported to possess potent free radical scavenging and antioxidant activity owing to its high polyphenolic content (Kaur *et al.*, 2004; Kaur *et al.*, 2008). The gall contains a high amount of tannins (or tannic acid) and gallic acid (Dar *et al.*, 1976). Bhat and Hadi (1994) have reported that tannic acid, which is a type of water-soluble polyphenol, could cause DNA breakage in calf thymus and supercoiled DNA in the presence of Cu (II) (Wu *et al.*, 2004).

Many researchers have probed into the effects of medicinal and herbal plants on human tissue and DNA. It has been revealed that animals possessing highest rates of oxidative damage induced by free radicals generally have the shortest life spans (Wan-Ibrahim *et al.*, 2010). Also, humans who have genetic diseases resulting in greater spontaneous DNA damage or inefficient DNA repair usually show signs of premature ageing. Exposure to external causes of damage, such as ultraviolet light and cigarette smoke have been shown to contribute to decreased lifespan (Friedberg *et al.*, 1995). Thus, one can possibly prolong lifespan by protecting the cell against DNA damage and by enhancing the efficiency of DNA repair.

The anti-genotoxic assessment of medicinal plant extracts is extremely important because they constitute very attractive sources of compounds that can be used in the development of novel pharmaceutical products (Simões *et al.*, 2000). Also, genotoxicity tests form an important part of risk assessment of potential carcinogens (Madhumita *et al.*, 2008).

Gonzalez-Avila *et al.* (2002) discovered that methanolic extracts of *Rhoeo discolor* was not genotoxic in liver cell culture even at concentrations as high as 166-fold of those needed for maximal chemoprotective activities. *Rhoeo discolor* is an endemic plant of south-eastern Mexico commonly used to treat cancer, venereal diseases and mycoses. Madhumita *et al.* (2008) extensively researched on the genoprotection of a wide array of dietary phytochemicals, including capsaicin from green chillies and capsicum, curcumin from rhizome of turmeric, ellagic acid from guava, fisetin from strawberries, gallic acid from ginger, limonene from lemon and orange peel, lycopene from tomato, quercetin from citrus fruits, broccoli, onions, red grapes, resveratrol from grapes, and rutin from apricots, cherries and citrus fruits. These phytochemicals were tested against arsenite induced genotoxicity and all were found to confer various levels of protection against DNA damage to normal mammalian V79 cells. However, curcumin gave better protection during simultaneous treatment while that of resveratrol was during pre-treatment and ellagic acid gave better results of genoprotection during repair experiments.

Ceasalpinia sappan Linn. is a medicinal plant cultivated in Northern Thailand and has been used for various disease treatment especially inflammatory symptom, arthritis

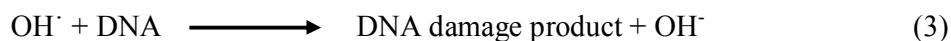
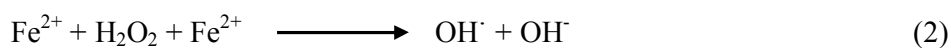
and cancer. Ethanolic extract of the heartwood of *C. sappan* extract which was studied for its protective effect on DNA damage-induced by hydroxyl radical. *C. sappan* extract exerted protective effect on DNA damage-induced by hydroxyl radical. According to the strong activity on free radical scavenging *in vitro* and protective effect on DNA damage-induced by hydroxyl radical, *C. Sappan* presented a high potential for chemoprevention (Saenjum *et al.*, 2010).

‘Douradinha’ is the popular name by which the medicinal plant - *Palicourea coriacea* is known in Brazilian Cerrado region. The plant has been used in folk medicine to treat kidney and urethral stones and kidney inflammation. The aqueous extract of the plant was examined for cytotoxic, genotoxic, and possible anti-genotoxic activities on somatic cells of *Drosophila melanogaster*, using the somatic mutation and recombination test. A third-stage larvae of *D. melanogaster* from a standard cross and a high bioactivation cross was used, and tested against 10 different doses of aqueous extract of *P. coriacea* (5, 15, 25, 35, 50, 65, 80, 95, 110, and 125 mg/mL). Doxorubicin (0.125 mg/mL) was used as a positive control and distilled water as a negative control. None of the doses showed lethality to the larvae. There was no genotoxic effect at 5, 10, or 15 mg extract/mL. However, a significant decrease in the frequency of spots induced by doxorubicin was observed when administered with *P. coriacea* aqueous extract at these same doses. These indicated clearly that the aqueous extracts of *P. coriacea* were not cytotoxic or genotoxic at these doses but did protect against the genotoxic action of doxorubicin.

An *in vivo* assessment of DNA damage and the protective effects of methanolic extracts of *Miconia* species, viz: *Miconia albicans*, *Miconia cabucu*, *Miconia rubiginosa*, *Miconia stenostachya* and the chloroformic extract of *M. albicans* indicated a protective effect against cyclophosphamide induced DNA damage (Serpeloni *et al.*, 2008). The study showed the advantages in using crude extracts than the application of isolated compounds in protecting the DNA.

The aqueous extract of leaf (LE), fruit (FE) and seed (SE) of *Moringa oleifera* was assessed to examine the ability to inhibit the oxidative DNA damage, antioxidant and anti-quorum sensing (QS) potentials. It was found that these extracts could significantly inhibit the OH[•]-dependent damage of pUC18 plasmid DNA (Singh *et al.*, 2009).

Damage to plasmid DNA results in a cleavage of one of the phosphodiester chains of the supercoiled DNA and produces a relaxed open circular form. Further cleavage near the first breakage results in linear double-stranded DNA molecules. The formation of circular form of DNA is indicative of single-strand breaks and the formation of linear form of DNA is indicative of double-strand breaks (Burrows and Muller, 1998). Since the Fenton's reagents are composed by ascorbic acid, H₂O₂ and Fe³⁺. They can produce OH[•] according to the following reaction (Lee *et al.*, 2002).



The formation of Fe^{2+} after reduction of Fe^{3+} in the presence of potent reducing agent ascorbic acid, initiate the decomposition of H_2O_2 leading to generate highly reactive free radical species $\text{OH}^\cdot$ via Fenton-like reactions above (equations 1 and 2) (Singh *et al.*, 2009).

Majority of the oxidative damage in the biological system is caused by the $\text{OH}^\cdot$ (Halliwell and Gutteridge, 1984). These $\text{OH}^\cdot$, bound on the DNA, would lead to strand breakage, de-oxy sugar fragmentation and base modification. In fact, pyrimidine dimers formed due to UV exposure of scDNA can be corrected by synthesis of the enzyme photolyase that cleaves this pyrimidine dimer. This is followed by ligation, which rectifies the damage. In that particular study, the possible role of the endophytic fungal metabolites may involve the activation of the locus called phr(photo-reactivation) locus by visible light, which results in the synthesis of the enzyme followed by ligation (Krishnan and Maru, 2004). The results of the study indicated that the crude endophytic metabolites prevented DNA damage under the test conditions. Efforts were being made in order to isolate and characterize the active components of the metabolites responsible for the phenomenon using LC-MS and gas chromatography (Bhagobaty and Joshi, 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Materials used for anti-oxidation experiments

Chemicals/Reagents

Petroleum ether, Methanol, α -tocopherol, Ascorbic acid, Carbon tetrachloride (CCl_4), Thiobarbituric acid (TBA), trichloro-acetic acid (TCA), Corn oil (Cash 'n' Carry, Lagos), Assay kits for catalase, glutathione peroxidase and superoxide dismutase (BioVision Inc., 155 South Milpitas Blvd, Milpitas, CA 95035, USA).

Except where otherwise stated, all chemicals were supplied by Zayo-Sigma Chemicals Limited, Zayo House, Yakubu Gowon Way, Jos, Plateau State.

Equipment

Spectrophotometer: 6405 UV/VIS Jenway laboratory equipment, Japan; Elisa plate reader ELX 800 BioTek, U.S.A.; Soxhlet extractor (500ml capacity); Centrifuge: Labofuge 300 Heraeus, UK; Tissue homogenizer.

3.1.2 Materials used for Anti-inflammation experiments

Chemicals/Reagents

Carrageenan, diclofenac sodium (diclofenac Na, as standard anti-inflammatory drug), plant extracts, Griess' reagent.

Equipment

Oral cannula, 1ml syringes, 1ml injection needles, plethysmometric tube, micrometer screw gauge, indelible paw marker.

3.1.3 Materials used for Hepatoprotection experiments

Chemicals/Reagents

Assay kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom.

Equipment

(Same as in anti-oxidation)

3.1.4 Materials used for Cytotoxicity experiments

Chemicals/Reagents

Sea salt, 0.1M NaOH, plant extracts.

Agarose gel electrophoresis kit, Quercetin standard, pUC18 plasmid, Fenton's reagent, plant extracts.

Equipment

Four (4) and twelve (12) outlet Air pumps and tubings, 30 air stones, 24 brine shrimp hatchery tanks, 2 short tube fluorescent light bulbs, 2 magnifying hand lens, magnetic plate (with stirrer), 10ml syringe, saucers (white inner surface). Electrophoresis machine, UV lamp, camera.

3.2 METHODOLOGY

3.2.1 Collection and authentication of plant sample

The different parts of *Morinda lucida* plant were collected from Idah Local Government Area of Kogi State. They were compressed in a plant press and sent to the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication. A specimen with voucher number: **1862** was deposited at the herbarium.

3.2.2 Plant extracts preparation and determination of percentage yield

Procedure for extract preparation was carried out as described by Atawodi *et al.* (2010). Fresh plant parts, namely, whole fruit, leaf, bark, and root were collected and dried under shade. The dried materials were then pulverized with a mortar and pestle. The pulverized samples were then extracted with petroleum ether for de-fatting, for 15 – 35 hours, with the more oily parts requiring longer hours then removed. The residues were spread on the filter paper to dry for 30 minutes then re-extracted three times with methanol for 5 hours each. Additional five hours was allowed until solvent in the delivery arm of the soxhlet extractor became as colourless as the fresh methanol. The extracts were then pooled together and concentrated with rotary evaporator at 60°C for 3 hrs. The extract with relatively less solvent was poured into an already dried and weighed crucible then allowed to dry over water bath at 45°C for 12 hours. The gummy extracts were then transferred to desiccators containing activated silica gel for between 6 – 8 weeks until extracts became dry, depicted by

constant weights recorded during the weekly weight measurements. Extracts yield were then quantified by method described by Mishra *et al.* (2005) as:

$$\text{Percentage (\%) yield} = \frac{(\text{Weight of extract} + \text{Weight of crucible}) - (\text{Weight of crucible})}{\text{Weight of plant powder used}}$$

3.3.1 Care of experimental rats

Cages: Sixteen (16) rat cages were obtained from Bingham University Animal house and six rats were housed per cage.

Animal feeds: Standard regular rodent pellet for rats was used for the animal model obtained from Vital Feeds depot, Gizzard plaza, Mararaba, Nasarawa State.

Animals: Two hundred male albino Wistar rats weighing 150 – 299g obtained from Afkol farms, Jos, Plateau state were used for the different experiments in this work.

Animal Management: Animal care was in compliance with the general guidelines for animal experimentation.

3.3.2 Choice of carbon tetrachloride for antioxidant and hepatotoxicity experiments

Carbon tetrachloride (CCl₄) can form the trichloromethyl radical (·CCl₃) which can bind to cellular molecules, damaging crucial cellular progressions. This radical also

react with oxygen to form the trichloromethylperoxy radical ($\text{CCl}_3\text{OO}\cdot$), which is a

3.3.4 Extract pre-treatment (Hepatoprotective effect) experiment

very reactive specie. It can induce liver damages for the study of hepatotoxicity in acute and chronic liver failure, as a single dose of CCl_4 can cause centrilobular cell necrosis of the liver and changes in lipid profiles within three (3) hours of administration (Dawkins 1963). These attributes makes CCl_4 a choice candidate for both anti-oxidant and hepatotoxicity studies.

3.3.3 Animal experiment groupings for Anti-oxidation experiment

A total of 96 male albino Wistar rats were used. They were broadly divided into extract pre-treatment (Protective effect) and extract post-treatment (Curative effect) groups of 6 animals each their sub-groups as follows:

3.3.5 Extract post-treatment (Hepatcourage effect) experiment

Group Treatments

- A1 Normal control: Received neither CCl₄ nor extract for the 7 days
- A2 CCl₄ (0.6ml /kg on day 1) only
- A3 α -Tocopherol (0.20mg/ kg daily, on days 1 - 7) + CCl₄ (0.6ml /kg on day 7)
- A4 Ascorbic acid 0.86mg/ kg on days 1 - 7) + CCl₄ (0.6ml /kg on day 7)
- B1 Fruit extract (100mg/ kg daily, on days 1 - 7) only
- B2 Leaf extract (100mg/ kg daily, on days 1 - 7) only
- B3 Bark extract (100mg/ kg daily, on days 1 - 7) only
- B4 Root extract (100mg/ kg daily, on days 1 - 7) only
- C1 Fruit extract (100mg/ kg daily, days 1 - 7) + CCl₄ (0.6ml /kg on day 7)
- C2 Leaf extract (100mg/ kg daily, days 1 - 7) + CCl₄ (0.6ml /kg on day 7)
- C3 Bark extract (100mg/ kg daily, days 1 - 7) + CCl₄ (0.6ml /kg on day 7)
- C4 Root extract (100mg/ kg daily, days 1 - 7) + CCl₄ (0.6ml /kg on day 7)

One (1) part of CCl₄ was mixed with two (2) parts of corn oil.

Group Treatments

- A1 Normal control: Received neither CCl₄ nor extract for the 7 days
- A2 CCl₄ (0.6ml /kg on day 1) only
- B1 Fruit extract (100mg/ kg daily, on days 1 - 7) only
- B2 Leaf extract (100mg/ kg daily, on days 1 - 7) only
- B3 Bark extract (100mg/ kg daily, on days 1 - 7) only
- B4 Root extract (100mg/ kg daily, on days 1 - 7) only
- C1 CCl₄ (0.6ml /kg b. w. on day 7) + Fruit extract (100mg/ kg daily, days 1 - 7)
- C2 CCl₄ (0.6ml /kg b. w. on day 7) + Leaf extract (100mg/ kg daily, days 1 - 7)
- C3 CCl₄ (0.6ml /kg b. w. on day 7) + Bark extract (100mg/ kg daily, days 1 - 7)
- C4 CCl₄ (0.6ml /kg b. w. on day 7) + Root extract (100mg/ kg daily, days 1 - 7)

One (1) part of CCl₄ was mixed with two (2) parts of corn oil.

3.3.6 Collection of animal samples

Rats were sacrificed after administration of the methanolic extracts of the fruit, leaf, bark and root of *M. lucida* for 7 days orally, and single dose of CCl₄ injection (*intra-peritoneally*) on the first or last day for the pre or post treatment groups respectively. On the 7th day, the rats were allowed to fast overnight and then sacrificed under

chloroform anesthesia by decapitation. Blood samples for determination of hematological and serobiochemical parameters were immediately collected. The haematological parameters studied were: Hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), white blood cells (WBC), Mean Corpuscular Volume (MCV), Platelets, Neutrophils, and Lymphocytes.

The serobiochemical parameters studied were Asparate aminotransferase (AST), Alanine aminotransferase (ALT), total protein, blood urea nitrogen (BUN), total creatinine, total bilirubin, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, and triglyceride concentration.

At necropsy, all rats were examined to identify gross lesions, and the specimen of the liver and kidney were quickly removed and fixed in 10% formol-saline for histopathological studies.

3.4.1 Preparation of Serum

Sera were prepared by allowing the collected blood to stand for 1 hour to clot. This was thereafter centrifuged at 1,000 x g for 15min to separate the serum which was collected and stored in a freezer.

3.4.2 Preparation of tissue homogenates

Liver and kidney portions were removed, blotted and weighed then processed for the relevant assays. These procedures have been previously described (Gyamfi *et al.*, 1999 and Usoh *et al.*, 2005).

3.4.3 Animal experiment groupings for Anti-inflammation experiment

A total of 90 male albino wistar rats were used. They were broadly divided fifteen

3.4.4 Carrageenan-induced rat paw oedema anti-inflammation groups

Group	Treatments
N	Normal control: Untreated and un-induced.
C	1% Carrageenan (0.1ml /kg) only
S	Diclofenac (standard drug) (5mg/kg) + 1% Carrageenan (0.1ml /kg)
F1	Fruit extract (100mg/kg) + 1% Carrageenan (0.1ml /kg)
F2	Fruit extract (300mg/kg) + 1% Carrageenan (0.1ml /kg)
F3	Fruit extract (500mg/kg) + 1% Carrageenan (0.1ml /kg)
L1	Leaf extract (100mg/kg) + 1% Carrageenan (0.1ml /kg)
L2	Leaf extract (300mg/kg) + 1% Carrageenan (0.1ml /kg)
L3	Leaf extract (500mg/kg) + 1% Carrageenan (0.1ml /kg)
B1	Bark extract (100mg/kg) + 1% Carrageenan (0.1ml /kg)
B2	Bark extract (300mg/kg) + 1% Carrageenan (0.1ml /kg)
B3	Bark extract (500mg/kg) + 1% Carrageenan (0.1ml /kg)
R1	Root extract (100mg/kg) + 1% Carrageenan (0.1ml /kg)
R2	Root extract (300mg/kg) + 1% Carrageenan (0.1ml /kg)
R3	Root extract (500mg/kg) + 1% Carrageenan (0.1ml /kg)

groups as follows:

3.4.5 LD₅₀ DETERMINATION

Stage 1:

The method described by Lorke (1983) was employed. Three different groups of three (3) rats each, were administered with different doses of the extracts, namely: 10, 100 and 1000mg/kg of extracts and observed for 24 hours for death or physical signs of toxicity.

Stage 2:

Fresh rats were used. Doses equivalent to the rats, based on results obtained in stage 1, were administered and the animals were observed for another 24 hours. Results obtained were recorded.

3.4.6 ANTI-OXIDATION EXPERIMENTS (*IN VIVO*)

Preparation of liver tissue homogenate for determination of Lipid peroxidation, Catalase, Glutathione peroxidase and SOD:

Liver tissues were removed immediately after sacrifice of rats, and washed in ice cold normal saline. They were then blotted, weighed and homogenized in 10vols of ice cold PBS buffer (pH=7.4) followed by centrifugation of homogenate at 11,180 x g for 30min (at 4°C). The supernatant was stored frozen until used for the determination of TBARS, SOD, Catalase and Glutathione peroxidase (Raja *et al.*, 2007; Gyamfi *et al.*, 1999; Usuh *et al.*, 2005).

3.4.6.1 Determination of lipid peroxidation

Principle and procedure:

The assay is based on the reaction of thiobarbituric acid reacting substances (TBARS) with thiobarbituric acid (TBA) to form a pink coloured product. The colour intensity measured at 535nm is directly proportional to TBARS concentration in the sample (Okhawa *et al.*, 1979).

For TBARS assay, 1ml of trichloroacetic acid (15%) was measured into a test tube, followed by 1ml of thiobarbituric acid and 50ul of the liver homogenate. The mixture was incubated at 80°C for 30 minutes in a water bath and allowed to cool for 2 minutes followed by centrifugation at 3000rpm for 10 minutes. The absorbance of a clear supernatant was read spectrophotometrically at 535nm. The same method was used in determination of TBARS concentration of the liver homogenates. TBARS concentrations were expressed in nmol/mg protein as follows:

$$\text{Concentration (nmol/mg protein)} = \frac{\text{Sample Absorbance}}{1.56 \times 10^{-5} \times \text{protein concentration (mg)}}$$

3.4.6.2 Determination of catalase (Cat) activity

Principle and procedure:

In the assay (using Biovision catalase assay kit), catalase first reacts with H₂O₂ to produce water and oxygen, the unconverted H₂O₂ reacts with OxiRed™ probe to produce a product, which can be measured at 570 nm.

Twelve (12) µl of fresh 1mM H₂O₂ was added to the microplate wells of each of the samples (homogenates) and standard. To start the reaction, they were incubated at 25°C for 30 min, followed by addition of 10µl of stop solution into each well to stop the reaction. Fifty (50) ul of developer mix made of 46µl of assay buffer, 2µl of OxiRed™ Probe, 2µl of lyophilized HRP solution was added to each test samples, controls, and standards. They were well mixed and incubated at 25°C for 10 min then, measure at 570 nm in a microplate reader. Catalase activity was calculated as follows:

$$\text{Catalase Activity (nmol/min/ml = mU/mL)} = \frac{B}{30 \times V} \times \text{Sample dilution factor}$$

[Where: B is the decomposed H₂O₂ amount from H₂O₂ Standard Curve (in nmol), V is the pretreated sample volume added into the reaction well (in ml), and 30 is the reaction time (30 min)].

3.4.6.3 Determination of Glutathione peroxidase (GPx) activity

Principle and procedure:

The assay (using Biovision's GPx Assay kit), GPx reduces Cumene Hydroperoxide while converting glutathione (reduced) (GSH) to glutathione (oxidized) (GSSG). The generated GSSG is reduced to GSH with consumption of NADPH by glutathione

reductase enzyme. The decrease of NADPH is proportional to GPx activity and measured at 340nm.

Into each sample, positive control and reagent control wells, 40µl Reaction Mix made of 33µl Assay Buffer, 3µl of 40mM NADPH solution, 2µl of glutathione reductase solution and 2µl GSH solution was added, mixed properly, and incubated for 15 minutes to deplete all GSSG in the samples. Absorbance (A0) is read at 340nm, and Cumene hydroperoxide (10µl) solution was then added to start the GPx reaction. After proper mixing, absorbance (A1) was read at 340nm at time (T1) followed by incubation at 25°C for 5 min and a second absorbance reading (A2) was taken at 340nm at time (T2). GPx activity was calculated as:

$$\text{GPx activity (nmol/min/ml = mU/mL)} = \frac{(B - B^0)}{(T2 - T1) \times V} \times \text{Sample dilution}$$

[Where: B is the NADPH amount that was decreased between T1 and T2 (in nmol), B⁰ is the background change (without Cumene Hydroperoxide) between T1 and T2; T1 is the time of first reading (A1) (in min); T2 is the time of second reading (A2) (in min); and V is the pretreated sample volume added into the reaction well (in ml)].

3.4.6.4 Determination of Superoxide dismutase (SOD) activity

Principle and procedure:

The assay (using Biovision SOD assay kit) is based on the generation of a water-soluble WST-1-formazan dye upon reduction reaction of WST-1 with superoxide

anion, which is linearly related to the xanthine oxidase (XO) activity, inhibited by SOD. The inhibition activity of SOD can be determined colorimetrically.

Twenty (20) µl of liver homogenate (sample solution) was added to each well followed by 200µl of the WST Working Solution. Distilled H₂O (20µl) was added to Blank 1 and Blank 3 wells. Then, 20µl of dilution buffer was added to Blank 2 and Blank 3 wells followed by 20µl of Enzyme Working solution to each sample and Blank 1 wells and thoroughly mixed.

Contents were incubated at 37°C for 20 minutes and absorbance read at 450 nm using a microplate reader. Calculation was made as follows:

$$\text{SOD activity (U/mL)} = 100 - \left[\frac{(A_{\text{blank1}} - A_{\text{blank3}}) - (A_{\text{sample}} - A_{\text{blank2}}) \times 100}{(A_{\text{blank1}} - A_{\text{blank3}})} \right]$$

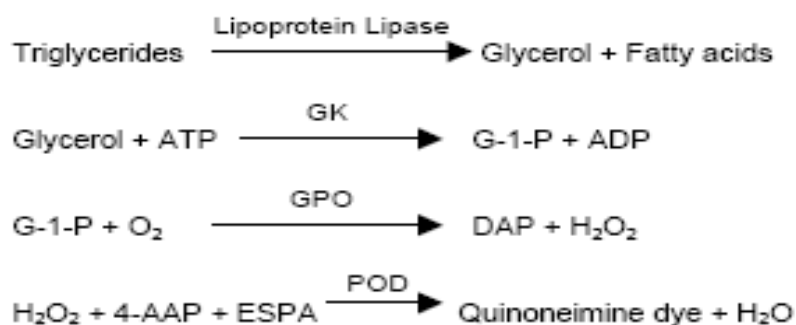
3.4.7 LIPID PROFILE STUDIES

Blood was collected into tubes containing EDTA and red cells were pelleted by centrifugation at 1,000 xg for 20 min and the plasma collected. Plasma was then centrifuged at 15,500 xg for 20 min at 12°C to separate chylomicrons. The infranatant was separated and used for isolation of other lipoprotein fractions i.e. LDL and HDL by sequential density ultra centrifugation (Salter *et al.*, 1998).

3.4.7.1 Total serum triglyceride (TG):

Principle and procedure:

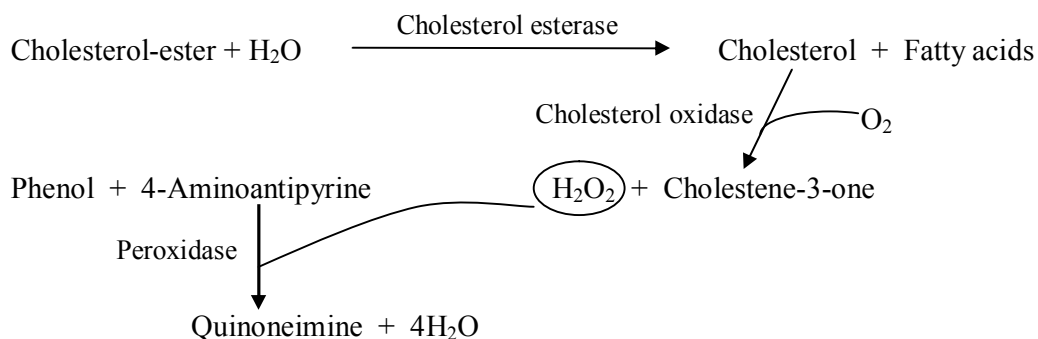
The assay (using Randox laboratories Ltd.) kit was based on enzymatic hydrolysis by lipase of the triglycerides to glycerol and free fatty acids. The glycerol produced was then measured by coupled enzyme reactions. Triglycerides were first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol was then phosphorylated by adenosine-5-triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P was then oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Peroxidase (POD) catalyzes the coupling of H_2O_2 with 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (ESPA) to produce a quinoneimine dye that shows an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to triglyceride concentration of the sample.



3.4.7.2 Total serum Cholesterol (TC)

Principle and procedure:

Method (using the Randox laboratoriesTM) was based on the formation of quinoneimine from the reaction between hydrogen peroxide and 4-Aminoantipyrine in the presence of phenol. The entire reaction is presented as follows:



Absorbance is read at 546nm and the concentration of cholesterol is thereby, calculated.

Into test tubes marked for samples and reagent blank, 10ul of sample and 10ul of distilled H₂O were respectively added. Reagent solution (1ml) was added to all the tubes, then, incubated for 10min. at 25°C. Absorbance was then measured at 546nm against the blank within 1 hour. Calculation of cholesterol in sample was done as follows:

$$\text{Cholesterol (mg/dL)} = \text{Absorbance} \times 840$$

3.4.7.3 High Density Lipoprotein (HDL)

Principle and procedure:

Determination (using Randox laboratoriesTM assay kit) is such that, very low density lipoprotein (VLDL) and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removal by centrifugation the clear supernatant containing high density lipoproteins (HDL) is used for the determination of HDL cholesterol.

Into a centrifuge tube, 100uL of a precipitating reagent made of 14mmol/L of phosphotungstic acid and 2mmol/L of magnesium chloride was added followed by 1ml of the sample (serum or plasma). This was mixed properly and allowed to stand for 10min at room temperature. Centrifugation was carried out at 1,789 xg for 2min). The supernatant of the liquid that separates out was collected for HDL test. Assay was done on test tubes marked as 'blank', 'standard' and 'sample' as required. Contents of each tube was mixed thoroughly and incubated for 5min at 37°C. Absorbance (A) was then read of the samples, standard, and blank at 505nm. Calculation was carried out as follows:

$$\text{HDL-cholesterol (mg/dL)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times (\text{Amount of Cholesterol Standard used})$$

3.4.7.4 Ligh Density Lipoprotein (LDL)

Principle and procedure:

Low density lipoproteins in serum precipitates after addition of a citrate buffered solution on their isoelectronic point. After centrifugation, the very low density lipoprotein (VLDL) and the HDL remains in the supernatant and is determined with the Centronic cholesterolTM reagent. From the difference of the concentration of the total cholesterol and the concentrations of the VLDL and HDL in the supernatant, the LDL-cholesterol concentration is calculated.

Into a test tube, 1ml of precipitation reagent was added followed by 0.1ml of serum then, mixed properly and incubated at room temperature for 10min. The mixture is then centrifuged for 15min. at 1,800 xg. The supernatant (0.1ml) was taken and 1ml of cholesterol reagent was added. This was incubated for 10min. at 25°C. Absorbance was finally read against blank at 500nm. The entire procedure was completed within 1hr.

3.4.8 ANTI-INFLAMMATORY TEST

3.4.8.1 Induced paw oedema assay

This assay was carried out following the procedures described by Raji *et al.* (2002), Arulmozhi *et al.* (2005), Onasanwo and Elegbe (2006), Perez and Solis (2009) and Domínguez-Ortiz *et al.* (2010).

Principle and procedure:

Inflammation was induced by injection of phlogistic agent (carrageenan) into the sub-plantar portion of rats left paw, and the differences in paw volumes of rats before and after injection (and treatment for test groups) between initial measurement of the left paw volume at time ($t=0$) and subsequent times was carried out, applying plethysmometric method based on Archimedes principle.

In order to assess the anti-inflammatory properties, three doses of the crude extracts suspended in sterilized distilled water, namely, 100mg/Kg , 300mg/Kg , and 500mg/Kg or 5mg/Kg of diclofenac Na (as standard anti-inflammatory drug) were administered orally. This was followed at exactly 30 minutes later with the administration of 0.10ml of 1% carrageenan equivalences, injected into the left hind paw of the rats. Measurements of the paw volume after inducing inflammation in the left hind paw were carried out at times 't' ($t=30$, $t=60$, $t=90$ and $t=120$ minutes) after administration of the inflammatory agent as earlier done, by the method of water displacement using a graduated tube operated plethysmometrically.

Control group also received only carrageenan, dissolved in sterilized distilled water instead of extract or diclofenac Na. Inflammatory inhibitory capacities of the extracts (or standard drugs) were generally calculated as percentage inhibition (%) using the formular:

$$\left[\frac{\text{Paw volume at } t=0 - \text{Paw volume at } t}{\text{Paw volume at } t=0} \right] \times 100$$

$$\text{Percentage inhibition (\%)} = \frac{(\text{Ct} - \text{Co})_{\text{control}} - (\text{Ct} - \text{Co})_{\text{standard or treated}}}{(\text{Ct} - \text{Co})_{\text{control}}} \times 100$$

[Where, Ct = paw size (at designated times) after induction in the treated or standard group; Co = paw size before induction].

3.4.8.2 Nitric Oxide assay

Principle and procedure:

This procedure is based on the oxidation of NO to nitric acid (HNO₃) which is converted to nitrite which is in turn converted to nitrous acid (HONO) and further to nitrite ions (NO₂⁻) that diazotizes with sulphanilic acid and couples with naphthylethylenediamine (Griess reagent) to produce a pink colour that is measurable at 546nm (Kumar *et al.*, 2008; Ljiljan *et al.*, 2009).

Nitric oxide is generated from sodium nitroprusside in aqueous solution of physiological pH, which interacts with oxygen to produce nitrite ions. This can be measured by the Griess reaction. Two (2ml) of 10mM Sodium nitroprusside in phosphate buffered saline (pH=7.4) was incubated with different concentrations of test compound (e.g. from 10 to 1000ug/ml) or vehicle (control), at room temperature (25°C) for 2hrs. One (1) ml of Griess reagent (1% Sulfanilamide and 0.1% Naphthylethylenediamine dihydrochloride in 2% H₃PO₄) was then added. Absorbance was read at 546nm. Percentage inhibition was then computed as follows,

$$\% \text{ Inhibition} = [(A_o - A_i) / A_o] \times 100$$

[Where, A_o = Absorbance of control, and A_i = Absorbance of test compound]

3.4.9 HEPATOPROTECTIVE/HEPATOCURATIVE TESTS

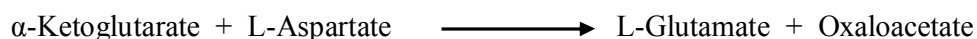
3.4.9.1 Induction of hepatotoxicity

Hepatotoxicity in rats was induced by the method described by Gyamfi *et al.* (1999) where single dose of 0.6ml/kg of tetrachloromethane (CCl_4) was injected intraperitoneally.

3.4.9.2 Determination of Aspartate aminotransferase (AST) activity

Principle and procedure:

AST determination (using the Randox laboratoriesTM assay kit) is based on description of Reitman and Frankel (1957). Aspartate aminotransferase (AST), also known as Serum Glutamate-oxaloacetate (SGOT) activities, catalyses the reaction:



Oxaloacetate so formed is coupled with 2, 4-Dinitrophenyl hydrazine, (pH 7.4) to give the corresponding hydrazone, which gives brown colour in alkaline medium which can be measured colorimetrically.

Buffered phosphate, aspartate and α -Ketoglutarate Substrate (0.25ml) was added to 0.05ml of serum, plus 0.05ml of distilled water, mixed properly and incubated at 37°C

for 30min. Solution of 2, 4-dinitrophenylhydrazine (0.25ml) was then added, mixed well and left to stand at room temperature for 20min. Then, (2.5ml) of NaOH was added, mixed and absorbance read against blank at 546nm. AST concentration was calculated as:

$$\text{ALT (U/I)} = (\text{Absorbance} \times 174) - 0.7333$$

3.4.9.3 Determination of Alanine aminotransferase (ALT) activity

Principle and procedure:

ALT determination (using the Randox laboratoriesTM assay kit) is based on description of Reitman and Frankel (1957). ALT also known as SGPT (Serum Glutamate-pyruvate transaminase) activities, catalyses the reaction:



Pyruvate so formed is coupled with 2, 4-Dinitrophenyl hydrazine, (pH 7.4) to give the corresponding hydrazone, which gives brown colour in alkaline medium which can be measured colorimetrically.

Same procedure as described for AST above, applied to ALT activity determination, and was followed).

3.4.9.4 Determination of bilirubin concentrations

Principle and procedure:

Direct bilirubin reacts with diazotised sulphanilic acid to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid indirect bilirubin was gotten by difference. Conjugated, unconjugated and total bilirubin was estimated as described by Jendrassik and Grof (1938) using assay kits (from Randox Laboratories Ltd).

3.4.9.5 Conjugated (Direct) bilirubin

One (1) drop (0.05ml) of reagent 2 was added into a clean test tube containing 0.20ml of reagent 1. Then, 2.00ml of sodium chloride (9 g/l) was added followed by 0.20ml of serum, mixed and allowed to stand for exactly 5 minutes at room temperature. The absorbance was read against the sample blank (ADB) at 530nm. Concentration of conjugated bilirubin was calculated as:

$$\text{Conjugated bilirubin (mg/dl)} = 14.4 \times \text{ADB}$$

3.4.9.6 Unconjugated (Indirect) bilirubin

This was obtained by difference between total and conjugated bilirubin concentrations as follows:

$$\text{Indirect bilirubin (mg/dl)} = \text{Total bilirubin} - \text{Conjugated bilirubin}$$

3.4.9.7 Total bilirubin:

One (1) drop (0.05ml) of reagent 2 made up of sodium nitrite was added into a clean test tube containing 0.20ml of reagent 1 which is made up of sulphanilic acid and hydrochloric acid and 1.00ml of reagent 3 made up of caffeine and sodium benzoate was added followed by 0.20ml of serum, mixed and allowed to stand for 10 minutes at room temperature. Exactly 1.00ml of reagent 4 made up of Tartrate and sodium hydroxide was also added, mixed and allowed to stand for 10 minutes at room temperature. The absorbance of the sample against sample blank (ATB) was read at 560nm. Concentration of total bilirubin was calculated as:

$$\text{Total bilirubin (mg/dl)} = 10.8 \times \text{ATB}$$

3.5.1 DETERMINATION OF NEPHROLOGICAL EFFECTS

3.5.1.1 Creatinine concentration

This was determined by method described for use of kit (Randox laboratoriesTM).

Principle and procedure:

This method utilizes picrate which forms a red colored complex with creatinine. The intensity of the color, measured at 525nm is directly proportional to creatinine concentration in the sample.

Three (3) ml of the unknown solution (4 or 5 x diluted urine or blood filtrate) are used. To this solution, add 1 ml of 0.04M picrate followed by 1 ml of standardized 0.75N (3%) NaOH. The color is allowed to develop for 15 minutes and remains stable for at least another 30 minutes then read at 525nm. The creatinine content of the solution is calculated from the standard curve (Bonsnes & Taussky, 1945).

3.5.1.2 Determination of blood Urea

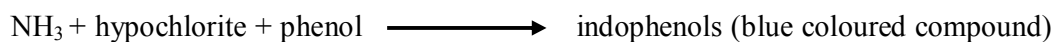
Urea was determined by method described for use of assay kit (Randox laboratoriesTM).

Principle and procedure:

Serum urea is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction below, using (Randox assay kit) according to Mackay and Mackay (1927).



I



II

With a micro pipette, 100µl reagent 1 which is made up of EDTA, sodium nitroprusside and urease was added in a clean test tube containing 10ul of serum.

They were mixed and incubated at 37°C for 10 minutes and 2.5ml of reagent 2 which is made up of phenol (diluted) and 2.5ml of reagent 3 which is made up of sodium hypochlorite (diluted) and sodium hydroxide were added, mixed immediately and incubated at 37°C for 15 minutes. Absorbance was taken at 540nm. Concentration of urea is calculated as follows:

$$\text{Urea concentration} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{standard concentration (mg/dl)}$$

3.5.1.3 Total Protein

Principle and procedure:

The protein assay (using the Randox laboratoriesTM assay kit) is based on the reaction of protein with an alkaline copper tartarate solution and Folin reagent. Two steps lead to colour development. First is the reaction between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation is due to amino acids that reduce the Folin reagent, yielding reduced species that imparts a characteristic blue colour with maximum absorbance at between 650 – 750nm. Bovine serum albumin is used as standard.

Serum sample (20ul) was added to 1ml of Biuret reagent then, mixed properly and followed by incubation for 30min at 25°C. Blank received 20ul of distilled water instead of sample.

Absorbance was read at 546nm, and total protein concentration was calculated as follows:

$$\text{Total Protein Concentration (g/L)} = 190 \times A_{\text{sample}}$$

3.6 CYTOTOXICITY TESTS

These were carried out by the brine shrimp assays as described by Ishrak *et al.* (2000) and Aseer *et al.* (2009) to evaluate cytotoxicity and hatchability. The brine shrimp cytotoxicity assay has been considered as a simple, inexpensive and convenient probe for preliminary assessment of toxicity, detection of fungal toxins, heavy metals, cytotoxicity testing of dental materials, to bio-direct phytochemical fraction of plant extracts and other natural products and is a predictor of anti-tumor and pesticidal activity (Ishrak *et al.*, 2000 and Aseer *et al.*, 2009). There are two types of cytotoxicity tests namely, hatchability test and lethality test.

3.6.1 Brine shrimp hatchability assay

Principle and procedure:

This test was based on the hatchability efficiency of *Artemia salina* against different concentrations of test compound. Over a period of 48hrs, the percentage of hatchability is calculated by comparing the number of newly hatched nauplii in chosen concentration with the number of nauplii in the control.

Two (2) milligrams of *Artemia salina* cysts was added to a 1.5L capacity bottle with open and inverted bottom, against different concentrations (12.5, 25.0, 50.0, 100.0, 200.0, 300.0 and 600.0 mg/L) of *Morinda lucida* extract dissolved in artificially prepared sea water at room temperature (25 – 29°C). The entire content was vigorously aerated using an electrically powered aquarium pump under constant bright fluorescent illumination through a 2mm tubes attached to the sealed cap which now serve as the bottom in this setup. The tank was supplemented with 5drops of egg yolk suspension. A control tank was similarly set up, with 2mg of artemia cysts in 1 litre of sea water but without extract. The assay was monitored over a period of 48 hours and the percentage hatchability was calculated by comparing the number of newly hatched nauplii in the chosen concentrations with the number of nauplii in the control.

3.6.2 Brine shrimp lethality assay

Principle and procedure:

This test is based on the ability of a test compound to destroy the hatched nauplii of *Artemia salina* at a given concentration. Over a period of 24hrs, the percentage dead

nauplii was calculated by comparing with that of the control. The concentration at which half of the nauplii were slain (LC₅₀) was computed by Probit analysis.

Procedure:

In this test, the newly hatched nauplii were grown to sizes enough to be seen without aid. Ten nauplii each were placed in 10ml test tubes in which different concentrations (150, 450, 750, 1050 and 1350 mg/L) of the *Morinda lucida* extracts have been prepared. Two drops of egg yolk suspension was added to each tube. The number of living nauplii was counted in each tube, 24 hours later. The LD₅₀ of each extracted was calculated.

3.6.3 Preparation of artificial sea water

The method described by Yoga *et al.* (2007) and Bogner *et al.* (2010) was used to prepare artificial sea water in the laboratory. Artificial sea water was prepared using 760gm of commercially sold sea salt - devoid of additives, dissolved in 20L of distilled water and adjusted to a pH of 8.5 and mixed properly using a stirring pole. This was then aerated for 72 hours through 2mm tubes with air stones attached to the tips in order to keep the tubes in place at the bottom of the jerry can.

3.6.4 Preparation of egg yolk suspension

To 5g of freshly boiled egg yolk, 10ml of artificial sea water was added and mixed. Using a Pasteur pipette, an equivalence of two (2) drops of the obtained suspension

was added per 10ml of sea water containing nauplii. Un-used portion was preserved in the refrigerator but allowed to attain room temperature before each use.

3.6.5 Genotoxicity test: Plasmid nicking assay

Into 0.5ml micro-centrifuge tubes, 250ng (0.5uL) of pUC18 plasmid DNA were added, followed by 10uL of Fenton's reagent, then 10uL of different concentrations of *Morinda lucida* extracts or PBS (phosphate buffered saline). The aliquots were raised to a final volume of 20.5uL then, incubated at 37°C for 30minutes. Three (3uL) of gel loading dye were added and electrophoresed in a horizontal slab gel apparatus in TAE gel buffer for 45minutes at 40V/20mA. Gels were stained with 0.6ug/ml of ethidium bromide for 30minutes followed by de-staining in de-ionized water for another 30minutes. Photographs of the gels were obtained under UV light and visually assessed. The extent of damages (or otherwise) were scored. Quercetin was used as positive control.

3.6.6 QUANTIFICATION OF FLAVONOIDS AND POLYPHENOLS

3.6.6.1 Total flavonoid content determination

Total flavonoid content was determined by procedures as described by Kalaivani *et al.* (2010):

Principle and procedure:

The reaction is based on the formation of a complex flavonoid–aluminium, showing maximum absorbance at 415 - 430nm.

The total flavonoid content was estimated as previously described (Konate *et al.*, 2010). Using a micropipette, 0.5ml of methanolic $AlCl_3$ (2%, w/v) was mixed with 0.5ml of methanolic extract solution (0.1 mg/ml). After 10 min, the absorbance was read at 415nm against a blank (mixture of 0.5ml methanolic extract solution and 0.5ml methanol) on a UV/visible spectrophotometer. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

3.6.8 Total Polyphenolic content determination

Principle and procedure:

Contents of the crude extracts were analyzed method described by Konate *et al.* (2010).

The total phenolic contents of methnolic extracts of parts of *Morinda lucida* were determined spectrometrically. Aliquots (125 μ l) of solution extracts in methanol (10mg/ml) were mixed with 625 μ l Folin-Ciocalteu reagent (0.2N). After 5 min, 500 μ l of aqueous Na_2CO_3 (75g/l) was added and the mixture well vortexed. After 2h of incubation in the dark at room temperature, the absorbance was measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu reagent + 1 ml Na_2CO_3) on a UV/visible light.

A standard calibration curve was plotted using gallic acid and the results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extract.

3.7 STATISTICS

Data was expressed as mean $\pm$ SD. Significance of difference was evaluated by ANOVA and Duncan Multiple Range Test.

CHAPTER 4

4.0 RESULTS

4.1 PERCENTAGE YIELD OF EXTRACTS

Samples were extracted using methanol, after defatting with petroleum ether. Each part was extracted for an average of six times in order to obtain enough extracts for the entire experiment. The percentage yields of each sample parts were measured after allowing the methanol to evaporate entirely and they were completely dried. Stem bark of *Morinda lucida* showed the highest percentage yield at 20.94%, followed by the fruit, leaf and roots at 19.01%, 17.14% and 13.19%, respectively (Table 4.1)

Table 4.1: The percentage yields of methanol extract of different plant parts of
Morinda lucida, following 12hrs of defatting with Petroleum ether.

S/No.	Plant part	Yield (%)
1	Fruit	19.01 $\pm$ 1.13
2	Leaf	17.14 $\pm$ 2.78
3	Stem bark	20.94 $\pm$ 1.09
4	Root	13.19 $\pm$ 1.17

4.2 Results of LD₅₀ dose determination

As presented in Table 4.2, the acute toxicity experiment of the methanolic extracts of the parts of *Morinda lucida* on rats showed no mortality after 24 hours. Also, there was no indication of adverse reaction after single oral administrations of 10, 100, and 1000mg/kg in the first stage and at high doses of 1,600, 2,900 and 5,000mg/kg in the second stage. Thus, in line with the finding of Lorke (1983), the LD₅₀ dose of all the methanol extracts of the fruit, leaf, bark and root of *Morinda lucida* was all above 5000mg/kg.

Table 4.2: LD₅₀ dose of the methanol extract of different parts of *M. lucida* in rats following oral administration of varying concentrations between 0 – 5000mg/Kg.

S/No.	Plant part	LD ₅₀ dose (mg/Kg)
1	Fruit	> 10, 100, 1000, 1600, 2900 and 5000*
2	Leaf	> 10, 100, 1000, 1600, 2900 and 5000*
3	Stem bark	> 10, 100, 1000, 1600, 2900 and 5000*
4	Root	> 10, 100, 1000, 1600, 2900 and 5000*

*According to Lorke (1983) the extracts are considered safe.

4.3 BODY AND ORGAN WEIGHT CHANGES IN RATS TREATED WITH METHANOLIC EXTRACTS OF PARTS OF *M. LUCIDA*

4.3.1 Relative body weight changes of rats pre-treated with extracts before CCl₄ administration

Table 4.3 shows that the relative body weight gain in the normal control group was significantly higher ($p < 0.05$) than among the rats administered with 'CCl₄ only'. The normal group also showed a significantly ($p < 0.05$) higher relative body weight gain than those that were administered with daily single doses of 100mg/kg b.w. of different parts of *M. lucida* before injection with CCl₄, except the group that was pre-treated with bark extract for 7 days.

The 'CCl₄ only' group showed a significantly ($p < 0.05$) higher relative body weight loss than those that were treated with 100mg/kg b.w. of the methanolic extracts of the parts of *M. lucida* for 7 days before the single dose of CCl₄ injection. The 'CCl₄ only' group also showed a significantly lower relative body weight ($p < 0.05$) than those that were pre-treated with vitamin E and Vitamin C for 7 days before the single dose of CCl₄ injection.

4.3.2 Relative liver/body weight ratio of rats pre-treated with extracts before CCl₄ administration

Also, table 4.3 shows that the relative liver/body weight ratio in the normal control group was significantly higher ($p < 0.05$) than among the rats administered with 'CCl₄

only'. The normal group also showed no significant difference from those that were pre-treated with daily single dose of 100mg/kg for 7 days with the extract of different parts of *M. lucida* before injection with CCl₄, except those that received root extract. Also, there was no significant ($p<0.05$) difference between the normal control group and those that were administered only with daily single doses of the different extract of parts of *M. lucida* for 7 days, except those that only received root extracts.

The 'CCl₄ only' group showed significantly lower relative liver/body weight ratio ($p<0.05$) than those that were pre-treated with 100mg/kg of the methanolic extracts of the parts of *M. lucida* for 7 days before the single dose of CCl₄ injection except the group pre-treated with root extract.

The groups pre-treated with vitamins E and C before CCl₄ injection showed significantly higher ($p<0.05$) liver/body weight ratio than the 'CCl₄ only' group. But there was no significant difference between the vitamins E and C groups.

4.3.3 Relative kidney/body weight ratio of rats pre-treated with extracts before CCl₄ administration

The relative kidney/body weight ratio in the normal control group (Table 4.3) was significantly higher ($p<0.05$) than among the 'CCl₄ only' group. The normal group also showed no significant difference from those that were pre-treated with daily single dose of 100mg/kg of extract of the different parts of *M. lucida* for 7 days before CCl₄ injection. Also, there was no significant difference between the normal control

group and those that were administered only with daily single dose of the different extract for 7 days without CCl₄ injection.

The 'CCl₄ only' group showed significantly ($p < 0.05$) lower relative kidney/body weight ratio ($p < 0.05$) than those that were pre-treated with 100mg/kg of the methanolic extracts of different parts of *M. lucida* for 7 days before the single dose injection of CCl₄. There was no significant difference among the groups that were pre-treated with daily single dose of 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days before CCl₄.

No significant difference ($p < 0.05$) in kidney/body weight ratio between the vitamins E (α -Tocopherol) and C (ascorbic acid) groups. There was also no significant difference in the kidney/body weight ratio among the groups that were administered only with daily single doses of 100mg/kg of the methanolic extract of the different parts of *M. lucida* for 7 days.

Table 4.3: Relative change in body weight and target organ/body weight ratios of extract pre- treated rats before CCl₄ administration.

Treatment group	Relative weight ratios					
	Body weight				Percentage Liver/body ratio	Percentage Kidney/body ratio
	Initial (g)	Final (g)	Change (g)	Percentage change		
Normal control	+187.33 ± 43.89	+189.22 ± 42.56	+2.33 ± 0.57	+1.45 ± 0.21 ^a	+3.64 ± 0.11 ^a	+0.34 ± 0.08 ^a
CCl ₄ only	+236.78 ± 29.37	+224.78 ± 30.92	-12.01 ± 3.67	-7.67 ± 1.00 ^b	+2.92 ± 0.08 ^b	+0.16 ± 0.05 ^b
Fruit only	+200.45 ± 41.67	+196.66 ± 40.06	+3.54 ± 0.72	+2.09 ± 0.34 ^a	+3.70 ± 0.71 ^a	+0.33 ± 0.09 ^a
Leaf only	+198.75 ± 55.11	+198.54 ± 53.45	-1.28 ± 0.59	-0.55 ± 0.07 ^c	+3.43 ± 0.43 ^a	+0.37 ± 0.14 ^a
Bark only	+200.04 ± 53.04	+200.04 ± 52.80	+2.27 ± 0.62	+1.38 ± 0.34 ^a	+3.70 ± 0.23 ^a	+0.29 ± 0.07 ^a
Root only	+223.53 ± 57.68	+222.52 ± 56.17	-2.34 ± 0.56	-0.89 ± 0.22 ^c	+3.03 ± 0.05 ^b	+0.32 ± 0.12 ^a
Vit. E + CCl ₄	+190.78 ± 37.03	+187.67 ± 37.56	-2.45 ± 0.67	-1.42 ± 0.33 ^c	+3.66 ± 0.12 ^a	+0.24 ± 0.07 ^a
Vit. C + CCl ₄	+184.81 ± 25.78	+183.33 ± 24.78	-2.32 ± 0.54	-1.23 ± 0.23 ^c	+3.46 ± 0.16 ^a	+0.26 ± 0.05 ^a
Fruit + CCl ₄	+223.33 ± 21.14	+215.73 ± 20.84	-6.54 ± 2.11	-2.71 ± 0.61	+3.31 ± 0.18 ^{ab}	+0.25 ± 0.03 ^a
Leaf + CCl ₄	+211.18 ± 45.08	+203.51 ± 44.30	-7.71 ± 1.91	-3.67 ± 0.89	+3.46 ± 0.25 ^a	+0.30 ± 0.04 ^a
Bark + CCl ₄	+205.24 ± 47.77	+205.46 ± 49.34	+1.53 ± 0.63	+0.78 ± 0.10 ^a	+3.47 ± 0.66 ^a	+0.31 ± 0.09 ^a
Root + CCl ₄	+235.53 ± 34.23	+215.24 ± 26.03	-8.45 ± 0.72	-3.65 ± 0.62	+3.10 ± 0.24 ^b	+0.25 ± 0.04 ^a

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.3.4 Relative body weight changes of rats post-treated with extracts after CCl₄ administration

The relative body weight in the normal control group was significantly higher than among the rats group injected with 'CCl₄ only' ($p < 0.05$) (Table 4.4). The normal group also presented a significantly higher relative body weight ($p < 0.05$) than those that were given a daily single dose of 100mg/kg b.w. of extracts of different parts of *M. lucida* after injection with CCl₄ except those that were treated with bark extract. But the normal group showed no significant difference in relative body weight gain from those that were administered daily with fruit and bark 'extracts only' for 7 days. The group treated with 'CCl₄ only' had a significantly lower ($p < 0.05$) relative body weight than those that were treated only with the extracts for 7 days, but no significant difference from the group that was treated with root extract for 7 days after 'CCl₄ administration.

4.3.5 Relative liver/body weight ratio of rats post-treated with extracts after CCl₄ administration

Table 4.4 also shows the relative liver/body weight ratio in the normal control group was significantly higher than the rats group injected with 'CCl₄ only' ($p < 0.05$). The normal group also presented a significantly higher relative liver/body weight ratio ($p < 0.05$) than those that were treated with fruit and root extracts for 7 days after injection with CCl₄. There was no significant difference between the normal control group and those that were treated with leaf and bark extracts for 7 days after the single dose of CCl₄ injection.

The 'CCl₄ only' group showed a significantly lower ($p<0.05$) relative liver/body weight ratio than those treated bark and leaf extracts for 7 days after CCl₄ administration. The 'CCl₄ only' group showed no significant difference from those treated with fruit and root extracts for 7 days after CCl₄ administration.

4.3.6 Relative kidney/body weight ratio of rats post-treated with extracts after CCl₄ administration

Table 4.4 also shows the relative kidney/body weight ratio in the normal control group was significantly higher than the rats group injected with 'CCl₄ only' ($p<0.05$). The 'normal control' group presented a significantly higher relative kidney/body weight ratio ($p<0.05$) than the group treated with fruit extracts for 7 days after a single dose injection with CCl₄. There was no significant difference between the normal control group and those that were treated with leaf, bark and root extracts for 7 days after the single dose of CCl₄ injection.

The 'CCl₄ only' group showed a significantly lower ($p<0.05$) relative liver/body weight ratio than those treated bark, leaf and root extracts for 7 days after CCl₄ administration. The 'CCl₄ only' group showed no significant difference from the group treated with fruit extracts for 7 days after CCl₄ administration.

Table 4.4: Relative change in body weight and target organ/body weight ratios of extract post- treated rats after CCl₄ administration.

Treatment group	Relative weights ratios					
	Body weight				Percentage Liver/body ratio	Percentage Kidney/body ratio
	Initial (g)	Final (g)	Change (g)	Percentage change		
Normal control	+187.33 ± 43.89	+189.22 ± 42.56	+2.33 ± 0.57	+1.45 ± 0.21 ^a	+3.64 ± 0.11 ^a	+0.34 ± 0.08 ^a
CCl ₄ only	+236.78 ± 29.37	+224.78 ± 30.92	-12.01 ± 3.67	-7.67 ± 1.00 ^b	+2.92 ± 0.08 ^b	+0.16 ± 0.05 ^b
Fruit only	+200.45 ± 41.67	+196.66 ± 40.06	+3.54 ± 0.72	+2.09 ± 0.34 ^a	+3.70 ± 0.71 ^a	+0.33 ± 0.09 ^a
Leaf only	+198.75 ± 55.11	+198.54 ± 53.45	-1.28 ± 0.59	-0.55 ± 0.07 ^c	+3.43 ± 0.43 ^a	+0.37 ± 0.14 ^a
Bark only	+200.04 ± 53.04	+200.04 ± 52.80	+2.27 ± 0.62	+1.38 ± 0.34 ^a	+3.70 ± 0.23 ^a	+0.29 ± 0.07 ^a
Root only	+223.53 ± 57.68	+222.52 ± 56.17	-2.34 ± 0.56	-0.89 ± 0.22 ^c	+3.03 ± 0.05 ^b	+0.32 ± 0.12 ^a
CCl ₄ + fruit	+260.71 ± 42.35	+251.45 ± 37.56	-11.82 ± 2.74	-4.36 ± 0.79 ^b	+3.08 ± 0.07 ^b	+0.23 ± 0.04 ^b
CCl ₄ + leaf	+197.67±36.82	+186.23 ± 34.48	-11.02 ± 2.53	-5.56 ± 0.31 ^b	+3.36 ± 0.15 ^a	+0.29 ± 0.02 ^a
CCl ₄ + bark	+221.50 ± 30.41	+219.67 ± 30.12	-1.26 ± 0.51	-0.63 ± 0.24 ^c	+3.42 ± 0.03 ^a	+0.33 ± 0.10 ^a
CCl ₄ + root	+249.34 ± 45.70	+235.51 ± 43.04	-11.78 ± 1.02	-5.35 ± 0.78 ^b	+3.15 ± 0.15 ^b	+0.27 ± 0.04 ^a

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.4 RESULTS OF BIOCHEMICAL ASSAYS

4.4.1 Anti-Oxidation Results: Lipid peroxidation

4.4.2 Concentration of thiobarbituric acid reactive substances in rats pre-treated with extracts before CCl₄ administration

As presented in table 4.5, no significant difference ($p < 0.05$) was also found in TBARS concentration between the normal control group and those pre-treated with methanolic extracts of the different parts of *M. lucida* before CCl₄ injection, nor with the groups administered with the methanolic extracts only. However, rats group injected with CCl₄ ‘only’, showed a higher concentration of TBARS compared to the normal control and all other groups ($p < 0.05$). There was no significant difference in TBARS concentration among all the groups pre-treated with the various extracts for 7days before injection with CCl₄.

Obtained results also showed no significant difference in TBARS concentration among the groups pre-treated with vitamin E and vitamin C, the normal, and those pre-treated with the extracts for 7days before CCl₄ ($p < 0.05$). These exhibited a mitigated liver damage. Hepatoprotective effect of methanolic extracts of parts of *Morinda lucida* on CCl₄ induced liver damage in rats was thus depicted.

4.4.3 Superoxide dismutase (SOD) enzyme activity in rats pre-treated with extracts before CCl₄ administration

The SOD activity in the normal control group was significantly higher ($p<0.05$) than the rats 'CCl₄ only' group (Table 4.5). There was no significant difference in SOD activity between the 'normal control' group and those that were administered with daily single dose of 100mg/kg of the leaf and bark extracts for 7 days before CCl₄ administration. There was no significant difference between the 'CCl₄ only' group and those that were pre-treated for 7 days with daily single dose of 100mg/kg of the methanolic extract of the different parts of *M. lucida* before CCl₄ injection.

The groups pre-treated with the vitamins before CCl₄ injection showed significantly higher ($p<0.05$) activity than the 'CCl₄ only' group and there was no significant difference ($p<0.05$) between them and from the 'normal control' group.

4.4.4 Glutathione peroxidase (GPx) enzyme activity in rats pre-treated with extracts before CCl₄ administration

Table 4.5 shows the GPx activity in the normal control group was significantly higher ($p<0.05$) than among the rats injected with 'CCl₄ only'. The normal group also showed a significantly higher GPx activity ($p<0.05$) than those that were pre-treated with daily single dose of 100mg/kg for 7 days with different parts of *M. lucida* before injection with CCl₄ except the bark extract group. But the normal group showed no significant difference in GPx activity than those that were administered only with

daily single dose of 100mg/kg for 7 days with bark extract before CCl₄. The 'CCl₄ only' group had significantly lower GPx activity ($p<0.05$) than those that were treated with 100mg/kg of the methanolic extracts of the bark for 7 days before the single dose of CCl₄ injection.

The groups administered with the vitamins before CCl₄ injection showed significantly higher ($p<0.05$) activity than the 'CCl₄ only' group and there was no significant difference between the vitamin groups and the 'normal control' group.

4.4.5 Catalase (CAT) activity in rats pre-treated with extracts before CCl₄ administration

Table 4.5 show the activity of CAT in the normal control group which was significantly higher ($p<0.05$) than among the rats administered with 'CCl₄ only'. The normal group also showed a significantly higher CAT activity ($p<0.05$) than those that were treated with daily single doses of 100mg/kg for 7 days with *M. lucida* leaf and root extracts before injection with CCl₄. But the normal group showed no significant difference in CAT activity from those that were pre-treated only with daily single dose of 100mg/kg for 7 days with bark and fruit extracts of *M. lucida* ($p<0.05$). The groups pre-treated with the vitamins before CCl₄ injection showed significantly higher ($p<0.05$) CAT activities than the 'CCl₄ only' group but there was no significant difference between the vitamin groups and the 'normal control' group.

4.4.6 Concentration of thiobarbituric acid reactive substances in rats post-treated with extracts after CCl₄ administration

As shown in table 4.6, the concentration of thiobarbituric acid reactive substances (TBARS) in the ‘normal control’ group was significantly lower in comparison to the ‘CCl₄ only’ group ($p < 0.05$). There was no significant difference between rat groups post-treated with 100mg/kg of the methanolic extracts of the leaf and bark of *M. lucida* for 7 days after injection of CCl₄.

4.4.7 Superoxide dismutase (SOD) activity in rats post-treated with extracts after CCl₄ administration

As shown in table 4.6, SOD activity in the normal control group indicated a significantly higher SOD activity ($p < 0.05$) than those that were treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* after injection with CCl₄ except the bark extracts. But the normal group showed no significant difference in SOD activity than those that were administered only with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* except those that were administered with root extracts only. Generally, the ‘CCl₄ only’ group showed a significantly lower ($p < 0.05$) SOD activity than those that were post-treated only with 100mg/kg of the methanolic extracts of parts of *M. lucida* for 7 days. The ‘CCl₄ only’ group also showed significantly lower SOD activity ($p > 0.05$) than those that were treated with 100mg/kg of the methanolic extracts of the bark of *M. lucida* for 7 days after the single dose of CCl₄ injection.

Table 4.5: TBARS concentration, and activities of Superoxide dismutase, Glutathione Peroxidase and Catalase enzymes in liver of rats pre-treated with methanolic extracts of different parts of *M. lucida* before CCl₄ administration.

Treatment group	TBARS (nMol/mg protein)	SOD (U/ml)	GPx (nmol/min/mL)	CAT (U mole/min/mg protein)
Normal control	94.3 ± 7.5 ^{a,c}	40 ± 3 ^a	25.9 ± 1.0 ^a	71.5 ± 7.3 ^a
CCl ₄ only	177.2 ± 15.0 ^b	22 ± 4 ^b	8.0 ± 1.7 ^b	48.5 ± 9.4 ^b
Fruit only	93.4 ± 6.3 ^{a,c}	32 ± 8	11.4 ± 1.5	79.3 ± 14.8 ^{ac}
Leaf only	63.8 ± 4.1 ^a	39 ± 4 ^a	23.3 ± 1.7 ^a	102.2 ± 8.2
Bark only	88.3 ± 10.0 ^{a,c}	37 ± 5	24.9 ± 2.0 ^a	70.5 ± 5.6 ^a
Root only	91.6 ± 14.2 ^{a,c}	27 ± 5	13.9 ± 1.7 ^d	56.5 ± 7.6 ^b
α-Tocopherol + CCl ₄	92.6 ± 7.1 ^{a,c}	43 ± 8 ^a	24.0 ± 1.3 ^a	78.1 ± 4.1 ^{ac}
Ascorbic acid + CCl ₄	37.1 ± 3.2 ^a	50 ± 4 ^c	34.4 ± 2.4 ^c	88.4 ± 8.0 ^c
Fruit + CCl ₄	76.7 ± 2.8 ^{a,c}	18 ± 6 ^b	10.7 ± 0.6	67.5 ± 8.7 ^a
Leaf + CCl ₄	58.9 ± 22.0 ^a	33 ± 7	13.3 ± 1.1 ^d	50.3 ± 11.8 ^b
Bark + CCl ₄	109.3 ± 8.6 ^c	30 ± 6	21.8 ± 1.0 ^a	67.6 ± 7.1 ^a
Root + CCl ₄	77.3 ± 9.1 ^{a,c}	17 ± 6 ^b	14.7 ± 1.0 ^d	55.8 ± 9.7 ^b

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.4.8 Glutathione peroxidase (GPx) activity in rats post-treated with extracts after CCl₄ administration

GPx activity in the normal control group was significantly higher than among the rats group injected with 'CCl₄ only' ($p<0.05$) (table 4.6). The normal group also presented a significantly higher GPx activity ($p<0.05$) than those that were treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* after injection with CCl₄. The 'CCl₄ only' group also showed significantly lower GPx activity ($p<0.05$) than those that were treated with 100mg/kg of the methanolic extracts of the bark of *M. lucida* for 7 days after the single dose of CCl₄ injection. There was no significant difference in GPx activity among the groups that were post-treated with daily single dose of 100mg/kg of the methanolic extract of the different parts of *M. lucida* for 7 days after injection with single dose of the CCl₄ hepatotoxin.

4.4.9 Catalase (CAT) activity in rats post-treated with extracts after CCl₄ administration

As presented in table 4.6, it was observed that CAT activity in the normal control group was significantly higher than among the rats group injected with 'CCl₄ only' ($p<0.05$). The normal group also presented a significantly higher CAT activity ($p<0.05$) than those that were treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* after injection with CCl₄ except those treated with

leaf and bark extracts. The 'CCl₄ only' group showed significantly lower CAT activity ($p>0.05$) than those that were treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days after a single dose of CCl₄ injection except the root and fruit extract groups.

Table 4.6: TBARS concentration and activities of Superoxide dismutase, Glutathione peroxidase and Catalase enzymes in liver of rats post -treated with methanolic extracts of different parts of *M. lucida* after CCl₄ administration.

Treatment group	TBARS (nMol/mg protein)	SOD (U/ml)	GPx (nmol/min/mL)	CAT (U/mole/min/mg protein)
Normal control	94.3 ± 7.5 ^{a,c}	40 ± 3 ^a	25.9 ± 1.0 ^a	71.5 ± 7.3 ^a
CCl ₄ only	177.2 ± 15.0 ^b	22 ± 4 ^b	8.0 ± 1.7 ^b	48.5 ± 9.4 ^b
Fruit only	93.4 ± 6.3 ^{a,c}	32 ± 8	11.4 ± 1.5	79.3 ± 14.8 ^{ac}
Leaf only	63.8 ± 4.1 ^a	39 ± 4 ^a	23.3 ± 1.7 ^a	102.2 ± 8.2
Bark only	88.3 ± 10.0 ^{a,c}	37 ± 5	24.9 ± 2.0 ^a	70.5 ± 5.6 ^a
Root only	91.6 ± 14.2 ^{a,c}	27 ± 5	13.9 ± 1.7 ^d	56.5 ± 7.6 ^b
CCl ₄ + fruit	148.5 ± 10.6 ^b	24 ± 7 ^b	14.6 ± 2.0 ^d	50.1 ± 8.2 ^b
CCl ₄ + leaf	117.1 ± 22.0 ^c	22 ± 6 ^b	17.2 ± 1.9	73.1 ± 10.6 ^a
CCl ₄ + bark	121.7 ± 8.3 ^c	34 ± 5	15.1 ± 1.3 ^d	64.6 ± 4.6 ^a
CCl ₄ + root	138.0 ± 19.3 ^b	23 ± 8 ^b	13.1 ± 1.3 ^d	45.9 ± 8.7 ^b

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.5 RESULTS OF ANTI-INFLAMMATION EXPERIMENTS

4.5.1 CARRAGEENAN-INDUCED RAT PAW OEDEMA

4.5.2 Inflammation inhibition by methanolic fruit extract

Methanolic fruit extracts of *Morinda lucida* delivered at 500mg/kg, exhibited a significantly higher ($p<0.5$) percentage inhibition on inflammation at 75.0% than at 100 and 300mg/kg within 30minutes of administration of phlogistic agent to the rats' left hind paws (Table 4.7). At 60minutes, 100% inhibition of inflammation was achieved at 500mg/kg, which was significantly higher ($p<0.5$) than at 100 and 300mg/kg which presented 22.2% and 11.1% respectively. At 120minutes however, 300mg/kg of methanolic fruit extract attained 72.7% inflammatory inhibition while 100mg/kg attained 54.5%. At all doses, inhibition of inflammation were significantly higher ($p<0.5$) than the standard drug, diclofenac sodium at the recommended dose of 5mg/kg

4.5.3 Inflammation inhibition by methanolic leaf extract

From Table 4.8, all the groups showed significant inflammatory inhibition ($p<0.5$) over the standard drug, diclofenac sodium at the recommended dose of 5mg/kg. There was no inhibition of paw inflammation shown by the groups administered with 100 and 300mg/kg of the leaf extracts within 30minutes of carrageenan administration. But there was a significant inflammatory inhibition of $87.5 \pm 0.11\%$ in the group administered with 500mg/kg of the leaf extract. One-hundred percent (100%)

inflammation inhibition was shown by the 500mg/kg of leaf extract administered group within 60minutes of inflammatory induction of the phlogistic agent to the rats' left hind paw. There was no significant difference in anti-inflammatory capacities between the 100 and 300mg/kg groups at the end of two (2) hrs.

4.5.4 Inflammation inhibition by methanolic bark extract:

Like the leaf, the methanolic bark extract showed (table 4.9) no inflammatory inhibition at the doses of 100 and 300mg/kg within 30minutes of carrageenan administration to the left hind paw of the rats. At 500mg/kg, inflammatory inhibition was 25%. There was a dose-dependent increase in the inflammatory inhibition among the different doses with time because, at 60 and 90 minutes, 100, 300 and 500mg/kg doses presented inflammation inhibitory effects at 11.1 ± 0.14 , 33.3 ± 0.09 , 58.3 ± 0.10 and 30.0 ± 0.10 , 75.0 ± 0.10 and 83.3 ± 0.06 , respectively. However, while at 120minutes there was no significant difference between the Percentage inflammatory inhibition shown by 300 and 500mg/kg doses, none attained a 100% inflammatory inhibition within 120minutes. All doses administered still showed better inhibitory effects on inflammation than diclofenac sodium in all the periods of examination.

4.5.5 Inflammation inhibition by methanolic root extract:

All the doses showed significantly higher inhibitory effects on inflammation than diclofenac sodium ($p < 0.5$). Root extract of *Morinda lucida* presented the best anti inflammatory properties with respect to both inhibitory time and extent of inhibition (table 4.10). Within 30minutes of carrageenan administration after treatment with

100mg/kg of root extract, $75.0 \pm 0.09\%$ inhibition of inflammation was achieved. The 300 and 500mg/kg dose groups presented 100% inflammatory inhibition within 30minutes of carrageenan administration to the rats' left hind paws, and these inflammatory inhibitions were maintained throughout the experimental period of 120minutes.

4.5.6 ANTI-INFLAMMATION RESULT: NITRIC OXIDE ASSAY

As shown in figure 4.6, the nitric oxide (NO) assay, which portrays the NO scavenging capacity of the extracts and as a consequence, the anti-inflammatory capacity of the extracts, indicated percentage (%) inhibitions from the highest to the lowest in the order: 88.28 ± 0.85 , 69.47 ± 2.98 , 53.38 ± 0.77 , 35.53 ± 1.02 presented by the bark, fruit, root and leaf extracts of *Morinda lucida*.

Table 4.7: Effect of methanolic fruit extract of *Morinda lucida* on carrageenan induced rat paw oedema.

Dose administered (mg/kg)	Percentage inhibition at time (t)			
	t=30min	t=60min	t=90min	t=120min
5.0 (Diclofenac Na) standard	0.4 ± 0.12 ^d	1.0 ± 0.15 ^d	20.0 ± 0.12 ^c	45.5 ± 0.15 ^b
100.0	12.5 ± 0.12 ^c	22.2 ± 0.08 ^c	40.0 ± 0.05 ^b	54.5 ± 0.08 ^b
300.0	0.0 ± 0.13 ^d	11.1 ± 0.14 ^c	30.0 ± 0.15 ^c	72.7 ± 0.16 ^a
500.0	75.0 ± 0.18 ^a	100.0 ± 0.18 ^a	100.0 ± 0.18 ^a	100.0 ± 0.18 ^a

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

Table 4.8: Effect of methanolic leaf extract of *Morinda lucida* on carrageenan induced rat paw oedema.

Dose administered (mg/kg)	Percentage inhibition at time (t)			
	t=30min	t=60min	t=90min	t=120min
5.0 (Diclofenac Na) standard	0.4 ± 0.12 ^d	1.0 ± 0.15 ^d	20.0 ± 0.12 ^c	45.5 ± 0.15 ^b
100.0	0.0 ± 0.08 ^d	22.2 ± 0.06 ^c	50.0 ± 0.08 ^b	81.8 ± 0.10 ^a
300.0	0.0 ± 0.08 ^d	22.2 ± 0.08 ^c	70.0 ± 0.05 ^a	90.9 ± 0.04 ^a
500.0	87.5 ± 0.11 ^a	100.0 ± 0.13 ^a	100.0 ± 0.13 ^a	100.0 ± 0.13 ^a

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

Table 4.9: Effect of methanolic bark extract of *Morinda lucida* on carrageenan induced rat paw oedema

Dose administered (mg/kg)	Percentage inhibition at time (t)			
	t=30min	t=60min	t=90min	t=120min
5.0 (Diclofenac Na) standard	0.4 ± 0.12 ^d	1.0 ± 0.15 ^d	20.0 ± 0.12 ^c	45.5 ± 0.15 ^b
100.0	0.0 ± 0.13 ^d	11.1 ± 0.14 ^c	30.0 ± 0.10 ^c	54.5 ± 0.13 ^b
300.0	0.0 ± 0.14 ^d	33.3 ± 0.09 ^c	75.0 ± 0.10 ^a	83.3 ± 0.10 ^a
500.0	25.0 ± 0.04 ^c	58.3 ± 0.10 ^b	83.3 ± 0.06 ^a	83.3 ± 0.06 ^a

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

Table 4.10: Effect of methanolic root extract of *Morinda lucida* on carrageenan induced rat paw oedema

Dose administered (mg/kg)	Percentage inhibition at time (t)			
	t=30min	t=60min	t=90min	t=120min
5.0 (Diclofenac Na) standard	0.4 ± 0.12^d	1.0 ± 0.15^d	20.0 ± 0.12^c	45.5 ± 0.15^b
100.0	75.0 ± 0.09^a	88.9 ± 0.12^a	90.0 ± 0.12^a	90.9 ± 0.12^a
300.0	100.0 ± 0.13^a	100.0 ± 0.13^a	100.0 ± 0.13^a	100.0 ± 0.13^a
500.0	100.0 ± 0.08^a	100.0 ± 0.08^a	100.0 ± 0.08	100.0 ± 0.08^a

Values are Mean $\pm$ SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

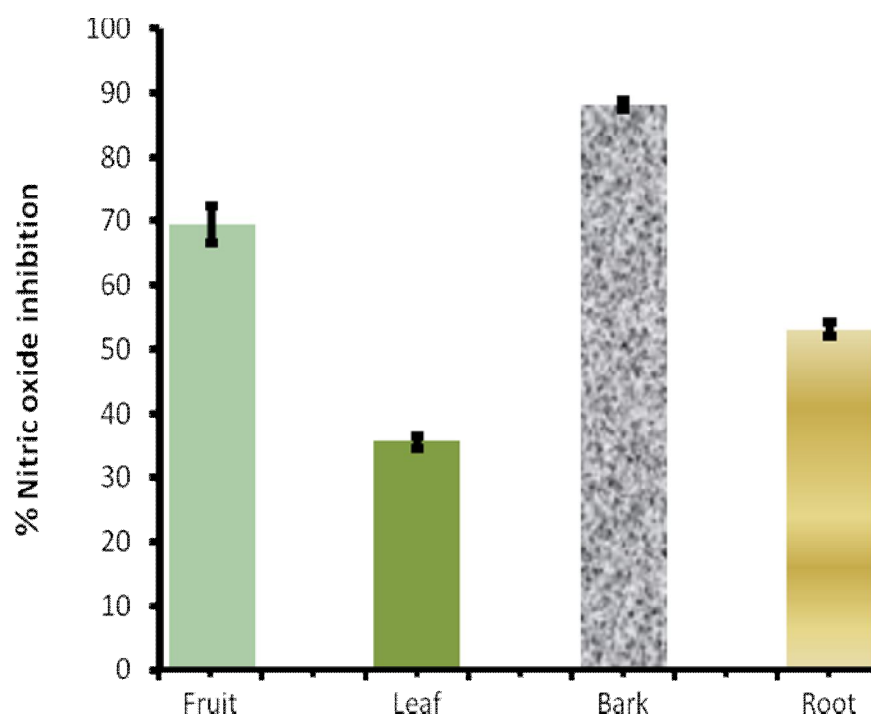


Figure 4.6: Percentage nitric oxide inhibition of different plant parts of *M. lucida*.

4.6 RESULTS OF LIVER FUNCTION EXPERIMENTS

4.6.1 Aspartate aminotransferase (AST) activity in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

As shown in table 4.11, there was no significant difference ($p < 0.05$) between normal control rats group and groups pre-treated with daily single doses of 100mg/kg of methanolic extracts of leaf, root and bark for 7 days before the single dose of CCl₄ administration. The normal control group also showed no significant difference from those that received daily single doses of 100mg/kg of *M. lucida* leaf and root 'extracts only' for 7 days. The group that received CCl₄ only presented a significantly higher ($p < 0.05$) serum AST activity than all the other groups. There was no significant difference ($p < 0.05$) that was shown between the groups pre-treated with vitamin E and vitamin C before the injection of a single dose of CCl₄.

4.6.2 Alanine aminotransferase (ALT) activity in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

The experiment showed a significantly higher ALT activity was observed in the 'CCl₄ only' group than the normal control group ($p < 0.05$). No significant difference in ALT activity between the normal control group and rat groups pre-treated with single daily dose of 100mg/kg of methanolic extracts of the leaf, fruit, vitamin E or vitamin C for 7 days before the single dose of CCl₄ administration (Table 4.11). There was also found to be no significant difference in the ALT activity among rats that received the

daily 100 mg/kg of the various extracts for 7 days only. Although there was no significant difference in the ALT activities between the vitamins (E or C) pre-treated groups and normal control groups, there was however, a significantly higher AST activity ($p < 0.05$) in the ' α -tocopherol + CCl₄' group than the 'ascorbic acid + CCl₄' group.

4.6.3 Direct bilirubin concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

The concentration of conjugated bilirubin was significantly lower in the normal control group than the CCl₄ only group (table 4.11). The normal control group also presented a significantly lower conjugated bilirubin concentration ($p > 0.05$) than the groups that were pre-treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days before CCl₄ injection. The group administered with only CCl₄ showed a significantly higher ($p < 0.05$) conjugated bilirubin concentration than both the normal control group and the groups administered with the different extracts before CCl₄ injection. There was no significant difference in conjugated bilirubin concentrations among the groups that were pre-treated with any of the vitamins, viz: α -Tocopherol and ascorbic acid before the injection of a single dose of CCl₄. The α -Tocopherol + CCl₄ and ascorbic acid + CCl₄ groups did not show significant difference in their conjugated bilirubin concentration when compared to the groups that were pre-treated with daily doses of 100mg/kg of the leaf and bark extracts of *M. lucida* before the injection of a single dose of CCl₄.

4.6.4 Indirect (unconjugated) bilirubin concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

As shown in table 4.11 the concentration of unconjugated bilirubin was significantly lower ($p>0.05$) in the normal control group when compared with the CCl₄ only group. The normal control group also presented no significant difference in conjugated bilirubin concentration when compared to the groups that were pre-treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days before CCl₄ injection. Also, the normal control group did not show any significant difference in the concentration of conjugated bilirubin when compared to the groups that were pre-treated with α -Tocopherol and ascorbic acid before the single dose of CCl₄ administration. The group administered with 'CCl₄ only' showed a significantly higher ($p<0.05$) unconjugated bilirubin concentration than the groups administered with any of the extracts before CCl₄ injection. The 'vitamin E + CCl₄' and 'vitamin C + CCl₄' groups did not show significant difference in their unconjugated bilirubin concentration when compared to the groups that were pre-treated with daily doses of 100mg/kg of the root and fruit extracts of *M. lucida* before the injection of the single dose of CCl₄.

4.6.5 Total bilirubin concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

As shown in table 4.11, the concentration of total bilirubin was significantly lower ($p>0.05$) in the normal control group when compared with the 'CCl₄ only' group. The normal control group also presented no significant difference in total bilirubin

concentration when compared to the groups that were pre-treated with 100mg/kg of the methanolic extracts of the leaf and bark of *M. lucida* for 7 days before CCl₄ injection. Also, the normal control group did not show any significant difference in total bilirubin concentration when compared to the groups that were pre-treated with vitamin E and vitamin C before the single dose of CCl₄ injection. The group administered with 'CCl₄ only' showed a significantly higher ($p < 0.05$) total bilirubin concentration than the groups pre-treated with the different extracts or vitamins before CCl₄ injection. There was no significant difference in total bilirubin concentrations between the groups that were pre-treated with either of the vitamins, viz: E and C, before the injection of a single dose of CCl₄.

Table 4.11: Activities of Aspartate aminotransferase and Alanine aminotransferase and Bilirubin (Direct, Indirect and Total) concentrations in rat serum tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage:

Treatment group	AST (U/I)	ALT (U/I)	Bilirubin (mg/dl)		
			Direct	Indirect	Total
Normal control	23.57 ± 3.07 ^{ab}	48.51 ± 17.76 ^a	0.12 ± 0.06 ^a	0.12 ± 0.05 ^a	0.24 ± 0.03 ^a
CCl ₄ only	90.73 ± 6.22 ^c	89.63 ± 10.49 ^c	0.51 ± 0.03 ^b	0.19 ± 0.03 ^b	0.62 ± 0.02 ^b
Fruit only	7.15 ± 2.85	29.34 ± 14.37 ^d	0.30 ± 0.02 ^c	0.07 ± 0.06 ^a	0.37 ± 0.07 ^c
Leaf only	26.47 ± 6.01 ^{ab}	28.32 ± 5.81 ^d	0.19 ± 0.08	0.11 ± 0.09 ^a	0.30 ± 0.10 ^a
Bark only	11.25 ± 3.82	20.38 ± 6.42 ^d	0.13 ± 0.05 ^a	0.10 ± 0.01	0.15 ± 0.02
Root only	12.66 ± 1.58 ^a	26.44 ± 8.20 ^d	0.28 ± 0.08 ^c	0.10 ± 0.05 ^a	0.38 ± 0.06 ^c
α-Tocopherol + CCl ₄	64.31 ± 8.03 ^d	52.37 ± 2.48 ^a	0.30 ± 0.03 ^c	0.12 ± 0.04 ^a	0.42 ± 0.05 ^c
Ascorbic acid + CCl ₄	62.87 ± 4.05 ^d	44.54 ± 14.92 ^{ab}	0.26 ± 0.03 ^c	0.13 ± 0.04 ^a	0.40 ± 0.05 ^c
Fruit + CCl ₄	10.95 ± 4.04	48.83 ± 7.04 ^a	0.16 ± 0.02 ^a	0.10 ± 0.06 ^a	0.26 ± 0.05 ^a
Leaf + CCl ₄	33.08 ± 8.44 ^b	53.09 ± 14.22 ^a	0.25 ± 0.06 ^c	0.14 ± 0.06	0.39 ± 0.08 ^c
Bark + CCl ₄	21.26 ± 3.56 ^{ab}	28.15 ± 18.41 ^d	0.25 ± 0.04 ^c	0.05 ± 0.03	0.30 ± 0.02 ^a
Root + CCl ₄	30.67 ± 2.49 ^b	32.26 ± 13.65 ^{bd}	0.36 ± 0.06	0.10 ± 0.02 ^a	0.46 ± 0.04

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.6.6 Aspartate aminotransferase (AST) activity in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

Obtained values as shown in table 4.12, presented no significant difference between ‘normal control’ group and those post-treated with daily single doses of 100mg/kg of methanolic extracts of root, bark and fruits for 7 days after the single dose of CCl₄ injection. The normal control group also showed no significant difference from those that received daily single doses of 100mg/kg of *M. lucida* leaf and root extracts only for 7 days. The group that received CCl₄ only presented a significantly higher ($p < 0.05$) serum AST activity than all the other groups.

4.6.7 Alanine aminotransferase (ALT) activity in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

Normal control rats group presented an ALT activity that was lower ($p < 0.05$) than the CCl₄ only group (table 4.12). ALT activity among the rats treated with CCl₄ only was significantly higher ($p < 0.05$) than those that were post-treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* extracts after CCl₄ administration and also higher ($p > 0.05$) than the normal control group. It was also found that there was no significant difference ($p < 0.05$) found among rat groups that were first administered with the CCl₄ hepatotoxin before the 7 days treatment with 100mg/kg daily of the methanolic extracts of different parts of *M. lucida*.

4.6.8 Direct bilirubin concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

Normal control rats group presented a concentration of conjugated bilirubin that was lower ($p < 0.05$) than the 'CCl₄ only' group. The concentration of direct (conjugated) bilirubin among the rats injected with CCl₄ only was significantly higher ($p < 0.05$) than those that were post-treated with daily single doses of 100mg/kg for 7 days with the different parts of *M. lucida* extracts after CCl₄ injection, and also significantly higher ($p < 0.05$) than the normal control group as shown in Table 4.12. There was no significant difference between the concentration of conjugated bilirubin in rats that were post-treated with daily single dose of 100mg/kg of the extract of the different parts of *M. lucida* for 7 days only, and those that were post-treated with daily single doses of 100mg/kg of the methanolic extract of the different parts of *M. lucida* for 7 days after CCl₄ injection. It was also found that there was no significant difference in conjugated bilirubin concentration found among rat groups that were post-treated with the extracts for 7 days after the single dose injection of CCl₄ hepatotoxin.

4.6.9 Indirect bilirubin concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

As shown in Table 4.12, the indirect bilirubin concentration of the group administered with only a single dose of CCl₄ also presented higher ($p < 0.05$) values than the normal control group. There was also a significantly higher ($p < 0.05$) concentration of the indirect bilirubin concentration in the 'CCl₄ only' group than those that were post-

treated with 100mg/kg of the methanolic extracts of parts of *M. lucida* for 7 days after injection with single dose of the CCl₄ hepatotoxin. The indirect bilirubin concentration among the rats treated with 'CCl₄ only' was significantly higher ($p<0.05$) than those that were administered with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* extracts only. Normal control rats group presented an indirect bilirubin concentration that was not significantly different from the groups that were administered only with daily single dose of 100mg/kg of the methanolic extract of the different parts of *M. lucida* for 7 days. There was no significant difference in the indirect bilirubin concentration among the groups that were post-treated with 100mg/kg of the methanolic extracts of parts of *M. lucida* for 7 days after injection with single dose of the CCl₄ hepatotoxin.

4.6.10 Total bilirubin concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

As presented in table 4.12, the total bilirubin concentration of the group injected with only single dose of CCl₄ was higher than the normal control group. There was also a significantly higher ($p<0.05$) concentration of the total bilirubin concentration in the 'CCl₄ only' group than those that were post-treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days after injection with single dose of the CCl₄ hepatotoxin. The total bilirubin concentration among the rats treated with 'CCl₄ only' was significantly higher ($p<0.05$) than those that were administered with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* extracts only. Normal control rats group presented a total bilirubin concentration that was

significantly lower ($p < 0.05$) than the groups that were administered only with daily single dose of 100mg/kg of the methanolic extract of the root and fruit extracts of *M. lucida* for 7 days. The total bilirubin concentration of the normal control rats group was not significantly lower ($p > 0.05$) than the groups that were post-treated with daily single dose of 100mg/kg of the methanolic extract of the leaf and bark extracts of *M. lucida* for 7 days after an initial injection of single doses of CCl_4 .

Table 4.12: Activities of Aspartate aminotransferase and Alanine aminotransferase and Bilirubin (Direct, Indirect and Total) concentrations in serum of rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

Treatment group	AST (U/I)	ALT (U/I)	Bilirubin (mg/dl)		
			Direct	Indirect	Total
Normal control	23.57 ± 3.07 ^{ab}	48.55 ± 17.71 ^a	0.12 ± 0.06 ^a	0.12 ± 0.05 ^a	0.24 ± 0.03 ^a
CCl ₄ only	90.73 ± 6.22 ^c	89.61 ± 10.52 ^c	0.51 ± 0.03 ^b	0.19 ± 0.03 ^b	0.62 ± 0.02
Fruit only	7.15 ± 2.85	29.34 ± 14.46 ^d	0.30 ± 0.02 ^c	0.07 ± 0.06 ^a	0.37 ± 0.07 ^c
Leaf only	26.47 ± 6.01 ^{ab}	28.18 ± 5.84 ^d	0.19 ± 0.08	0.11 ± 0.09 ^a	0.30 ± 0.10 ^a
Bark only	11.25 ± 3.82	20.36 ± 6.43 ^d	0.13 ± 0.05 ^a	0.10 ± 0.01	0.15 ± 0.02
Root only	12.66 ± 1.58 ^a	26.44 ± 8.26 ^d	0.28 ± 0.08 ^c	0.10 ± 0.05 ^a	0.38 ± 0.06 ^c
CCl ₄ + fruit	35.38 ± 7.18 ^b	30.83 ± 7.35 ^d	0.19 ± 0.04	0.11 ± 0.06 ^a	0.30 ± 0.08 ^a
CCl ₄ + leaf	45.74 ± 9.79	28.53 ± 6.45 ^d	0.25 ± 0.04 ^c	0.06 ± 0.02 ^b	0.31 ± 0.06 ^a
CCl ₄ + bark	28.97 ± 3.48 ^b	21.48 ± 3.04 ^d	0.32 ± 0.02	0.04 ± 0.02	0.36 ± 0.04 ^c
CCl ₄ + root	28.17 ± 6.52 ^b	17.55 ± 5.62 ^d	0.33 ± 0.10	0.04 ± 0.02	0.37 ± 0.11 ^c

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.7 RESULTS OF LIPID PROFILE EXPERIMENTS

4.7.1 Total triglyceride concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

The group administered with only CCl₄ showed a significantly higher cholesterol concentration than the normal control group and the groups administered with only 100mg/kg of extracts daily for 7 days. Among the groups that were pre-treated with daily doses of 100mg/kg of parts of *M. lucida* extracts before the injection of a single dose of CCl₄, the bark pre-treated before CCl₄ group showed a significantly higher triglyceride concentration ($p < 0.05$). The normal control group did not show significant difference in the total triglycerides concentration when compared to those that received 7 days pre-treatment with daily doses of 100mg/kg of *M. lucida* leaf, root and fruit extracts before the injection of a single dose of CCl₄ (Table 4.13).

4.7.2 Total cholesterol (TC) concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

The total cholesterol concentration in the 'normal control' group was significantly lower than in the groups pre-treated with the different parts of *M. lucida* extract for 7 days before CCl₄ injection (Table 4.13). The normal control group presented cholesterol concentration that did not significantly differ from the groups that received 7 days administration of 100mg/kg/day of the 'leaf only', 'bark only' and 'fruit only' methanolic extracts. But the root and fruit extracts pre-treated groups were

significantly higher ($p < 0.05$) in cholesterol concentration than the leaf and bark pre-administered groups.

The *M. lucida* 'root only' extract group however, showed a significantly high cholesterol concentration ($p < 0.05$) above all the other 'extract only' groups, an indication that it was not an efficient anti-hypercholesterolemic extract. This is evidenced by the fact that the same trend was displayed in the group administered first with 7 days of the root extract before injection of the single dose of CCl_4 (Table 4.23). The cholesterol concentration in the normal control group was significantly lower than the CCl_4 only group ($p > 0.05$) but there was no significant difference between the 'CCl₄ only' group and the group that was pre-treated for 7 days with a daily 100mg/kg of the fruit extract before CCl_4 .

4.7.3 High density lipoprotein (HDL) concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl_4 damage

As in table 4.13, the concentration of HDL was significantly lower ($p < 0.05$) in the 'CCl₄ only' group than the normal control group. The 'CCl₄ only' group also presented significantly lower ($p > 0.05$) HDL concentration when compared to the groups that were administered only with 100mg/kg of the methanolic extracts of different parts of *M. lucida* for 7 days. Also, the 'CCl₄ only' group presented significantly lower HDL concentration ($p > 0.05$) than the groups that were pre-treated with 100mg/kg of the methanolic extracts of different parts of *M. lucida* for 7 days

before the CCl₄ injection. The normal control group did not show any significant difference in HDL concentration when compared to the groups that were pre-treated with α -Tocopherol and ascorbic acid before the single dose of CCl₄ administration. There was no significant difference in the HDL concentration within the groups that were pre-treated with any of the vitamins, viz: α -Tocopherol and ascorbic acid prior to the injection of a single dose of CCl₄. Also, the α -Tocopherol + CCl₄ and ascorbic acid + CCl₄ groups did not show significant difference in their total HDL concentrations when compared to the groups that were administered with only daily doses of 100mg/kg of the root and fruit extracts of *M. lucida*. LDL/HDL ratios showed that the values obtained for leaf and bark extracts were low, comparable to the normal while the CCl₄ only group was the highest and comparable with the root + CCl₄ and fruit + CCl₄ groups.

4.7.4 Low density lipoprotein (LDL) concentration in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage

As in table 4.13, the concentration of LDL was significantly higher ($p < 0.05$) in the 'CCl₄ only' group than the normal control group. The 'CCl₄ only' group also presented significantly higher ($p < 0.05$) LDL concentration when compared to the groups that were administered only with 100mg/kg of the methanolic extracts of different parts of *M. lucida* for 7 days. Also, the 'CCl₄ only' group presented significantly higher LDL concentration ($p < 0.05$) than the groups that were pre-treated with 100mg/kg of the methanolic extracts of different parts of *M. lucida* for 7 days before the injection of a single dose of CCl₄ except in the '*M. lucida* root extract +

CCl₄' and '*M. lucida* fruit extract + CCl₄' groups. The normal control group did not show any significant difference in LDL concentration when compared to the group that was pre-treated with α -tocopherol before the single dose of CCl₄ administration. There was a significantly higher LDL concentration shown among the rats groups that were pre-treated with α -Tocopherol than those that received ascorbic acid prior to the injection of a single dose of CCl₄. However, the α -Tocopherol + CCl₄ and ascorbic acid + CCl₄ groups showed significantly lower LDL concentrations when compared to the groups that were pre-treated with daily doses of 100mg/kg of the different parts of extracts of *M. lucida* before CCl₄ injection, except those that received the leaf extracts. LDL/HDL ratios indicated that the 'bark or leaf + CCl₄', presented values similar to the Vitamin E or Vitamin C + CCl₄' extracts were low and comparable to the normal control whereas.

Table 4.13: Concentration of cholesterol, triglyceride, HDL and LDL in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage:

Treatment group	Triglyceride mg/dl	Total Cholesterol (U/I)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL Ratio
Normal control	79.60 ± 7.07 ^a	31.12 ± 19.37 ^a	40.44 ± 0.75 ^{ad}	34.95 ± 9.51 ^a	0.86
CCl ₄ only	277.85 ± 18.88 ^b	319.28 ± 41.82 ^b	35.35 ± 1.45 ^b	95.57 ± 5.23 ^b	2.70
Fruit only	78.03 ± 9.56 ^a	6.74 ± 2.33 ^a	42.02 ± 0.72 ^c	10.80 ± 2.64 ^d	0.26
Leaf only	69.30 ± 5.88 ^a	13.41 ± 3.45 ^a	44.12 ± 0.78	7.60 ± 1.11 ^d	0.17
Bark only	78.03 ± 9.56 ^a	10.92 ± 2.34 ^a	42.55 ± 0.64	14.62 ± 1.88 ^{cd}	0.34
Root only	87.11 ± 21.49 ^a	211.71 ± 93.76 ^c	46.06 ± 0.89	71.93 ± 14.61	1.56
Vitamin E + CCl ₄	90.26 ± 14.35 ^a	273.8 ± 73.98 ^d	41.78 ± 0.89 ^c	27.27 ± 7.36 ^{ac}	0.65
Vitamin C + CCl ₄	169.56 ± 69.02 ^c	6.68 ± 4.83 ^a	41.24 ± 0.96 ^{acd}	10.14 ± 5.49 ^d	0.25
Fruit + CCl ₄	78.73 ± 13.51 ^a	30.22 ± 8.17 ^a	40.11 ± 1.71 ^{ad}	25.72 ± 9.39 ^{ac}	2.03
Leaf + CCl ₄	110.35 ± 27.43 ^d	243.63 ± 34.04 ^d	44.22 ± 1.40	90.93 ± 8.88 ^b	0.64
Bark + CCl ₄	153.31 ± 28.90 ^c	152.91 ± 42.34 ^c	46.27 ± 0.86	51.85 ± 9.86	1.12
Root + CCl ₄	85.19 ± 9.22 ^a	275.45 ± 30.92 ^{bd}	42.11 ± 0.71 ^c	85.33 ± 9.01 ^b	2.06

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.7.5 Total triglyceride concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

The concentration of triglycerides in the normal control group indicated no significant difference with the groups that were post-treated with 100mg/kg of methanolic extracts of all the different parts of *M. lucida* after an earlier single dose of CCl₄ injection. Also, the normal control group showed no significant difference in triglyceride concentrations from the rat groups that were administered with 100mg/kg methanolic extracts of the leaf, root, bark and fruit ‘only’ of *M. lucida* for 7 days. However, there was a significantly higher concentration ($p<0.05$) of triglyceride in the group injected with an only dose of CCl₄ than both the normal control and the groups that were pre-treated with 100mg/kg of methanolic extracts of the various parts of *M. lucida* for 7 days after CCl₄ injection. It was indicative of the triglyceride lowering effects of *M. lucida* in the absence or presence of the CCl₄ – hepatotoxin (Table 4.14).

4.7.6 Total cholesterol (TC) concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

The concentration of cholesterol in the normal control group was significantly lower ($p<0.05$) than in the CCl₄ only group (table 4.14). Also, the CCl₄ only group presented a higher total cholesterol concentration than all the rat groups that received daily single doses of 100mg/kg of methanolic extracts of the different parts of *M. lucida* for 7 days after a single dose of CCl₄. There was no significant difference in the cholesterol concentrations in the only leaf, bark and fruit extracts except for the only root extract group that presented a very high cholesterol concentration. Similarly,

the group that was treated for 7 days with daily doses of 100mg/kg of the methanolic fruit extract presented a very high ($p<0.05$) cholesterol concentration after the initial CCl_4 administration. Contrariwise, among the group that received single daily doses of 100mg/kg of methanolic extracts of the various plant parts after an initial CCl_4 administration, the bark extract presented the least cholesterol concentration.

4.7.7 High density lipoprotein (HDL) concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl_4 damage

HDL concentration as presented in table 4.14, showed a higher value ($p<0.05$) in the normal control group than among the rats treated with ‘ CCl_4 only’. The normal group also indicated a significantly lower HDL concentration ($p<0.05$) than those that were administered with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* extracts only. The ‘ CCl_4 only’ group showed a significantly lower ($p<0.05$) HDL concentration than those that were post-treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days after injection with single dose of the CCl_4 hepatotoxin. The ‘ CCl_4 only’ group also showed a significantly lower ($p<0.05$) HDL concentration than those that were treated with 100mg/kg of the methanolic extracts of parts of *M. lucida* only for 7 days. Among the groups that were post-treated with 100mg/kg of the methanolic extracts of parts of *M. lucida* for 7 days after injection with single dose of the CCl_4 hepatotoxin, those that received *M. lucida* leaf extract presented the lowest HDL concentration. The ‘ CCl_4 + bark’ presented a low LDL/HDL ratio among the post treatment groups. The ratio was comparable to the normal control.

4.7.8 Low density lipoprotein (LDL) concentration in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage

As shown in table 4.14, LDL concentration in the normal control group was lower than among the rats treated with 'CCl₄ only' ($p < 0.05$). The normal group also indicated a significantly lower LDL concentration ($p < 0.05$) than those that were treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* after injection with CCl₄ except the bark extracts which was lower. The 'CCl₄ only' group also showed higher LDL concentration than those that were post-treated with 100mg/kg of the methanolic extracts of the different parts of *M. lucida* for 7 days after the single dose of CCl₄ injection. There was no significant difference among the groups that were administered with daily single dose of 100mg/kg of the methanolic extract of the different parts of *M. lucida* for 7 days after injection with single dose of the CCl₄ hepatotoxin, except the 'CCl₄ + bark extract' group which showed significantly lower ($p > 0.05$) LDL concentration. The LDL/HDL ratios obtained in all the post treatment groups except in the 'CCl₄ + bark' group were higher than the normal control group.

Table 4.14: Concentration of cholesterol, triglyceride, HDL and LDL in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage:

Treatment group	Triglyceride (mg/dl)	Total Cholesterol (U/l)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL Ratio
Normal control	79.60 ± 7.07 ^a	31.11 ± 19.44 ^a	40.44 ± 0.75 ^a	34.95 ± 9.51 ^a	0.86
CCl ₄ only	277.85 ± 18.88 ^b	319.24 ± 41.78 ^b	35.35 ± 1.45 ^b	95.57 ± 5.23 ^b	2.70
Fruit only	78.03 ± 9.56 ^a	6.67 ± 2.33 ^a	42.02 ± 0.72 ^c	10.80 ± 2.64 ^d	0.26
Leaf only	64.41 ± 8.14 ^a	13.39 ± 3.45 ^a	44.12 ± 0.78 ^c	7.60 ± 1.11 ^d	0.17
Bark only	87.11 ± 21.49 ^a	10.92 ± 2.27 ^a	42.55 ± 0.64 ^c	14.62 ± 1.88 ^{cd}	0.34
Root only	69.30 ± 5.88 ^a	211.66 ± 93.78 ^c	46.06 ± 0.89 ^c	71.93 ± 14.61	1.56
CCl ₄ + fruit	86.94 ± 6.07 ^a	280.56 ± 33.82 ^d	42.90 ± 0.24 ^c	73.97 ± 9.04	1.72
CCl ₄ + leaf	113.66 ± 32.02 ^a	120.09 ± 41.19 ^c	38.75 ± 0.78	74.72 ± 13.24	1.93
CCl ₄ + bark	83.97 ± 11.68 ^a	13.36 ± 3.49 ^a	46.60 ± 0.59 ^c	25.35 ± 6.47 ^{ac}	0.54
CCl ₄ + root	88.09 ± 11.59 ^a	146.18 ± 52.56 ^c	44.17 ± 1.94 ^c	81.20 ± 3.54	1.82

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.8 RESULTS OF KIDNEY FUNCTION EXPERIMENTS

4.8.1 Creatinine Concentration in rats pre-treated with extracts before CCl₄ administration

As presented in Table 4.15, the serum creatinine concentration on the ‘normal control’ group was significantly lower than in the ‘CCl₄ only’ group. All the groups presented significantly lower creatinine concentration ($p > 0.05$) than the group injected with ‘CCl₄ only’ without pre-treatment with *M. lucida* extracts. There was no significant difference in creatinine concentration among the groups pre-treated with single daily doses of 100mg/kg b.w. of extracts of *M. lucida* for 7 days before the injection of CCl₄ except the ‘bark extract + CCl₄’ group which was higher ($p < 0.05$). Also, they showed significantly higher ($p < 0.05$) creatinine concentration than the groups administered only with daily doses 100mg/kg b.w. for 7 days.

4.8.2 Total urea concentration in rats pre-treated with extracts before CCl₄ administration

As presented in Table 4.15, the ‘normal control’ group showed a significantly lower urea concentration than the ‘CCl₄ only’ group ($p < 0.05$). There was also a significantly lower urea concentration ($p < 0.05$) in the normal control group and those pre-treated with daily single doses of 100mg/kg b.w. of methanolic extracts of the various parts of *M. lucida* before CCl₄ injection. The ‘CCl₄ only’ group presented a significantly higher ($p < 0.05$) urea concentration than those that were administered with the daily dose of 100mg/kg b.w. of methanolic extracts of *M. lucida* for 7 days before CCl₄ injection. There was no significant difference shown between urea concentration

among the group pre-treated with vitamin E and the 'CCl₄ only' group. There was a significantly lower ($p < 0.05$) urea concentration presented by the vitamin C pre-treated group than the 'Vitamin E + CCl₄' group. Among the groups that were pre-treated with daily doses of 100mg/kg b.w. of parts of *M. lucida* extracts before the injection of a single dose of CCl₄, the root extracts pre-treated group showed significantly lower ($p > 0.05$) urea concentration.

4.8.3 Total protein concentration in rats pre-treated with extracts before CCl₄ administration

There was no significant difference (table 4.15) in the total protein concentration between the normal control group and the groups that were administered only with 100mg/kg b.w. of the methanolic extracts of the different parts of *M. lucida* for 7 days. Also, the normal control group did not show significant difference in the total protein concentration when compared to those that received 7 days pre-treatment with daily doses of 100mg/kg b.w. of *M. lucida* leaf and root extracts before the injection of a single dose of CCl₄. The group administered with only CCl₄ showed a significantly lower ($p > 0.05$) protein concentration than the normal control group and the groups administered with only 100mg/kg b.w. of extracts daily for 7 days ($p < 0.05$). Among the groups that were pre-treated with daily doses of 100mg/kg b.w. of parts of *M. lucida* extracts before the injection of a single dose of CCl₄, the bark and fruit extracts pre-treated groups before CCl₄ injection showed significantly lower ($p > 0.05$) total protein concentration but not significantly different from the group that was injected with CCl₄ only.

Table 4.15: Concentration of creatinine, urea and total protein in rats pre-treated with methanol extracts of *M. lucida* before CCl₄ administration.

Treatment group	Creatinine (nMol/mg protein)	Urea (mg/dL)	Total protein (g/L)
Normal control	0.8 ± 0.26 ^a	40.3 ± 5.20 ^{ab}	23.01 ± 6.14 ^a
CCl ₄ only	2.2 ± 0.24 ^b	51.8 ± 3.83 ^b	11.21 ± 2.16 ^b
Fruit only	1.1 ± 0.51 ^a	15.0 ± 5.19 ^c	23.23 ± 3.00 ^a
Leaf only	1.0 ± 0.21 ^a	19.6 ± 8.07 ^c	28.44 ± 4.33 ^a
Bark only	0.6 ± 0.23 ^a	17.3 ± 5.78 ^c	24.47 ± 4.69 ^a
Root only	1.1 ± 0.38 ^a	12.7 ± 2.83 ^c	20.55 ± 4.03 ^{ab}
Vitamin E + CCl ₄	1.0 ± 0.41 ^a	44.2 ± 3.77 ^b	21.62 ± 2.19 ^a
Vitamin C + CCl ₄	1.3 ± 0.35 ^c	23.5 ± 6.24 ^a	29.54 ± 4.72 ^a
Fruit + CCl ₄	1.0 ± 0.23 ^a	34.5 ± 6.18 ^a	23.27 ± 3.54 ^a
Leaf + CCl ₄	1.1 ± 0.54 ^a	40.3 ± 10.15 ^{ab}	25.59 ± 4.32 ^a
Bark + CCl ₄	1.3 ± 0.56 ^c	32.2 ± 5.59 ^{ab}	14.07 ± 2.35 ^b
Root + CCl ₄	1.1 ± 0.45 ^a	17.3 ± 3.80 ^{cd}	14.72 ± 2.07 ^b

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.8.4 Creatinine Concentration in rats post-treated with extracts after CCl₄ administration

As presented in Table 4.16 the ‘normal control group’ showed a significantly lower ($p<0.05$) creatinine concentration than the ‘CCl₄ only’ group. There was a significantly higher concentration of creatinine among the rats group that were injected with ‘CCl₄ only’ than those that were post-treated with 100mg/kg b.w. of methanolic extracts of leaf, root, bark and fruit of *M. lucida* after CCl₄ injection ($p<0.05$). There was no significant difference among all the groups post-treated with the different extracts after CCl₄ injection.

4.8.5 Total urea concentration in rats post-treated with extracts after CCl₄ administration

As shown in Table 4.16, the urea concentration of the group administered with only a single dose of CCl₄ also presented higher ($p<0.05$) urea concentration than the normal control group. Urea concentration among the rats injected with single dose of CCl₄ was significantly higher ($p<0.05$) than those that were post-treated with daily single dose of 100mg/kg b.w. for 7 days with different parts of *M. lucida* extracts only. There was no significant difference between the urea concentration of the ‘CCl₄ only’ group and those that were post-treated with 100mg/kg b.w. of the methanolic extract of the leaf and fruit of *M. lucida* for 7 days after CCl₄ injection. It was also found that the urea concentration of the rat groups that were first administered with single dose of the CCl₄ hepatotoxin before the 7 days treatment with 100mg/kg b.w. daily of the

methanolic extracts of the leaf and fruit, were higher ($p<0.05$) than those that received root and bark extracts after CCl_4 injection.

4.8.6 Total protein concentration in rats post-treated with extracts after CCl_4 administration

There was no significant difference between the protein concentration in the normal control group and all groups administered with only 100mg/kg b.w. of extracts daily for 7 days. Also, the normal control group did not show significant difference in the total protein concentration when compared to the group that received 7 days treatment with daily doses of 100mg/kg b.w. of *M. lucida* root or bark extracts after the injection of a single dose of CCl_4 . The group administered with only CCl_4 showed a significantly lower total protein concentration than the normal control group and the groups administered with only 100mg/kg b.w. of extracts daily for 7 days ($p>0.05$). Among the groups that received the injection of a single dose of CCl_4 prior to treatment for 7 days with daily doses of 100mg/kg b.w. of parts of *M. lucida* extracts, groups that received the fruit and leaf extracts after CCl_4 showed significantly lower total protein concentration ($p<0.05$) (table 4.16).

Table 4.16: Concentration of creatinine, urea and total protein in rats post-treated with methanol extracts of *M. lucida* after CCl₄ administration.

Treatment group	Creatinine (nMol/mg protein)	Urea (mg/dL)	Total protein (g/L)
Normal control	0.81 ± 0.26 ^a	40.3 ± 5.22 ^a	23.01 ± 6.14 ^a
CCl ₄ only	2.24 ± 0.17 ^b	51.8 ± 3.78 ^b	11.21 ± 2.16 ^b
Fruit only	1.08 ± 0.53 ^a	15.0 ± 5.18 ^c	20.55 ± 4.03 ^{ab}
Leaf only	1.04 ± 0.20 ^a	19.6 ± 8.08 ^c	23.23 ± 3.00 ^a
Bark only	0.64 ± 0.22 ^a	17.3 ± 5.76 ^c	24.47 ± 4.69 ^a
Root only	1.07 ± 0.44 ^a	12.7 ± 2.80 ^c	28.44 ± 4.33 ^a
CCl ₄ + fruit	1.39 ± 0.37 ^c	48.3 ± 8.71 ^b	15.44 ± 5.52 ^b
CCl ₄ + leaf	1.25 ± 0.35 ^c	44.2 ± 6.23 ^b	25.06 ± 2.18 ^a
CCl ₄ + bark	1.42 ± 0.48 ^c	12.7 ± 5.18 ^c	32.42 ± 7.18 ^a
CCl ₄ + root	1.73 ± 0.41 ^c	17.9 ± 3.75 ^c	13.33 ± 2.53 ^b

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column.

4.9 RESULTS OF CYTOTOXICITY EXPERIMENTS

4.9.1 Inhibitory effect of extracts on *Artemia* cyst

The findings showed that the fruit extract of *M. lucida* possessed the highest capacity to inhibit the *Artemia* cysts. The IC₅₀ obtained in their order of decreasing capacities were: fruit > root > leaf > bark (Table 4.17).

4.9.2 Lethal effect of extracts on *Artemia* nauplii:

The lethality assay using brine shrimps showed that the leaf extracts of *M. lucida* possessed the highest capacity to slay the *Artemia* nauplii (Table 4.18). The LC₅₀ obtained in their order of decreasing capacities were: leaf > root > bark > fruit.

Table 4.17: The IC₅₀ dose of methanolic extracts different parts of *Morinda lucida* obtained from the hatchability assay on *Artemia* cysts.

S/No.	Plant part	IC ₅₀ dose
1	Fruit	180.0mg/L
2	Leaf	230.0mg/L
3	Bark	400.0mg/L
4	Root	190.0mg/L

Table 4.18: The LC₅₀ dose of methanolic extracts different parts of *Morinda lucida* obtained from the lethality assay on *Artemia* nauplii.

S/No.	Plant part	LC ₅₀ dose
1	Fruit	631.0mg/L
2	Leaf	316.2mg/L
3	Bark	501.2mg/L
4	Root	398.1mg/L

4.9.3 RESULTS OF GENOTOXIC EFFECTS OF *MORINDA LUCIDA* EXTRACTS ON pUC18 PLASMID DNA

4.9.3.1 Genotoxic effects of *M. lucida* fruit extract

Plate 4 shows the plasmid nicking effect of Fenton's reagent on the pUC18 plasmid DNA (lane G) where only the plasmid DNA and Fenton's reagent were incubated, as very little or no traces of the DNA substance were visible as against lane H (pUC18 plasmid DNA + PBS) where the supercoiled pUC18 plasmid DNA was intact and clearly visible, and lane C where the protective effect of 200µg/10µl of quercetin standard was observed. Lanes D to F showed the protective effect of the methanolic fruit extract of *M. lucida* on the pUC18 DNA, where the pUC18 DNA was incubated with 100µg/10µl, 200µg/10µl and 500µg/10µl of the methanolic fruit extracts of *M. lucida* respectively, plus 10µl of Fenton's reagent. Also, the intensity of the supercoiled band was relatively more visible with lower concentrations (Plate 4). The highest visibility of the supercoil was observed in lane D where 100µg/10µl of the methanolic fruit extract of *M. lucida* was used, and the intensity was similar to that observed in lane C (quercetin standard at 200µg/10µl) and lane H where only the pUC18 plasmid DNA and PBS were loaded. But surprisingly, there was no band in lane B where only the pUC18 plasmid DNA was incubated with 500µg/10µl of the root extract, depicting the genotoxic effect of the methanolic fruit extract of *M. lucida* at the highest concentration used (500µg/10µl). Lane A showed the bands of the DNA marker (ladder) composed of varying kilobytes of DNA.



Plate 4: Effect of *M. lucida* methanolic fruit extract on the integrity of supercoiled pUC18 plasmid DNA (2686bp) in the presence of Fenton's reagent.

Lane A: Marker DNA or blank.

Lane B: pUC18 DNA + 500ug *M. lucida* fruit only.

Lane C: pUC18 DNA + 200ug quercetin (standard) + Fenton's reagent.

Lane D: pUC18 DNA + 100ug *M. lucida* fruit extract + Fenton's reagent.

Lane E: pUC18 DNA + 200ug *M. lucida* fruit extract + Fenton's reagent.

Lane F: pUC18 DNA + 500ug *M. lucida* fruit extract + Fenton's reagent.

Lane G: pUC18 DNA + Fenton's reagent (DNA damage control).

Lane H: pUC18 DNA + PBS.

4.9.3.2 Genotoxic effects of *M. lucida* leaf extract

Incubation of pUC18 plasmid DNA with Fenton's reagent for 15 minutes at 150V resulted in the cleavage of the supercoil to yield open circular and linear forms of plasmid DNA due to the attack of OH[•] (hydroxyl) radicals generated in the reaction mixture, on the nitrogenous bases or the deoxyribosyl backbone of DNA. Plate 5 showed the plasmid nicking effect of Fenton's reagent on the pUC18 plasmid DNA (lane G) where only the plasmid DNA and Fenton's reagent were incubated, as very little or no trace of the DNA substance were visible as against lane H where the supercoiled pUC18 plasmid DNA was intact/visible, and lane C where the protective effect of 200µg/10µl of quercetin standard was observed. Lanes D to F presents the absence of protective effect of the methanolic leaf extract of *M. lucida* on the pUC18 DNA, where the pUC18 DNA was incubated with 100µg/10µl, 200µg/10µl and 500µg/10µl of the methanolic leaf extracts of *M. lucida* respectively, plus 10ul of Fenton's reagent. However, the intensity of the supercoiled band was relatively more visible with lower concentrations (Plate 5). The highest visibility of the supercoil was observed in lane D where 100µg/10µl of the methanolic leaf extract of *M. lucida* was used. The non protective effect of the methanolic leaf extract of *M. lucida* was clearly depicted in lane B where the pUC18 plasmid DNA was incubated with 500µg/10µl of the leaf extract, as nicked circular form, indicative of single-strand breaks became more evident. Lane A (blank) where only 20µl of PBS was loaded showed no band, as expected.

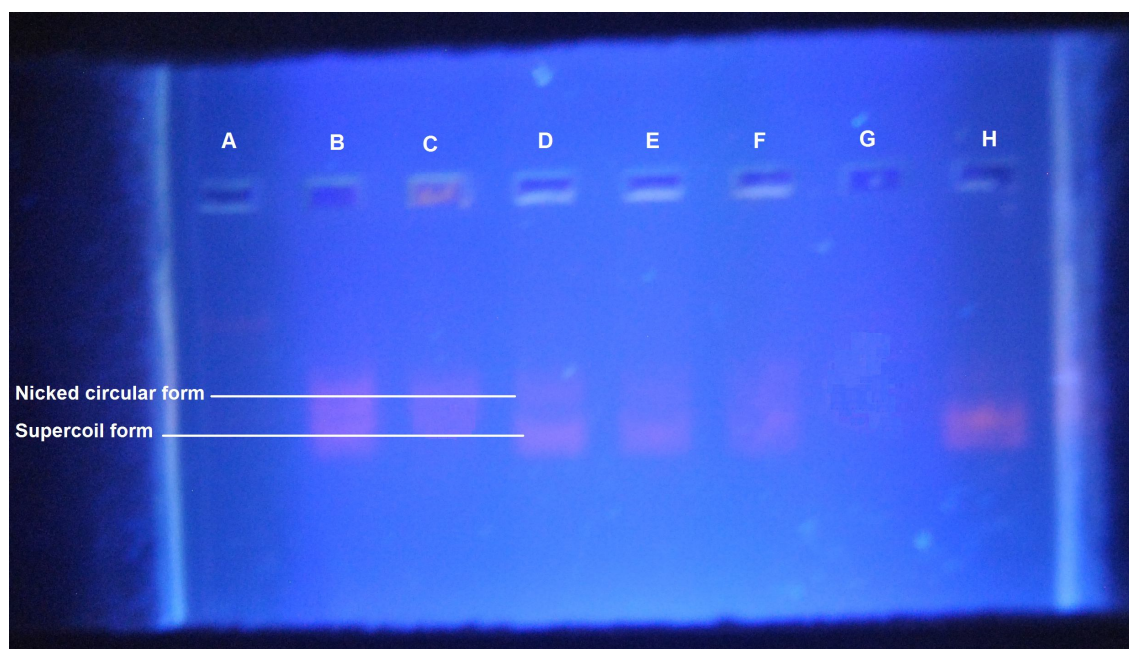


Plate 5: Effect of *M. lucida* methanolic leaf extract on the integrity of supercoiled pUC18 plasmid DNA (2686bp) in the presence of Fenton's reagent.

Lane A: Marker DNA or blank.

Lane B: pUC18 DNA + 500ug *M. lucida* leaf only.

Lane C: pUC18 DNA + 200ug quercetin (standard) + Fenton's reagent.

Lane D: pUC18 DNA + 100ug *M. lucida* leaf extract + Fenton's reagent.

Lane E: pUC18 DNA + 200ug *M. lucida* leaf extract + Fenton's reagent.

Lane F: pUC18 DNA + 500ug *M. lucida* leaf extract + Fenton's reagent.

Lane G: pUC18 DNA + Fenton's reagent (DNA damage control).

Lane H: pUC18 DNA + PBS.

4.9.3.3 Genotoxic effects of *M. lucida* bark extract

Plate 6 shows the plasmid nicking effect of Fenton's reagent on the pUC18 plasmid DNA (lane G) where only the plasmid DNA and Fenton's reagent were incubated, as very little or no traces of the DNA substance were visible as against lane H (pUC18 plasmid DNA + PBS) where the supercoiled pUC18 plasmid DNA was intact and clearly visible, and lane C where the protective effect of 200µg/10µl of quercetin standard was observed. Lanes D to F showed the protective effect of the methanolic bark extract of *M. lucida* on the pUC18 DNA, where the pUC18 DNA was incubated with 100µg/10µl, 200µg/10µl and 500µg/10µl of the methanolic bark extracts of *M. lucida* respectively, plus 10ul of Fenton's reagent. Also, the intensity of the supercoiled band was relatively more visible with lower concentrations (Plate 6). The highest visibility of the supercoil was observed in lane D where 100µg/10µl of the methanolic bark extract of *M. lucida* was used, and the intensity was similar to that observed in lane C (quercetin standard at 200µg/10µl) and lane H where only the pUC18 plasmid DNA and PBS were loaded. But surprisingly, there was no band in lane B where only the pUC18 plasmid DNA was incubated with 500µg/10µl of the root extract, depicting the genotoxic effect of the methanolic bark extract of *M. lucida* at the highest concentration used (500µg/10µl). Lane A showed the bands of the DNA marker (ladder) composed of varying kilobytes of DNA.

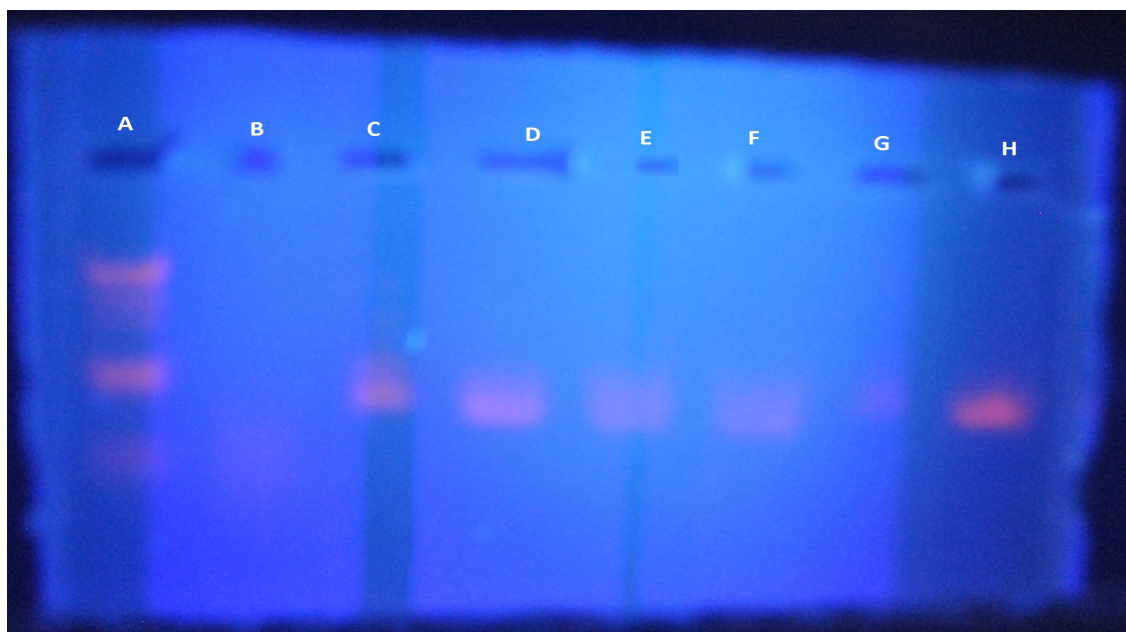


Plate 6: Effect of *M. lucida* methanolic bark extract on the integrity of supercoiled pUC18 plasmid DNA (2686bp) in the presence of Fenton's reagent.

Lane A: Marker DNA or blank.

Lane B: pUC18 DNA + 500ug *M. lucida* bark only.

Lane C: pUC18 DNA + 200ug quercetin (standard) + Fenton's reagent.

Lane D: pUC18 DNA + 100ug *M. lucida* bark extract + Fenton's reagent.

Lane E: pUC18 DNA + 200ug *M. lucida* bark extract + Fenton's reagent.

Lane F: pUC18 DNA + 500ug *M. lucida* bark extract + Fenton's reagent.

Lane G: pUC18 DNA + Fenton's reagent (DNA damage control).

Lane H: pUC18 DNA + PBS.

4.9.3.4 Genotoxic effects of *M. lucida* root extract

Plate 7 shows the plasmid nicking effect of Fenton's reagent on the pUC18 plasmid DNA (lane G) where only the plasmid DNA and Fenton's reagent were incubated, as very little or no traces of the DNA substance were visible as against lane A (pUC18 plasmid DNA + PBS) where the supercoiled pUC18 plasmid DNA was intact and clearly visible, and lane C where the protective effect of 200µg/10µl of quercetin standard was observed. Lanes D to F showed the protective effect of the methanolic root extract of *M. lucida* on the pUC18 DNA, where the pUC18 DNA was incubated with 100µg/10µl, 200µg/10µl and 500µg/10µl of the methanolic root extracts of *M. lucida* respectively, plus 10µl of Fenton's reagent. Furthermore, the intensity of the supercoiled band was relatively more visible with lower concentrations (Plate 7). The most visible supercoil was observed in lane D where 100µg/10µl of the methanolic root extract of *M. lucida* was used, and the intensity was similar to that observed in lane C (quercetin standard at 200µg/10µl) and lane H where only the pUC18 plasmid DNA and PBS were loaded. The protective effect of the methanolic root extract of *M. lucida* was thus, clearly depicted. However, in lane B where only the pUC18 plasmid DNA was incubated with 500µg/10µl of the root extract there were no clearly visible band. Lane A showed the bands of the DNA marker (ladder) made of varying kilobytes of DNA.

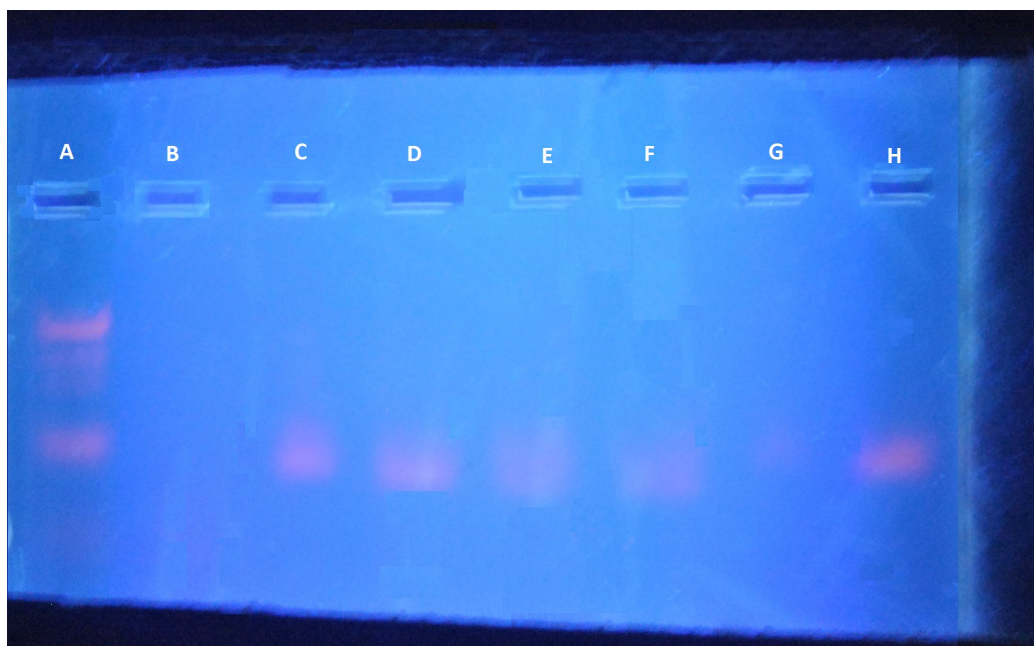


Plate 7: Effect of *M. lucida* methanolic root extract on the integrity of supercoiled pUC18 plasmid DNA (2686bp) in the presence of Fenton's reagent.

Lane A: Marker DNA or blank.

Lane B: pUC18 DNA + 500ug *M. lucida* root only.

Lane C: pUC18 DNA + 200ug quercetin (standard) + Fenton's reagent.

Lane D: pUC18 DNA + 100ug *M. lucida* root extract + Fenton's reagent.

Lane E: pUC18 DNA + 200ug *M. lucida* root extract + Fenton's reagent.

Lane F: pUC18 DNA + 500ug *M. lucida* root extract + Fenton's reagent.

Lane G: pUC18 DNA + Fenton's reagent (DNA damage control).

Lane H: pUC18 DNA + PBS.

4.10 HISTOPATHOLOGY OF LIVER AND KIDNEY TISSUES OF RATS TREATED WITH *MORINDA LUCIDA* EXTRACTS

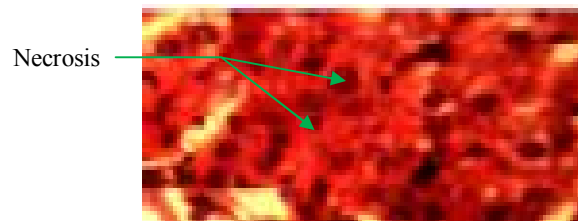
4.10.1 Liver histopathology in CCl₄ induced rats

The observation with the fruit extracts was that, the rats group that received 7 days administration of 100mg/kg of *M. lucida* fruit before CCl₄, showed only slight necrosis and distortions (Plate 8). The vitamins treated groups showed slides with good preservation of hepatocytes with negligible necrosis for α -tocopherol pre-treated group, and some necrosis and infiltration of adipocytes in the ascorbic acid pre-treated group (Plate 12 and 13).

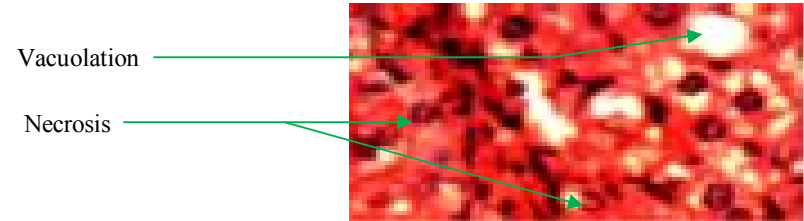
Liver tissues histopathological slides of rats pre-treated for 7 days with 100mg/kg with *M. lucida* leaf extract before and after the single dose of CCl₄ presented necrotic damages to the hepatocytes although, more severe in the later (Plate 9).

Plate 10 showed normal hepatocytes in both the tissue slide of rats that received 7 days administration of 100mg/kg of *M. lucida* bark extracts at both after and before the single dose of CCl₄. The histopathological pictures of the slides showed normal hepatocytes in both cases, similar to the slide of the 'normal control' and '*M. lucida* bark only', but contrasting sharply with the highly necrotic or damaged tissues of the 'CCl₄ only' slide.

In the groups treated with 100mg/kg of root extracts of *M. lucida* (Plate 11) there was less extensive vacuolation and necrosis of hepatocytes observed in the rats pre-treated with 100mg/kg of *M. lucida* for 7 days before CCl₄ injection, than those that were post-treated with the root extract after CCl₄ injection.

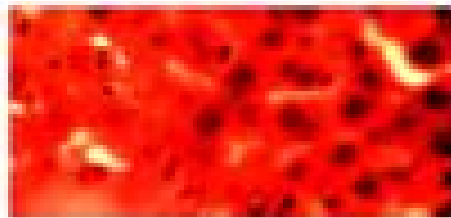


a. Fruit + CCl₄
- Slight necrosis and distortion
- Onset of vacuolation

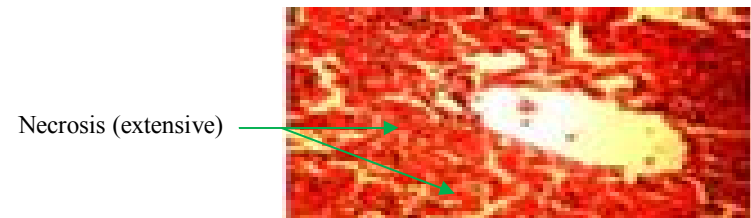


b. CCl₄+Fruit
- Necrosis of hepatocytes

c. Normal control
*Normal



d. Fruit only
Normal



e. CCl₄ only
- Portal necrosis of hepatocytes

Plate 8: Photomicrographs of liver sections of CCl₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of *M. lucida* fruit extracts (H & E stain x250), showing a = fruit pre-treatment before CCl₄ administration, b = fruit post-treatment after CCl₄ administration, c = normal control, d = fruit only and e = CCl₄ only.

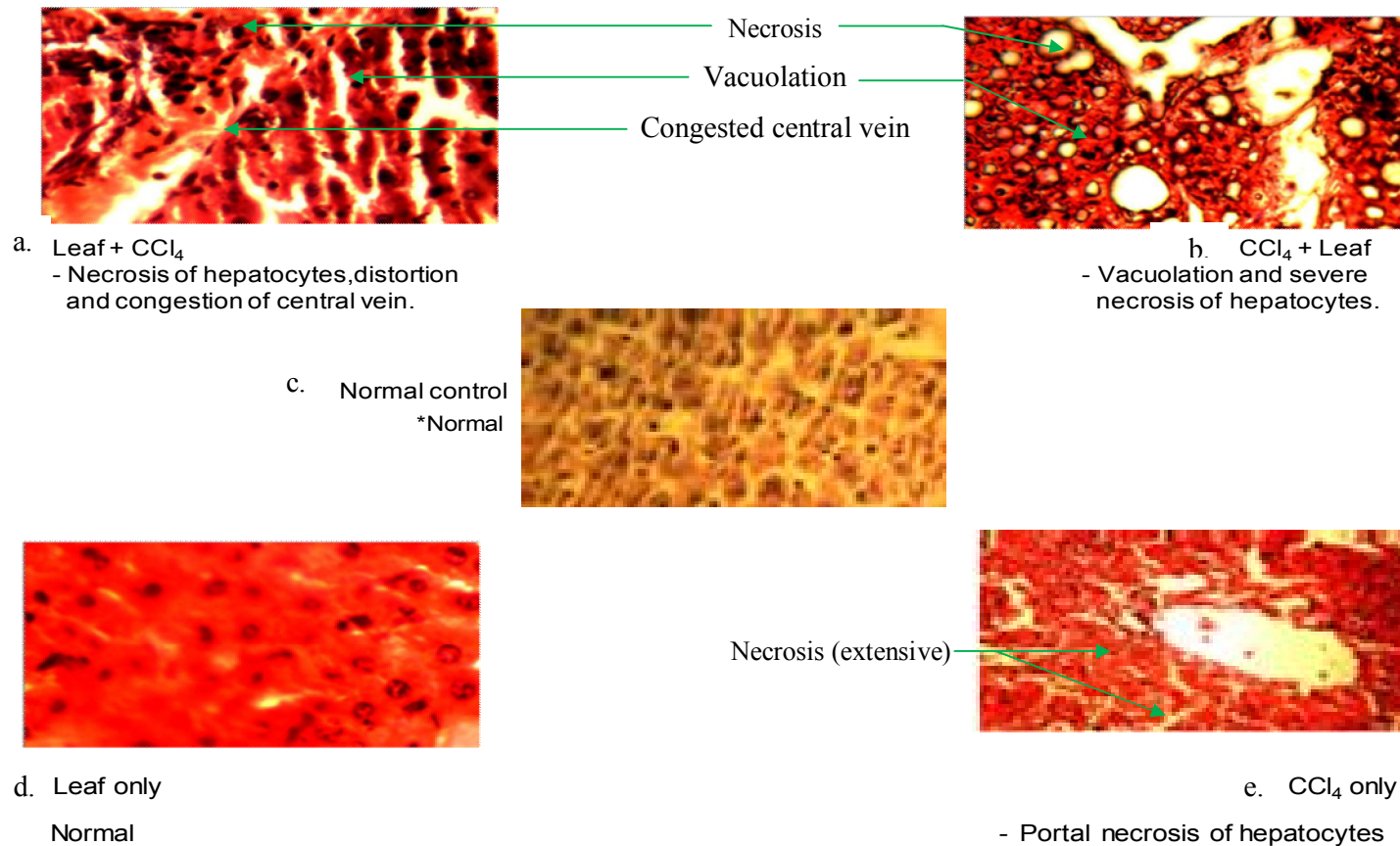
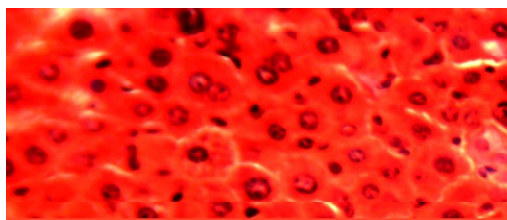
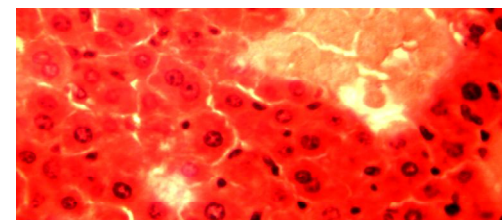


Plate 9: Photomicrographs of liver sections of CCl_4 - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg body weight of *M. lucida* leaf extracts (H & E stain x250), showing a = leaf pre-treatment before CCl_4 administration, b = leaf post-treatment after CCl_4 administration, c = normal control, d = leaf only and e = CCl_4 only.

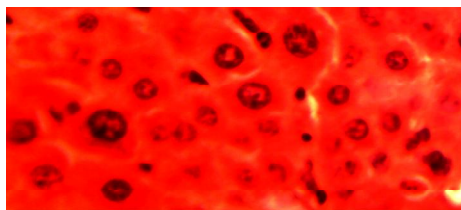
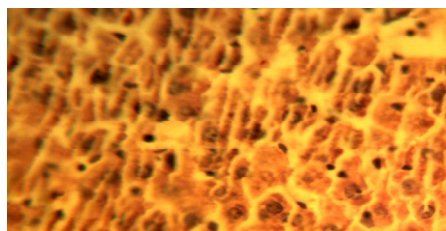


a. Bark + CCl₄
- Well protected hepatocytes



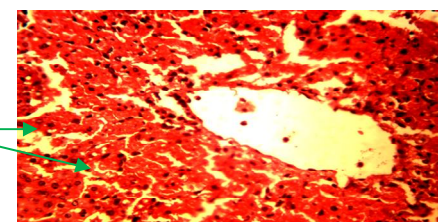
b. CCl₄ + Bark
- Normal with processing artefacts

c. Normal control
* Normal



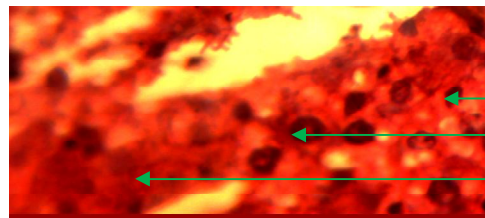
d. Bark only
- Preserved hepatocytes

Necrosis (extensive)



e. CCl₄ only
- Portal necrosis of hepatocytes

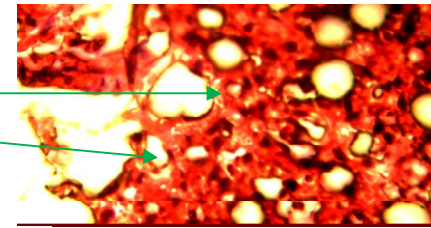
Plate 10: Photomicrographs of liver sections of CCl₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg of *M. lucida* bark extracts (H & E stain x250), showing a = bark pre-treatment before CCl₄ administration, b = bark post-treatment after CCl₄ administration, c = normal control, d = bark only and e = CCl₄ only.



a. Root + CCl₄

- Necrosis of hepatocytes, distortion,
- Congestion of central vein

Necrosis
Vacuolation
Congested central vein

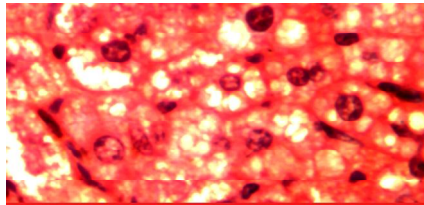
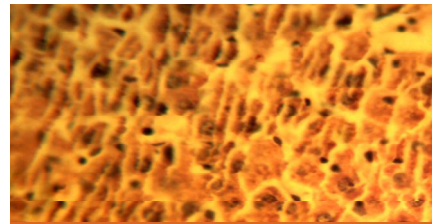


b. Cl₄ + Root

- Extensive vacuolation
- Necrosis of hepatocytes

c. Normal control

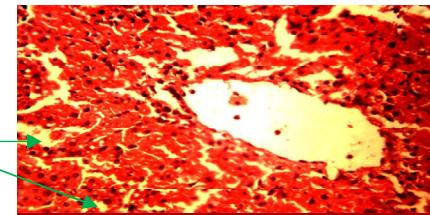
- Normal



d. Root only

- Preserved hepatocytes

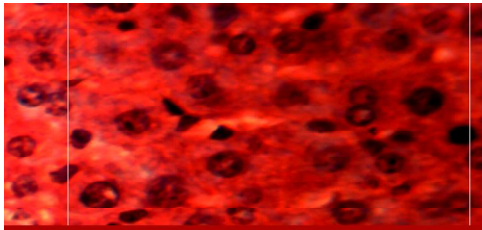
Necrosis (extensive)



e. Cl₄ only

- Portal necrosis of hepatocytes

Plate 11: Photomicrographs of liver sections of CCl₄ - induced hepatotoxicity in rats pre-and-post treated with 100mg/kg of *M. lucida* root extracts (H & E stain x250), showing a = root pre-treatment before CCl₄ administration, b = root post-treatment after CCl₄ administration, c = normal control, d = root only and e = CCl₄ only.

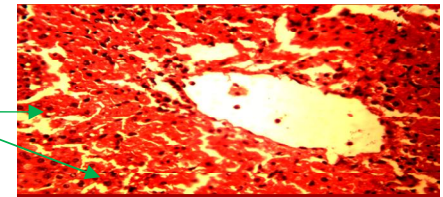
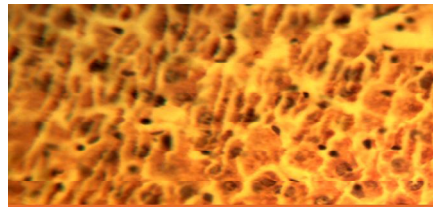


a. α -Tocopherol + CCl_4

*Good preservation of hepatocytes
Negligible necrosis of hepatocytes

b. Normal control

* Normal

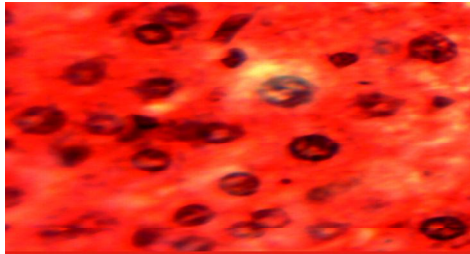


Necrosis (extensive)

c. CCl_4 only

*Portal necrosis of hepatocytes

Plate 12: Photomicrographs of liver sections of CCl_4 - induced hepatotoxicity in rats pre-administered with α -tocopherol (Vitamin E) (H & E stain x250), showing a = α -tocopherol pre-treatment before CCl_4 administration, b = normal control and c = CCl_4 only.



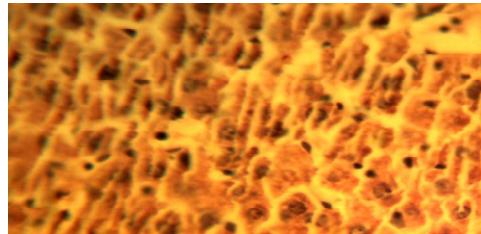
a. Ascorbic acid + CCl₄

*Preservation of hepatocytes

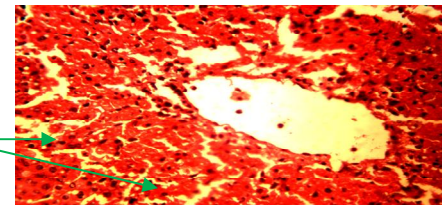
*Some infiltration of adipocytes with hepatic necrosis

b. Normal control

*Normal



Necrosis (extensive)



c. CCl₄ only

*Portal necrosis of hepatocytes

Plate 13: Photomicrographs of liver sections of CCl₄ - induced hepatotoxicity in rats pre-administered with ascorbic acid (Vitamin C) (H & E stain x250), showing a = ascorbic acid pre-treatment before CCl₄ administration, b = normal control and c = CCl₄ only.

4.10.2 Kidney histopathology in CCl₄ induced rats

The histopathological slides of kidney tissues of rats treated with single dose of 'CCl₄ only' showed extreme tubular necrosis, contrary to the 'normal control' and 'extract only' of fruit, leaf, bark and root of *M. lucida*. Groups that received 7 days treatment with 100mg/kg of *M. lucida* fruit extract before or after single dose of CCl₄ injection showed mild lymphocytic infiltration but with appreciable level of preservation of the glomerulocytes and slight necrosis (Plate 14). The α -tocopherol and ascorbic acid vitamins treated groups showed slides with good preservation of glomerulocytes (Plates 18 and 19).

The tissue slide of the group injected with CCl₄ before or after 7 days treatment with 100mg/kg of *M. lucida* leaf extract presented confluent tubular necrosis of the glomerulocytes, but more severe in the former (Plate 15).

There was an observed protective and/or curative effect of the bark extract against CCl₄ damage on the glomerulocytes showed by the normal histopathological slides of groups that received 7 days treatment with 100mg/kg of *M. lucida* bark extracts at both before and after the single dose of CCl₄ injection (Plate 16).

Like in the leaf, extensive damage was observed in the groups treated with 100mg/kg of root extracts of *M. lucida* (Plate 17) where extensive necrosis and lymphatic infiltrations were observed in the rats administered with CCl₄ before or after 7 days treatment with 100mg/kg of *M. lucida* root extract.

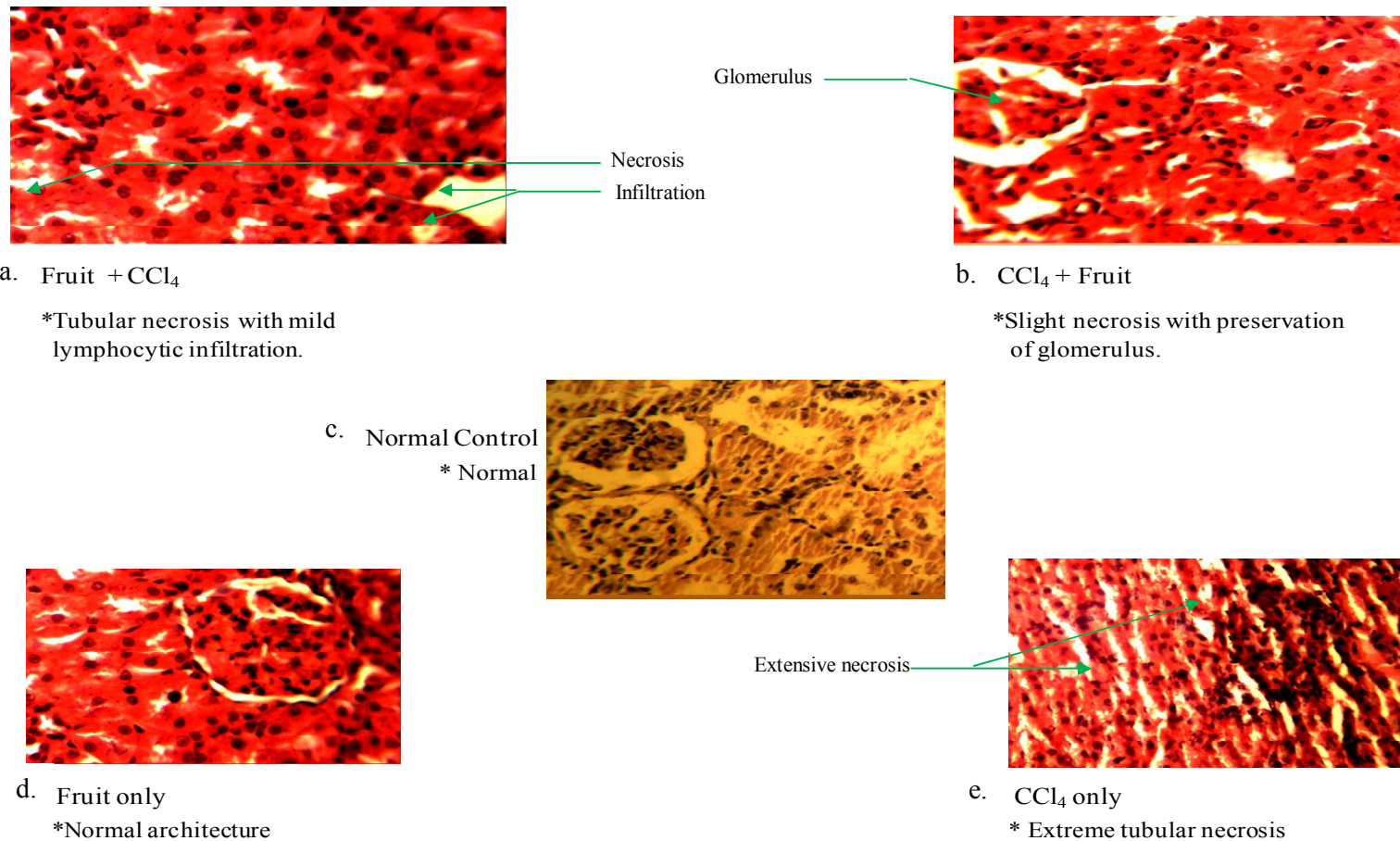


Plate 14: Photomicrographs of kidney sections of rats pre-and-post treated with 100mg/kg of *M. lucida* fruit extracts upon injection of CCl₄ (H & E stain x250), showing a = fruit pre-treatment before CCl₄ administration, b = fruit post-treatment after CCl₄ administration, c = normal control, d = fruit only and e = CCl₄ only.

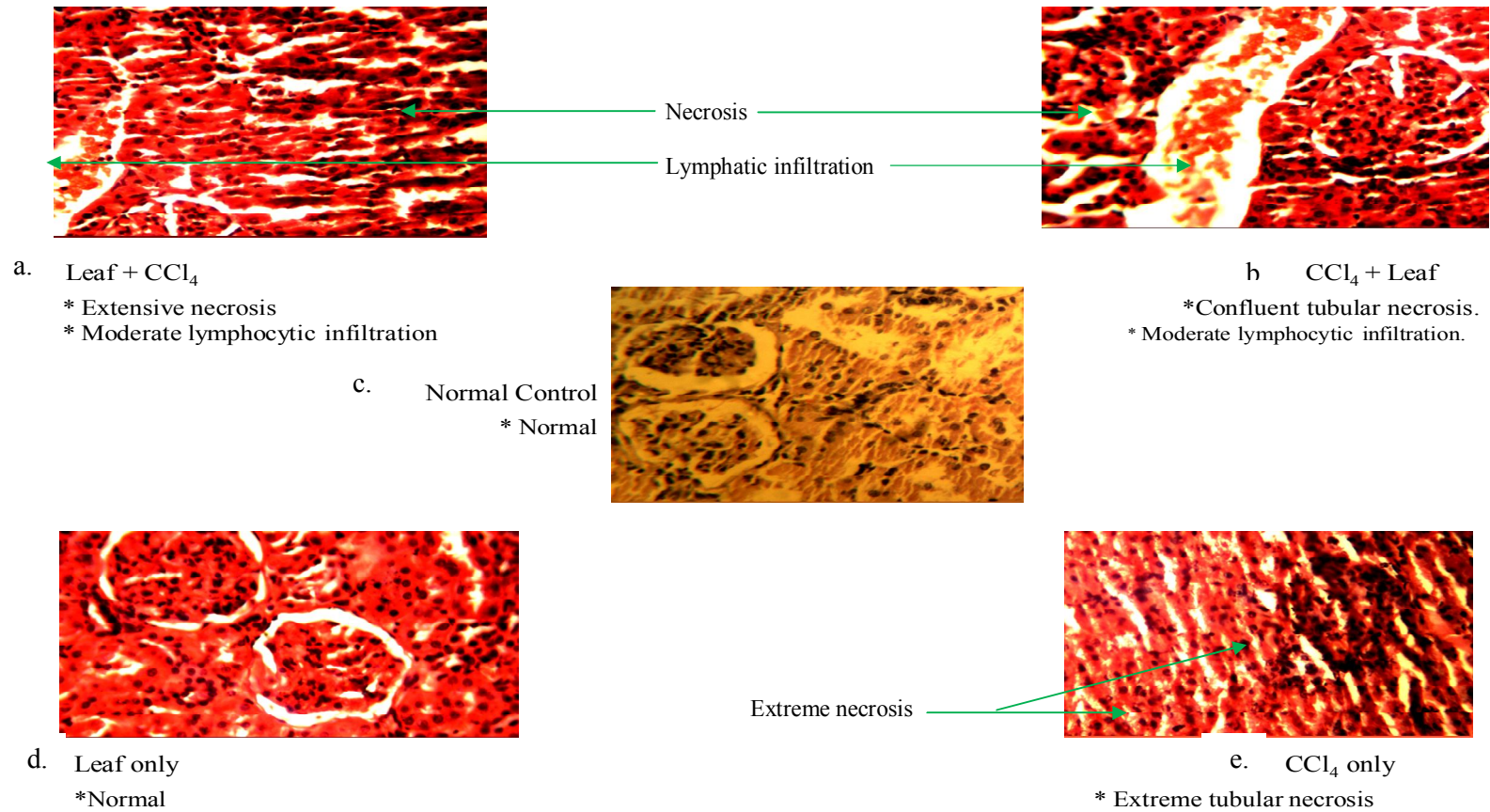
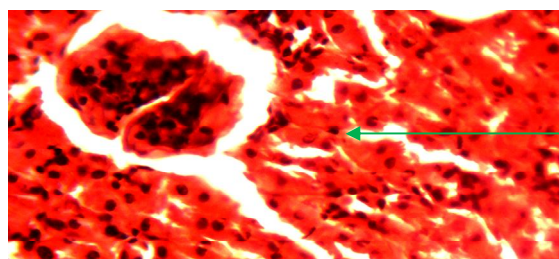
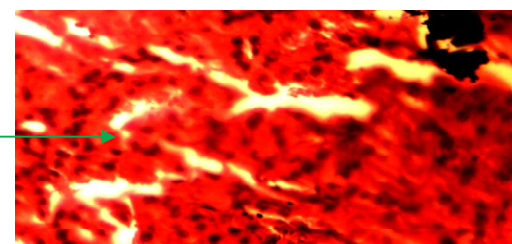


Plate 15: Photomicrographs of kidney sections of rats pre-and-post treated with 100mg/kg of *M. lucida* leaf extracts upon injection of CCl₄ (H & E stain x250), showing a = leaf pre-treatment before CCl₄ administration, b = leaf post-treatment after CCl₄ administration c = normal control d = leaf only and e = CCl₄ only



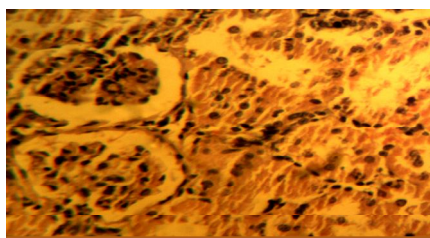
a. Bark + CCl₄

*Normal with mild lymphatic infiltration.



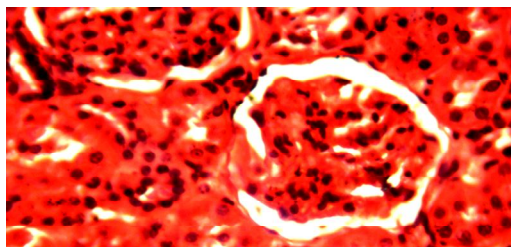
b. CCl₄ + Bark

*Normal with processing artefacts.



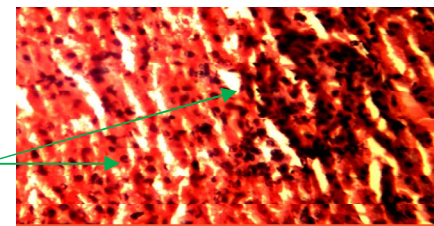
c. Normal Control

* Normal



d Bark only

*Normal with processing artefacts



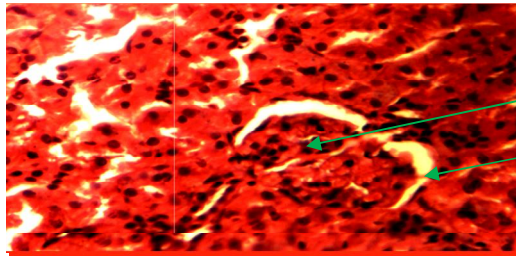
e. CCl₄ only

* Extreme tubular necrosis

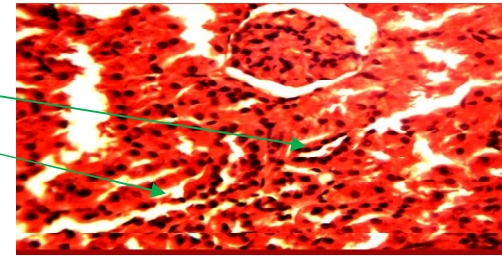
Lymphatic infiltration (mild)

Extensive necrosis

Plate 16: Photomicrographs of kidney sections of rats pre-and-post treated with 100mg/kg *M. lucida* bark extracts upon injection of CCl₄ (H & E stain x250), showing a = bark pre-treatment before CCl₄ administration, b = bark post-treatment after CCl₄ administration, c = normal control, d = bark only and e = CCl₄ only.

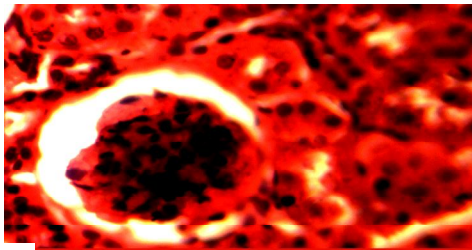
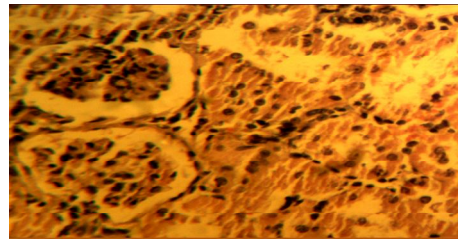


- a. Root + CCl₄
 * Extensive necrosis.
 * Moderate lymphocytic infiltration.

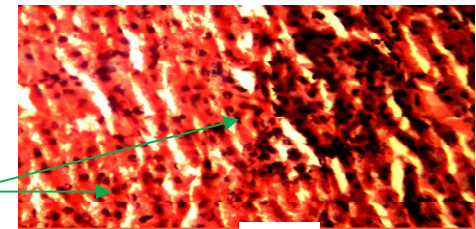


- b. CCl₄ + Root
 * Extensive necrosis
 * Steatosis with moderate lymphocytic infiltration.

- c. Normal Control
 * Normal



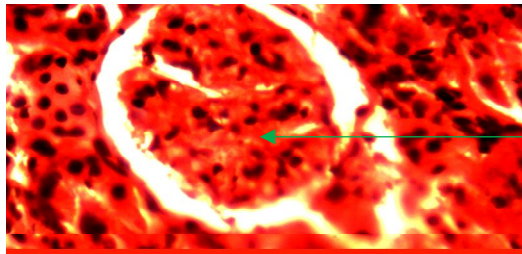
- d Root only
 * Normal with processing artefacts



Extreme necrosis

- e. CCl₄ only
 * Extreme tubular necrosis.

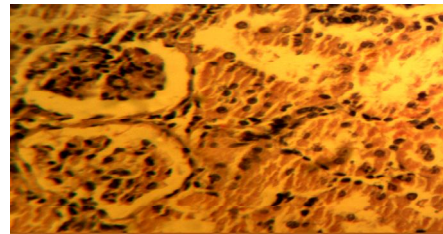
Plate 17: Photomicrographs of kidney sections of rats pre-and-post treated with 100mg/kg of *M. lucida* root extracts upon injection of CCl₄ (H & E stain x250), showing a = root pre-treatment before CCl₄ administration, b = root post-treatment after CCl₄ administration, c = normal control, d = root only and e = CCl₄ only.



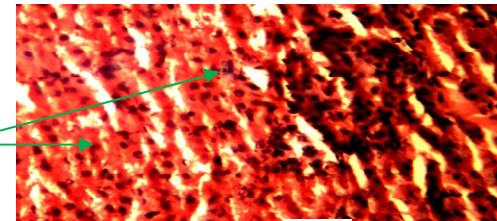
Glomerus

- a. α -Tocopherol + CCl_4
*Good preservation of glomerulus.

- b. Normal Control
*Normal

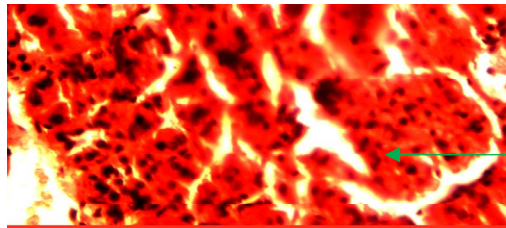


Extensive necrosis



- c. CCl_4 only
*Extreme tubular necrosis

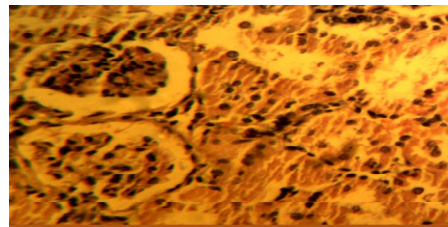
Plate 18: Photomicrographs of kidney sections of rats pre-administered with α -tocopherol (Vitamin E) after CCl_4 injection (H & E stain x250), showing a = α -tocopherol pre-treatment before CCl_4 administration, b = normal control and c = CCl_4 only.



Glomerulus (preserved)

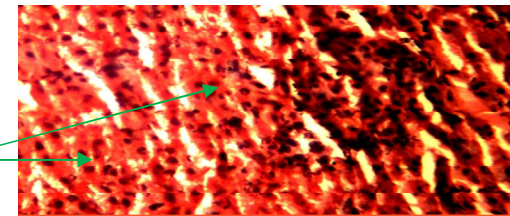
a. Ascorbic acid + CCl_4

*Slight necrosis with good preservation of glomerulus.



b. Normal Control

*Normal



Extensive necrosis

c. CCl_4 only

*Extreme tubular necrosis

Plate 19: Photomicrographs of kidney sections of rats pre-administered with ascorbic acid (Vitamin C) after CCl_4 injection (H & E stain x250), showing a = ascorbic acid pre-treatment before CCl_4 administration, b = normal control and c = CCl_4 only.

4.11 RESULTS OF HAEMATOLOGICAL STUDIES

4.11.1 Red blood cell count

As presented in Table 4.19, hematological investigations did not reveal any significant changes in RBC values among the various groups.

Table 4.20 showed that hematological investigations did not reveal any significant changes in RBC values among the various groups. However, the groups administered with 100mg/kg of the methanolic extracts of the leaf or fruits of *M. lucida* for 7 days before injection of a single dose of CCl₄ presented significantly higher ($p < 0.05$) RBC values than the CCl₄ only group (Table 4.19).

4.11.2 Packed cell volume

Table 4.19 and 4.20 showed that the hematological investigations did not reveal any significant changes in PCV values among all the various groups in this experiment, either among the extracts pre-treated before or post-treated after CCl₄ administration.

4.11.3 Haemoglobin count

Hematological investigations (tables 4.19) revealed that all the groups that were treated with daily single doses of 100mg/kg for 7 days with different parts of *M. lucida* before injection with CCl₄, presented significantly higher ($p < 0.05$) haemoglobin (Hb) concentration values than the 'CCl₄ only' group. There was also a significant difference between the 'CCl₄ only' and normal control group. But there was no significant difference among the *M. lucida* extracts pre-treated groups or among the 'extract only' groups.

4.11.4 White blood cell count

Hematological investigations as presented in Table 4.19 did not reveal any significant changes among the groups particularly, between the normal control and 'CCl₄ only' group. However, the group administered with 100mg/kg of the methanolic extracts of the bark and fruit of *M. lucida* for 7 days before injection of a single dose of CCl₄, presented significantly lower ($p < 0.05$) WBC values than the 'CCl₄ only' group (Table 4.19). The same groups also showed lower values than those that received the leaf and root extracts before CCl₄.

The group administered with 100mg/kg of the methanolic extracts of the bark of *M. lucida* for 7 days after injection of a single dose of CCl₄, and the groups that only received daily administration of 100mg/kg of the methanolic extracts of the bark and fruit of *M. lucida* for 7 days presented significantly lower ($p < 0.05$) values than the CCl₄ only group (Table 4.20).

4.11.5 Lymphocytes count

There was significantly lower lymphocyte value among the groups administered with the bark and fruit extracts before CCl₄ injection (Table 4.19) than those that received leaf and root extracts after CCl₄ injection. Table 4.20 showed that groups that were treated with daily single doses of 100mg/kg for 7 days with bark and fruit extracts of *M. lucida* after injection with CCl₄, presented significantly lower ($p < 0.05$) amount of lymphocytes than the 'CCl₄ only' group. There was also a significant difference between the 'CCl₄ only' and normal control group.

Experimental findings (table 4.19), showed that groups that were treated with daily single doses of 100mg/kg for 7 days with extracts of all the different parts of *M. lucida* before injection with CCl₄, presented significantly lower ($p<0.05$) amount of lymphocytes than the 'CCl₄ only' group. There was also a significant difference between the 'CCl₄ only' and normal control group. There was also a slightly lower lymphocyte value among the normal control group than the 'CCl₄ only' group.

4.11.6 Neutrophil count

In Table 4.19, the groups that were treated with daily single doses of 100mg/kg for 7 days with extracts of all the different parts of *M. lucida* before injection with a single dose of CCl₄, presented significantly higher ($p<0.05$) amount of neutrophils than the 'CCl₄ only' group but not significantly different from the normal control. Also, there was a significant difference between the 'CCl₄ only' and normal control group. But there was no significant difference between the '*M. lucida* root or bark extract only' and 'CCl₄ only' group.

The groups that were treated with daily single doses of 100mg/kg for 7 days with bark and fruit extracts of *M. lucida* after injection with CCl₄, presented significantly higher ($p<0.05$) amount of neutrophils than the 'CCl₄ only' group but not significantly different from the normal control (Table 4.20). There was a significant difference between the 'CCl₄ only' and normal control group. There was also, significantly higher amount of neutrophils among the groups administered with the leaf or fruit extracts only, than those that received root or bark extracts only.

4.11.7 Platelet count

As presented also in Table 4.19, the group that was treated with daily single doses of 100mg/kg for 7 days with bark extract of *M. lucida* before injection with a single dose of CCl₄, presented platelet value that was not significantly different from the normal control group. The normal control group was significantly higher than the 'CCl₄ only' group ($p<0.05$).

Findings revealed that only the group that was treated with daily single doses of 100mg/kg for 7 days with root and fruit extracts of *M. lucida* after injection with a single dose of CCl₄, presented significantly higher ($p<0.05$) amount of platelets than the 'CCl₄ only' group but not significantly different from the normal control (table 4.20). Also, there was a significant difference between the 'CCl₄ only' and normal control group. But there was no significant difference between the '*M. lucida* root, fruit or bark extract only' and 'normal control' group.

4.11.8 Mean corpuscular volume

As presented in Table 4.19, there was no significant difference between the normal control and 'CCl₄ only' groups. The groups that were treated with daily single doses of 100mg/kg for 7 days with leaf or bark extracts of *M. lucida* before injection with a single dose of CCl₄, presented significantly lower ($p<0.05$) MCV than the 'CCl₄ only' group and the normal control.

There was no significant difference between the normal control and 'CCl₄ only' groups (Table 4.20). All the groups that were treated with daily single doses of 100mg/kg for 7 days with root, bark and fruit extracts of *M. lucida* after injection with a single dose of CCl₄, did not presented any significantly different ($p < 0.05$) mean corpuscular volume (MCV) than the 'CCl₄ only' group, except the 'CCl₄ + leaf' group.

Table 4.19: Haematological changes in rats tested for hepatoprotective effect of *M. lucida* extracts against CCl₄ damage.

Treatment group	RBC ($\times 10^6/\text{mm}^3$)	PCV (%)	Hb (g/dL)	WBC ($\times 10^3/\text{mm}^3$)	Lymphocytes (%)	Neutrophils (%)	Platelets ($\times 10^9/\text{L}$)	MCV (mm^3)
Normal control	7.94 $\pm$ 0.85 ^a	49.83 $\pm$ 8.01 ^a	14.00 $\pm$ 1.71	12.60 $\pm$ 1.81 ^a	69.50 $\pm$ 8.44 ^{ab}	27.60 $\pm$ 9.29 ^a	819.17 $\pm$ 230.33 ^a	55.55 $\pm$ 18.75 ^a
CCl ₄ only	7.87 $\pm$ 0.66 ^a	45.33 $\pm$ 2.16 ^a	13.05 $\pm$ 0.66 ^b	11.88 $\pm$ 1.10 ^{ab}	83.00 $\pm$ 12.36 ^b	13.17 $\pm$ 5.27 ^b	640.17 $\pm$ 197.47 ^b	57.85 $\pm$ 2.66 ^a
Fruit only	8.47 $\pm$ 0.56 ^{ab}	51.50 $\pm$ 3.99 ^a	14.2 $\pm$ 0.72	8.63 $\pm$ 0.92 ^b	61.00 $\pm$ 12.13 ^a	33.00 $\pm$ 12.95 ^a	762.17 $\pm$ 76.12 ^a	60.67 $\pm$ 3.06
Leaf only	7.66 $\pm$ 1.12 ^a	49.67 $\pm$ 4.08 ^a	13.30 $\pm$ 1.23	13.77 $\pm$ 1.55 ^a	68.83 $\pm$ 6.82 ^{ab}	29.50 $\pm$ 6.05 ^a	1015.60 $\pm$ 101.06 ^c	65.82 $\pm$ 4.56
Bark only	7.76 $\pm$ 0.49 ^a	47.17 $\pm$ 2.64 ^a	13.18 $\pm$ 0.37	8.42 $\pm$ 0.92 ^b	81.83 $\pm$ 11.72 ^b	16.33 $\pm$ 3.44 ^b	845.00 $\pm$ 94.36 ^a	53.32 $\pm$ 9.21 ^a
Root only	8.24 $\pm$ 1.36 ^{ab}	48.67 $\pm$ 7.34 ^a	13.73 $\pm$ 1.73	13.63 $\pm$ 1.34 ^a	80.33 $\pm$ 3.83 ^b	17.00 $\pm$ 2.37 ^b	973.20 $\pm$ 73.69 ^a	59.33 $\pm$ 3.75 ^a
α -Tocopherol + CCl ₄	8.05 $\pm$ 0.18 ^{ab}	51.00 $\pm$ 1.90 ^a	14.43 $\pm$ 0.33	12.33 $\pm$ 1.68 ^{ab}	70.50 $\pm$ 6.80 ^{ab}	30.00 $\pm$ 6.78 ^a	819.17 $\pm$ 77.79 ^a	63.27 $\pm$ 2.33
Ascorbic acid + CCl ₄	8.17 $\pm$ 0.38 ^{ab}	49.67 $\pm$ 2.94 ^a	14.20 $\pm$ 0.62	11.82 $\pm$ 1.32 ^{ab}	70.00 $\pm$ 5.18 ^{ab}	27.33 $\pm$ 5.01 ^a	640.17 $\pm$ 87.37 ^b	59.27 $\pm$ 4.01 ^a
Fruit + CCl ₄	9.03 $\pm$ 0.48 ^b	53.17 $\pm$ 3.31 ^a	14.52 $\pm$ 0.91	9.02 $\pm$ 0.98 ^b	63.00 $\pm$ 11.52 ^a	33.33 $\pm$ 8.48 ^a	753.00 $\pm$ 88.8 ^b	54.42 $\pm$ 6.95
Leaf + CCl ₄	8.79 $\pm$ 0.62 ^b	54.33 $\pm$ 5.61 ^a	14.65 $\pm$ 1.46	11.50 $\pm$ 1.50 ^{ab}	58.17 $\pm$ 8.52 ^a	39.17 $\pm$ 7.05 ^a	588.83 $\pm$ 52.11 ^b	47.33 $\pm$ 10.49 ^a
Bark + CCl ₄	7.67 $\pm$ 0.09 ^a	48.33 $\pm$ 2.16 ^a	13.18 $\pm$ 0.63	9.40 $\pm$ 1.36 ^b	61.17 $\pm$ 1.47 ^a	36.00 $\pm$ 4.56 ^a	975.40 $\pm$ 190.88 ^a	47.07 $\pm$ 5.59 ^a
Root + CCl ₄	7.45 $\pm$ 0.91 ^a	45.33 $\pm$ 5.75 ^a	13.17 $\pm$ 1.56	15.38 $\pm$ 1.52 ^a	66.17 $\pm$ 1.47 ^a	31.00 $\pm$ 2.19 ^a	720.20 $\pm$ 95.82 ^b	60.62 $\pm$ 1.58

Values are Mean $\pm$ SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

Table 4.20: Haematological changes in rats tested for hepatocurative effect of *M. lucida* extracts against CCl₄ damage.

Treatment group	RBC (x10 ⁶ mm ³)	PCV (%)	Hb (g/dL)	WBC (x10 ³ mm ³)	Lymphocytes (%)	Neutrophils (%)	Platelets (x10 ⁹ /L)	MCV (mm ³)
Normal control	7.94±0.85 ^a	49.83±8.01 ^a	14.00±1.71	12.60±1.81 ^a	69.50±8.44 ^{ab}	27.60±9.29 ^a	819.17±77.79 ^a	55.55±8.07 ^a
CCl ₄ only	7.87±0.66 ^a	45.33±2.16 ^a	13.05±0.66 ^b	11.88±1.10 ^{ab}	83.00±12.36 ^b	13.17±5.27 ^b	640.17±87.37 ^b	57.85±2.66 ^a
Fruit only	8.47±0.56 ^{ab}	51.50±3.99 ^a	14.2±0.72	8.63±0.92 ^b	61.00±12.13 ^a	33.00±12.95 ^a	762.17±76.12 ^a	60.67±3.06 ^a
Leaf only	7.66±1.12 ^a	49.67±4.08 ^a	13.30±1.23	13.77±1.55 ^a	68.83 ± 6.82 ^{ab}	29.50±6.05 ^a	1015.60±101.06 ^c	65.82±4.56
Bark only	7.76±0.49 ^a	47.17±2.64 ^a	13.18±0.37	8.42±0.92 ^b	81.83±11.72 ^b	16.33±3.44 ^b	845.00±94.36 ^a	53.32±9.21 ^a
Root only	8.24±1.36 ^{ab}	48.67±7.34 ^a	13.73±1.73	13.63±1.34 ^a	80.33±3.83 ^b	17.00±2.37 ^b	973.20±73.69 ^a	59.33±3.75 ^a
CCl ₄ + fruit	8.27±0.22 ^{ab}	50.60±4.10 ^a	14.20±0.90	10.70±0.60 ^{ab}	66.60±8.96 ^a	30.00±4.90 ^a	816.33±67.51 ^a	62.45±1.49
CCl ₄ + leaf	8.26±1.58 ^{ab}	51.67±7.89 ^a	13.65±1.78	10.88±0.62 ^a	80.00±3.22 ^b	17.00±4.82 ^b	616.67±80.44 ^b	41.02±6.41 ^a
CCl ₄ + bark	8.44±0.49 ^{ab}	51.50±2.59 ^a	14.67±0.79	7.37±1.61 ^b	66.33±7.37 ^a	31.17±7.11 ^a	724.33±87.90 ^b	61.00±3.02
CCl ₄ + root	8.16±0.74 ^{ab}	49.50±4.46 ^a	13.20±1.24	11.70±0.72 ^{ab}	75.00±3.29 ^b	22.33±3.93 ^b	847.20±53.13 ^a	60.68±2.93

Values are Mean±SD. (n=6). No significant difference between values with same superscripts down the column (p<0.05).

4.12 RELATIVE TOTAL FLAVONOID AND TOTAL POLYPHENOL CONTENT

As presented in tables 4.21 and 4.22, there were corresponding relative flavonoid and total polyphenol contents portrayed by the different parts of *M. lucida*. The bark extract contained the highest flavonoid and total polyphenol followed by the leaf extract. But, while the root extract showed a higher total polyphenol content than fruit, it contained the least amount of flavonoid.

Table 4.21: Total flavonoid contents of methanolic extracts of parts of *M. lucida*.

Plant Part	Total Flavonoid content in mg Quercetin
	equivalent/g dry weight of extract
<i>M. lucida</i> Fruit	89.09 $\pm$ 5.10
<i>M. lucida</i> Leaf	166.89 $\pm$ 4.28
<i>M. lucida</i> Bark	314.12 $\pm$ 9.10
<i>M. lucida</i> Root	56.58 $\pm$ 7.14

Table 4.22: Total polyphenol contents of methanolic extracts of parts of *M. lucida*

Plant Part	Total polyphenol content in mg Gallate equivalent/g dry weight of extract
<i>M. lucida</i> Fruit	108.25 ± 4.13
<i>M. lucida</i> Leaf	218.83 ± 10.26
<i>M. lucida</i> Bark	737.75 ± 6.85
<i>M. lucida</i> Root	125.58 ± 3.22

CHAPTER 5

5.0

DISCUSSION

5.1 Percentage of extracts yield

The growing attention in polyphenolic compounds from plant sources has reached a high level today. Reasons are due to their roles in preservation of manufactured food products and ability to offer protection from debilitating diseases linked with oxidative stress (Claudine *et al.*, 2004). The extent of yield of methanolic extract of plant material containing polyphenols is directly related to the amount of polyphenolic compound obtainable. Extracts yields of the different parts of *M. lucida* were found to be highest in the stem bark at 20.9% and least in the root at 13.2%. But, Ogunlana *et al.* (2008) obtained as low as 4.9% methanol crude extract from *M. lucida* powdered stem bark. Nweze and Okafor (2010) obtained crude extracts yields of 14%, 18% and 15% from the leaf, stem bark and roots of *M. lucida*, using ethanol. A similar extraction carried out on fresh flowers of *Hibiscus tiliaceus* gave an extract yield of only 5.76 % w/w of fresh drug (Kumar *et al.*, 2008). Adam *et al.* (2011) reported a methanol extract of the aerial part of *Argemone mexicana* and obtained a yield of 17%w/w of the freeze dried matter.

5.2 LD₅₀ determination

The need to determine LD₅₀ is very necessary in order to prevent eventualities of drug or compounds overdose which may interfere with experimental results (Prohp and Onoagbe, 2012). These studies are also useful in understanding toxicity profiles of plant extracts (Ozbek *et al.*, 2004). The result of acute oral toxicity study in the albino wistar rats recorded an LD₅₀ of

the methanolic extracts of all the parts of *M. lucida* plant as being above 5000 mg/kg, as the experimental rats tolerated the extracts without any symptoms of acute toxicity (no mortality, skin changes, aggressiveness, diarrhoea, restiveness, seizures, dizziness, weakness, or withdrawal from either food or water) even at larger doses of extracts administered. Lower LD₅₀ implies a more toxic extract being tested. According to Lorke (1983), an LD₅₀ value beyond 5000 mg/kg is of no toxicological significance. Tanko *et al.* (2007), reported LD₅₀ of beyond 5000 mg/kg for the leaves of *Cissampelos mucronata* (Menispermaceae) in rats (Wistar strain).

5.3 EFFECT OF EXTRACTS AGAINST CCL₄ ON RATS BODY AND ORGAN WEIGHTS

5.3.1 Body weight changes

When there is an introduction of unpalatable drugs or chemical agents that cause injuries to the internal organs, sharp or sudden physiological changes usually occur in animals, including the loss of appetite (anorexia). The injection of 'CCL₄ only' must have led to body weight changes as depicted in tables 4.12 and 4.13, where the highest relative body weight loss was recorded in that group. In contrast, the normal control group presented the lowest body weight changes. A few members of the normal control group actually gained some weight over the experimental period. In the groups treated with extracts after CCL₄ injection, only the group treated with *M. lucida* bark extracts seemed to have completely nullified negative influence of CCL₄ on the body weight. Body weight changes were not significantly different (statistically), from the normal control group or the groups administered with the various extracts of *M. lucida* only for

7 days. The same pattern was observed in the hepatoprotective experiment where extracts were administered prior to CCl₄ injection where value of relative body weight changes (loss) observed in the '*M. lucida* + CCl₄' was neither significantly different from the normal control nor the vitamins (E or C) + CCl₄ groups. Prabhat *et al.* (2009) had earlier reported body weight losses to about -12.83 ± 1.13 grammes among rats administered with aqueous extracts of *Argemone Mexicana* (Linn.) administered to albino wistar rats over a period of 7 days. Body weight loss is usually attributed to anorexia, precipitated by the extract administration (Aniagu *et al.*, 2004).

5.3.2 Relative liver/body ratio

There was a relative reduction in the liver/body ratio in the group injected with CCl₄ only than all other groups (Table 4.14). Also, the groups treated with *M. lucida* root extract after CCl₄ or received *M. lucida* root extract only showed reduction in liver /body ratio comparable to the 'CCl₄ only' group. The groups that were treated with *M. lucida* leaf or bark extracts after CCl₄ injection seemed to have maintained a relative increase in liver/body ratio comparable to the normal control group. Similarly, Table 4.15 shows the higher relative liver/body ratio of rat groups treated with the leaf or bark extracts of *M. lucida* before CCl₄ injection, than the 'CCl₄ only' group but comparable to the normal control, *M. lucida* extracts only, and α -Tocopherol or ascorbic acid prior to CCl₄ injected groups. These observed trends point to the hepatoprotective and hepatocurative abilities of *M. lucida* extracts, particularly the bark and leaf portions. Lal *et al.* (2007) reported the hepatoprotective effect of an herbal mixture administered against CCl₄ hepatotoxin injection in male mice. The protection was depicted either by the increase or maintenance of their relative liver/body ratio.

5.3.3 Relative kidney/body ratio

While CCl₄ is known to cause kidney damage (Zimmerman *et al.*, 1983), the increased creatinine concentration and total urea concentration (Table 4.27) in the ‘CCl₄ only’ groups confirms the same fact. As shown in table 4.13, the relative kidney/body ratio in the group injected with CCl₄ only was lower than all the other groups, which was indicative of the nephrotoxic effect of CCl₄ on the rats’ kidneys.

Apart from the groups treated with *M. lucida* fruit extract after CCl₄ injection, all the others, namely, leaf, root and bark have shown kidney/body ratio comparable to the normal control, which is indicative of the ability to protect the kidney from the toxicity effects of CCl₄ on the nephrons. Also, the groups that were treated with *M. lucida* leaf or bark extracts before CCl₄ injection have proven to maintain a relatively higher kidney/body ratio, comparable to the normal control group and the groups that received only the extracts of the different parts of *M. lucida* (Table 4.13). These trends of events pointed to the nephroprotective and nephrocurative properties of *M. lucida* extracts, particularly the bark and leaf. Lal *et al.* (2007) have reported the protective effect of a herbal mixture which was administered against CCl₄ injection in male mice. The protection against the nephrotoxic effect of CCl₄ was portrayed by the relative stability in their kidney/body ratios.

5.4 ANTIOXIDANT EFFECT OF EXTRACTS AGAINST CCL₄ IN RATS

5.4.1 Lipid peroxidation

The concentration of reactive oxygen species (ROS) measured as malondialdehyde (MDA) equivalents, which is an index of lipid peroxidation was increased in rats administered with only CCl₄ but this was significantly decreased in all the rats pre-treated for 7 days with the different parts of *M. lucida* extracts. Also, among the rats treated with the different parts of extracts of *M. lucida* after administration of CCl₄, there was a significant decrease in the MDA concentration among the groups treated with leaf and bark extracts. There was a clear evidence of the intervention of the extracts against the generation of free radicals.

The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Tabassum *et al.*, (2009) studied the protective effect of *Ocimum sanctum* on lipid peroxidation against restraint stress in male albino rats. Rats were subjected to restraint/immobilization stress 3h/day for 6 consecutive days. Post administration of 100 mg/kg of aqueous extract of *O. sanctum* for 6 consecutive days was given following restraint stress. MDA, a marker of lipid peroxidation was estimated in cerebrum, cerebellum and brain stem. Exposure to restraint stress caused a significant elevation in the rate of lipid peroxidation, as compared to the controls in all three regions of brain of male albino rats. Post treatment of aqueous extract of *O. sanctum* prevented the stress induced changes in these biochemical parameters. The results of the study indicate the protective nature of *O. sanctum* on different regions of brain against the detrimental effect of restraint stress. This finding is in agreement with both the present findings on *M. lucida* and with the observation that *O. sanctum* possesses significant antioxidant activity (Devi, 2000; Geetha and Vasudevan, 2004).

The anti-oxidant action of an orally administered 80% ethanolic extract of dried flowers of *Hibiscus sabdariffa*, L. on lipid peroxidation against sodium arsenite model was able to significantly ($p < 0.05$) reduce the sodium arsenite-induced MDA formation in the liver, which suggest the role of the extract in protection against pro-oxidant induced membrane damage (Usoh, 2005).

5.4.2 Superoxide dismutase (SOD)

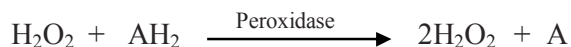
The activity of SOD, one of the physiological antioxidants was quantified. It was revealed that only the group that was treated with daily single dose of 100mg/kg for 7 days with the bark extracts of *M. lucida* after injection with CCl_4 showed significantly higher activity of SOD than the 'CCl₄ only' group, indicating a rehabilitative effect of the bark extract after the liver damage caused by the CCl_4 assault. The liver rejuvenating activities of the bark extract is believed on the premise that the groups that received the various extracts 'only' showed significantly higher values of SOD activity than the 'CCl₄ only' and 'CCl₄ + any of the extract' groups (Table 4.14). Also, it was observed that there was higher SOD activity in the groups that were pre-treated for 7 days with the *M. lucida* leaf and bark extracts before CCl_4 injection (Table 4.15). The SOD activities in these groups were significantly higher than in the 'CCl₄ only' group, indicating the protective effect of these two extracts against the assault of the CCl_4 . SOD possesses toxicity quenching effect against superoxide anions. SOD accelerates dismutation of superoxide radicals to hydrogen peroxide (H_2O_2), which is removed by catalase

(Aebi, 1984). H_2O_2 which is the product of the SOD reaction inactivates SOD and in the presence of H_2O_2 , SOD acts as a pro-oxidant (Yim *et al.*, 1990; Bast *et al.*, 1991).

It was observed that the administration of CCl_4 alone decreased SOD activity in the liver homogenates. This may likely be related to a response to an increased production of ROS induced by the hepatotoxin. The pharmacokinetics of CCl_4 may play a significant role in the difference observed in the SOD activity in the liver. The decreased SOD activity in the liver is thus, attributed to the high distribution of the toxicant in the liver, a major target organ for most xenobiotics. The high activity therefore in the liver and the resulting damage must be responsible for the decreased SOD activity observed in the ‘ CCl_4 only’ group in this study. The affinity of xenobiotics for plasma protein determines the volume of distribution in this medium as well as the activity of free (active) drug. The experiment had revealed the significantly lower plasma protein (Tables 4.20 and 4.21) in the ‘ CCl_4 only’ group and significantly higher SOD activity in the ‘extract only’ and ‘ CCl_4 + extract groups’ had clearly shown the curative and protective capacities of *M. lucida*, particularly the bark and leaf extracts.

5.4.3 Glutathione peroxidase (GPx)

GPx catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids and hemoglobin against oxidation by peroxides.



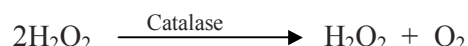
The reduction in liver GPx activity in the 'CCl₄ only' group as observed in this study indicates the damage to the hepatic cells. The administration of daily single dose of 100mg/kg for 7 days with the extracts of the different parts of *M. lucida* after injection with CCl₄ must have likely conferred some curative phenomenon on the liver, evidenced by the significantly higher activities observed in these groups than the 'CCl₄ only' group. A trend in the GPx activity was observed as follows: Normal control group > 'extracts only' ≥ CCl₄ + bark' group > CCl₄ only group. The trend presented a clear evidence of the decreased GPx activity after CCl₄ injection, followed by a replenishing of the GPx due to the 7 days administration of single doses of 100mg/kg of the methanol extracts of the different parts of *M. lucida*. The administration of daily single doses of 100mg/kg for 7 days with the extracts of the different parts of *M. lucida* before the injection of CCl₄ must have likely offered some curative phenomenon on the liver, evidenced by the significantly higher activities observed in these groups than the 'CCl₄ only' group. The reversal of the GPx activity towards normalcy after the administration of the various extracts was due to the antioxidant activity by both scavenging and detoxifying the endogenous metabolic peroxides generated after CCl₄ injury in the liver tissue.

5.4.4 Catalase (CAT)

Activity of CAT, one of the physiological antioxidants was also determined. It was found that only the groups that were treated with daily single doses of 100mg/kg for 7 days with the bark and leaf extracts of *M. lucida* after injection with CCl₄ showed significantly higher activity of CAT than the 'CCl₄ only' group, indicating a curative effect of the bark and leaf extracts after the liver damage caused by the CCl₄ assault. The liver rejuvenating activities of the bark extract is likely based on the observation that the groups that received the various

extracts ‘only’ showed significantly higher values of CAT activity than the ‘CCl₄ only’ and ‘CCl₄ + any of the extract’ groups (Table 4.14). Also, it was observed that there was higher CAT activity in the groups that were pre-treated for 7 days with the *M. lucida* bark and fruit extracts before CCl₄ injection (Table 4.15). The CAT activities in these groups were significantly higher than in the ‘CCl₄ only’ group, indicating the protective effect of these two extracts against CCl₄ assault. CAT possesses toxicity quenching effect against superoxide anions. CAT removes the H₂O₂ generated from the dismutation of superoxide radicals as a result of the activities of SOD (Aebi, 1984).

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H₂O₂ as a substrate electron donor and another molecule of H₂O₂ as an oxidant or electron acceptor.



Under most conditions *in vivo*, the peroxidation activity of catalase seems to be favored. Catalase is found in the blood, bone marrow, mucous membranes, kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases (Murray *et al.*, 2000).

The administration of CCl₄ only decreased CAT activity in the liver homogenates. This may likely be related to a response to an increased production of ROS induced by the hepatotoxin. The pharmacokinetics of CCl₄ may play a significant role in the difference observed in the CAT activity in the liver. The decreased CAT activity in the liver is thus, attributed also to the high distribution of the toxicant in the liver, a major target organ for most xenobiotics. The high activity therefore in the liver and the resulting damage must be

responsible for the decreased CAT activity observed in this study. The affinity of xenobiotics for plasma protein determines the volume of distribution in this medium as well as the activity of free (active) drug. The experiment which had revealed the significantly lower plasma protein (Tables 4.20 and 4.21) in the 'CCl₄ only' group and significantly higher CAT activity observed in the 'extract only' and 'CCl₄ + extract' groups' had clearly shown the curative capacities of the bark and leaf extracts *M. lucida*, and the protective capacity of the bark and fruit.

5.5 ANTI-INFLAMMATORY EFFECTS OF EXTRACTS

5.5.1 *M. lucida* methanolic fruit extract

At the highest dose of 500mg/kg of the methanol fruit (and seed) extract of *M. lucida*, there was a 75.02% inflammatory inhibition, compared to the 12.51% observed with the least dose of 100mg/kg within the first 30minutes of injection of the phlogistic agent (Table 4.8). The anti-inflammatory effect of the extract at 100mg/kg within 30 and 90 minutes remained significantly higher statistically ($p<0.05$), than observed with the 300mg/kg for unclear reasons. However, at the full period of 120minutes of the experiment, there was a clear dose-dependent effect of the extract on inflammatory inhibition on the experimental rats' paw, originally induced with carrageenan (Table 4.8).

In a related research, the results of the carrageenan-induced edema test on animal experiment treated with 200mg/kg hexane extract of *Prosthechea michuacana* showed maximum anti-inflammatory activity (74.02%) as compared to indomethacin the reference drug, which showed 79.21% inhibition (Perez *et al.*, 2009). These values were comparable to 72.74%

inflammatory inhibition shown by methanolic fruit extract at 300mg/kg (Table 4.8). The same hexane extract of *Prosthechea michuacana* at a dose of 150 mg/kg produced 40.25% of inhibition, similar to the inhibitory effect shown by *M. lucida* methanol fruit extract at 100mg/kg within 90minutes. In both experiments, obtained inhibitory values were higher than the reference drugs used.

In yet another study employing carrageenan-induced paw edema method, ethanolic extract of *Balanites aegyptiaca* at 300 mg/kg per day when given orally as a suspension, reduced paw volume by 55.03% whereas at 600 mg/kg per day, a 65.54% inhibition after 3h was shown, which indicated that the effect of ethanolic extract of *Balanites aegyptiaca* was dose-dependent (Gaur *et al.*, 2008), similar to the findings in this present study, with *M. lucida* fruit extract (Table 4.8) in all the selected doses.

5.5.2 *M. lucida* methanolic leaf extract

Inflammatory inhibition was elicited up to $87.5 \pm 0.11\%$ at the highest dose of 500mg/kg of *M. lucida* leaf extract within 30minutes of induction of inflammation and promptly reached $100.0 \pm 0.13\%$ in 60minutes which was maintained throughout the 120minutes duration. While there seemed to be no significant difference in inflammatory inhibition between the doses of 100 and 300 mg/kg at 60 minutes after induction, the later dose showed a better inhibitory effect ($p < 0.05$) on inflammation than the former at the 90th minute. Essentially, there was a dose-dependent trend in the rate and extent of inhibition of oedema paw inflammation observed in the leaf extract. The level of inflammatory inhibition which was achievable at the 120th minute at the selected lower doses was obtained within the 30th minute at the highest dose of

500mg/kg Also, the paw edema which decreased by $45.5 \pm 0.15\%$ with the standard drug (diclofenac sodium) at the 120th minute was already achieved by the least dose of the leaf extract used in this experiment within the 90th minute. The present study established the anti-inflammatory activity of methanolic extract of *Morinda lucida*. It is earlier reported that carrageenan induced inflammation is useful for the detection of orally active anti-inflammatory agents (Rosa *et al.*, 1971). Vinegar *et al.* (1969) reported that edema formation due to carrageenan in the rat paw is a biphasic event, where the initial phase is attributed to the release of histamine & serotonin and the second phase of edema is as a result of liberation of prostaglandins, lysosomes, bradykinins and protease (Crunkhon and Meacock, 1971). Dirosa *et al.* (1971) had earlier stated that the second phase is sensitive to most clinically active anti-inflammatory drugs. The observation that the methanolic leaf extract of *M. lucida* revealed dose dependent inhibitory activity over a period ranging from half our to two hours indicating that it attenuates both early and delayed phases of carrageenan induced inflammation, similar to the situation with ethyl acetate fraction of *Pterospermum acerifolium* Linn. (Santanu *et al.*, 2010).

5.3.3 *M. lucida* methanolic bark extract

Methanol bark extract of *M. lucida* presented the most gradual and dose-dependent incremental capacity of inflammatory inhibition. Within the first 30 minutes, there was no significant difference in the extent of inflammatory inhibition between the lowest and intermediate doses used and the standard drug, while the highest dose of 500mg/kg had elicited up to a quarter anti-inflammatory effects. Also, at the 500mg/kg dose, there was a slightly more than 50% inflammatory inhibition within 60minutes. This value was significantly higher ($p < 0.05$) than

the two lower doses of 100 and 300mg/kg and the standard drug. At the same 60minutes period and also 90minutes, there was a dose-dependent influence on the extent of inflammatory inhibition as 100, 300 and 500mg/kg doses of the methanol bark extract inhibited inflammation in the repective order: $11.1 \pm 0.14 < 33.3 \pm 0.09 < 58.3 \pm 0.10$ and $30.0 \pm 0.10 < 75.0 \pm 0.10 < 83.3 \pm 0.06$. At the maximum period of experimentation of 120minutes however, the highest and intermediate doses have both peaked at 83.3% in their anti-inflammatory effects which were significantly higher ($p < 0.05$) than the least dose of 100mg/kg and the standard drug. The methanol bark extract did not confer 100% inhibition on inflammation even at the highest dose of 500mg/kg within the experimental period of 120minutes. The anti-inflammatory effect of the bark extract of *M. lucida* even at the least dose of 100mg/kg appeared to have performed much better (54.50%) within 2 hours than the chloroform extract of *Trichodesma indicum* root where at the dose of 200mg/kg there was 48.12% inflammatory inhibition in 3 hrs (Perianayagam *et al.*, 2006). The inflammatory inhibitory performance of 100mg/kg of *M. lucida* bark was also observed to be more notable than the ethyl acetate extracts of *Seseli gummiferum* subsp. *corymbosum*, *Seseli petraeum*, *Seseli resinosum* and *Seseli tortuosum* that inhibited carrageenan-induced hind paw edema model at the same concentration ranging between 24.5–29.7, 28.1–33.3, 17.4–27.5 and 27.9–31.3%, respectively (Kupeli *et al.*, 2006).

5.5.4 *M. lucida* methanolic root extract

The root extract of *M. lucida* appeared to have inhibited the inflammatory effect of the administered carrageenan the most. An inhibition of $75.0 \pm 0.09\%$ was already achieved with the smallest dose of 100mg/kg of *M. lucida* root extract within 30minutes of induction of inflammation and the trend increased gradually through the 60th minute ($88.9.0 \pm 0.12\%$) and

peaked off through the 90th and 120th minutes at $90.0 \pm 0.12\%$. There was no significant difference in inflammatory inhibition between the doses of 300 and 500 mg/kg right from the first 30 minutes after induction, as both doses totally inhibited inflammation ($100\% \pm \text{SD}$) all through to the 120th minute. Comparatively, the paw edema which decreased by $45.5 \pm 0.15\%$ with the standard drug (diclofenac sodium-5mg/kg) at the 120th minute was by far significantly less ($p < 0.05$) than the decremental effect of the least dose of *M. lucida* on inflammation within 30 minutes.

Carrageenan induced hind paw edema in the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The methanol extract of *M. lucida* produced significant inhibition of carrageenan induced paw edema. The inhibition was much more, with onset being faster than that of the standard drug.

5.5.5 Anti-inflammatory test (Nitric oxide assay)

Findings revealed that the methanolic extracts of the different parts of *M. lucida* presented different levels of NO inhibition and in the order: bark > fruit > root > leaf (figure 4.6). With the aim to understanding the behavior of some compounds with respect to their potential as inhibitors of the NO release, Kumar *et al.* (2008) carried out NO scavenger activity assay to determine whether the inhibition of NO liberation within J-774 cells was produced by inhibition of some molecular process in the cellular machinery (such as inhibition of expression and activity of inducible Nitric Oxide Synthase (iNOS), or whether the inhibition is due to scavenger activity. Results showed that some of the tested compounds, did not exhibit any

scavenger activity, suggesting the possibility that these compounds may inhibit iNOS or other routes that influence the enzyme (Correa *et al.*, 2009).

5.6 EFFECT OF EXTRACTS AGAINST CCL₄ ON LIVER FUNCTION

5.6.1 AST and ALT levels and Bilirubin concentration

Literature is replete on the fact that serum enzymes particularly, alanine aminotransferase, (ALT) and aspartate aminotransferase (AST), are present in the hepatic and biliary cells (Jensen *et al.*, 2004). These enzymes are usually released from the hepatocytes and leak into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells resulting also, in an elevation of the alkaline phosphatase (ALP) levels (Jensen *et al.*, 2004). Serum levels of these enzymes are particularly high in acute hepatocellular damage caused by drug toxicity and xenobiotics (Norman, 1998). The extent of the enzyme changes is related to the nature, closeness to toxic agent and duration of toxicity (Shi *et al.*, 2003; Song *et al.*, 2003; Bruckner *et al.*, 1984).

In an inflammatory condition, there is a leakage of cytoplasmic enzymes into circulation, hence ALT levels increased above that of AST. Thus, when there is gross cellular necrosis, as in carbon tetrachloride or paracetamol poisoning, the level of AST may rise higher than that of ALT (Recknagel, 1987). This is because ALT level is increased in the serum solely due to conditions where cells of the liver have been inflamed or undergo cell death, and is specific for the liver cells (Jensen *et al.*, 2004) but the AST levels can be triggered in other conditions such as myocardial infarction apart from hepatocellular damage (Jensen *et al.*, 2004).

In the present study, there was conformity in the levels of the enzymes with the available literature. The high levels of AST observed may be attributed to the fact that carbon tetrachloride may have caused some injury to other organs, in addition to that caused to the liver. However, the increase in aminotransferase is generally produced by cellular necrosis (Bruckner *et al.*, 1986).

Carbon tetrachloride administration is observed to be associated with increased serum enzyme activities (Iga *et al.*, 1977; Wettstein *et al.*, 1990). Hence, the elevated levels of the enzymes namely alanine aminotransferase (ALT), aspartate aminotransferase (AST) generally, was attributed to injury caused to the hepatocytes by carbon tetrachloride which now affects the normal functions of the liver, since these enzymes are indisputably, markers of liver injury. As stated earlier, they are localized in the cytoplasm under normal conditions and are released into the circulation under abnormal conditions (e.g. cellular damage) (Mourelle *et al.*, 1987, Romero *et al.*, 1999).

The increased levels of ALT and AST in rats treated with toxicant only was a clear indication of a kind of injury or the other, caused by carbon tetrachloride toxicity (Hsiao *et al.*, 2003). It is well documented from histological studies on the liver that necrosis in the centrilobular zone is a major cause of carbon tetrachloride - induced acute liver injury (Shi *et al.*, 2003). This hepatic necrosis in the rat involves a loss of mitochondrial functions (Brabec *et al.*, 1978). There is a possibility that apoptosis has a role in the development of carbon tetrachloride - induced liver injury (Olatunde, 2000). Cell death is thought to take place by at least two distinct processes, viz: apoptosis and necrosis (Prifchard and Butler, 1989). However both necrosis and apoptosis can also be caused by drugs and toxins (Shi *et al.*, 2003). These effects are present because they

contain some bioactive compounds (Shimkim and Anderson, 1963). The presence of saponins in a variety of herbal preparations administered to humans proved to be potent against cancer and hepatic cell proliferation (Lipkin, 1995). In a study by Hsiao (2003), PML (2, 2, 5, 7, 8-pentamethyl-6-hydroxy chromane), a derivative of α -tocopherol, dose-dependently (1-10mg/kg), ameliorated the increase in plasma AST and ALT levels caused by chronic repeated carbon tetrachloride induced hepatotoxicity *in vivo* in mice.

Bilirubin is a metabolite (product of breakdown) of hemoglobin present in the bloodstream and liver of all mammals. In the liver, bilirubin is converted into a water soluble form (known as “direct bilirubin”). Bilirubin is excreted by the liver; hence its level in the blood is an index of liver function. The determination of serum bilirubin is an important marker for the diagnosis of several diseases; elevated levels of bilirubin are strongly associated with hemolysis, blockage of the biliary tract, and liver disease (BIOO Scientific Corp., 2009). Serum bilirubin (direct, indirect and total) levels were significantly elevated by CCl₄ injection (Tables 4.18 and 4.19). However, these were remarkably reduced by treatment with the methanolic extracts of the different parts of *M. lucida* before and after CCl₄ injection, suggesting the capabilities of the extracts to confer both hepatoprotection and hepatocuration (Tables 4.18 and 4.19). These findings have shown that the methanol extracts of *M. lucida* protected the rat livers against CCl₄-induced damage and lowered the serum bilirubin *in vivo*.

In this study, all the methanol extracts of the different parts of *M. lucida* that were administered before or after CCl₄ administration, ameliorated the increases in serum AST, ALT and bilirubin (direct, indirect and total) levels caused by acute CCl₄ induced hepatotoxicity *invivo*, although, the active principle that actually initiated this effect is not known. Reduced levels of ALT and

AST in rats treated with *M. lucida* methanol extracts, from the result, could also be attributed to the ability of the extracts to prevent the metabolism of carbon tetrachloride into more toxic metabolite and minimized the production of free radicals and/or, were able to boost the activities of the scavengers of free radicals and mopping up already generated free radicals in order to promptly attenuate the damages they may have caused (Chung *et al.*, 1999, Searle and Wilson, 1980) thus, minimizing hepatocellular injury produced.

5.7 EFFECT OF EXTRACTS AGAINST CCL₄ ON LIPID PROFILE

5.7.1 Total Cholesterol concentration

Statistically significant decrease in total cholesterol among the rat groups administered with daily single doses of 100mg/kg of methanolic bark extracts of *M. lucida* for 7 days after a single dose of CCl₄ injection indicated a strong cholesterol lowering effect of the bark, contrary to observations in those that received root, leaf and fruit extracts after CCl₄. A similar trend was observed but this time, the rats group administered with daily single doses of 100mg/kg of methanolic leaf extracts of *M. lucida* for 7 days before a single dose of CCl₄ injection indicated a strong cholesterol lowering effect. Total cholesterol concentration among the ‘CCl₄ only’ group was significantly very high, indicating the establishment of the hypercholesterolemic effect of the hepatotoxin.

5.7.2 Total triglyceride concentration

The administration of daily single doses of 100mg/kg of methanol leaf, root, bark and fruit extracts of *M. lucida* for 7 days after a single dose of CCl₄ injection showed triglyceride-

lowering effects of all the plant parts. The extents of the triglyceride-lowering among the different parts were not significantly different from one another or the normal group but significantly lower than the 'CCl₄ only' group. This was an indication that the extracts were capable of normalizing the concentration of triglyceride despite the pre-injection of CCl₄ which must have triggered hypertriglyceridemia. A similar trend was observed when the rat groups were administered with daily single doses of 100mg/kg of methanolic leaf, root, bark and fruit extracts of *M. lucida* for 7 days prior to the single dose injections of CCl₄. There was significant triglyceride lowering effect in all the extracts groups. The fact that triglyceride concentration among the 'CCl₄ only' group was significantly high, gave an indication of the anti-hypertriglyceridemic effects of the methanol extracts of all the different parts of *M. lucida* under the present study. However, only the leaf and fruit extracts were capable of lowering the concentration of triglyceride to the levels that were significantly indifferent from the normal group.

5.7.3 High density lipoprotein (HDL) concentration

The observed increase in HDL among the rat groups administered with daily single doses of 100mg/kg of methanolic bark extracts of *M. lucida* for 7 days after a single dose of CCl₄ injection indicated the hyperlipidemia - lowering effect of the different methanol extracts of *M. lucida*. It was equally observed that the methanol root, bark and fruit extracts showed significantly higher HDL concentration than the 'normal control' and the 'CCl₄ only' groups. Also, the rats group administered with daily single doses of 100mg/kg of methanol extracts of all the different parts of *M. lucida* for 7 days before the single dose of CCl₄ injection indicated a high concentration of HDL among all the groups. HDL concentration among the 'CCl₄ only'

group was significantly low, indicating the establishment of low HDL concentration due to the effect of CCl₄.

5.7.4 Low density lipoprotein (LDL) concentration

It was observed that the administration of daily single doses of 100mg/kg of methanol extracts of different parts of *M. lucida* for 7 days after a single dose of CCl₄ injection showed LDL-lowering effects of all the plant parts (table 4.22). The extents of the LDL-lowering among the different parts were however, significantly different from one another as the methanol bark extract presented the lowest LDL concentration after CCl₄ injection. The evidence of significantly high concentration of LDL in the 'CCl₄ only' group compared to the other groups that received an extract or the other after CCl₄ administration was indicative of the extracts' capabilities of lowering the concentration of LDL despite the pre-injection of CCl₄ which must have triggered high LDL concentration. A similar trend was observed when the rat groups were administered with daily single doses of 100mg/kg of methanolic leaf, root, bark and fruit extracts of *M. lucida* for 7 days prior to the single dose injections of CCl₄ (table 4.23). There was significant LDL lowering effect in the leaf and bark extract groups. The fact that LDL concentration among the 'CCl₄ only' group was significantly high, gave an indication of the LDL lowering effects of the methanol extracts of the leaf and bark extracts of *M. lucida* in this study.

In a similar lipid profile study, Jahan *et al.* (2009) attempted to investigate the effect of 'Garbha Cintamani Rasa', an Ayurvedic formulation on lipid profile and other parameters of rat plasma after chronic administration. They found a significant decrease of TG of both male and female

subjects. There was also a significant decrease of VLDL content in plasma of male and female subjects. However, there were no significant changes in the total cholesterol, LDL and HDL both of the sexes.

Like other plants constituents (Lee *et al.*, 2000) the methanol extracts of parts of *M. lucida* (particularly the bark and leaf) reduced TG level and it could be suggested that these extracts increased lipase activity which hydrolyzed TG. Among the lipids, increased blood level of TC, LDL and VLDL as well as lowered level of HDL has been identified as contributors in the development of hyperlipidemia (Ross, 1999) which is as a result of pathological conditions like liver disease and diabetes mellitus. (Pushparaj *et al.*, 2000; Pepato *et al.*, 2003; Sharma *et al.*, 1983). The elevation of lipid components is a risk factor for coronary heart disease (Mironova *et al.*, 2000). Thus, *M. lucida* may act as inhibitor for enzyme such as hydroxyl-methyl-glutaryl-CoA reductase, which is the key enzyme in *de novo* cholesterol biosynthesis as has been suggested for some plants earlier (Gebhardt and Beck, 1996; Eidi *et al.*, 2006). This reduction could be beneficial in improving lipid metabolism and complications arising from same (Cho *et al.*, 2002).

The present investigation has shown that extracts of parts of *M. lucida* improved liver synthetic activity, reduced serum lipid level and increased kidney function parameters. Regulation of HDL-cholesterol and LDL-cholesterol plays a central role in various disease developments. It is well known that low levels of HDL and high level of LDL are associated with an increased risk of cardiovascular events.

5.8 EFFECT OF EXTRACTS AGAINST CCL₄ ON KIDNEY FUNCTION

5.8.1 Creatinine and Urea Concentration

Although, most xenobiotics target the liver, it is quite usual that the kidney shares the burden of xenobiotic exposure because, they are the two organs that largely carry out the role of biotransformation of xenobiotics. The kidney is also involved with the maintenance of the normal physiological conditions of the *milieu interieur*. Besides these, the kidney helps in the elimination of metabolic waste. As such, a direct or indirect exposure to nephrotoxic agents may result in certain nephritic damages (Frank, 2002). Kidney function tests were carried out in the experimental rats by determining the creatinine and urea concentration. The ‘CCl₄ only’ group showed significantly higher concentration of both creatinine and urea (Tables 4.16 and 4.17). Elevated concentration of creatinine and urea are indicative of damages to the glomerular tissues although, the later may be as a result of nutritional deficiencies or liver toxicity due to toxicants. Urea in the blood is obtained through normal protein metabolism and it is excreted in the urine while creatinine, which is a metabolite of creatine is also excreted completely in the urine via glomerular filtration. Thus, elevated concentration of these two compounds in the blood presents a most likelihood condition of kidney function impairment (Frank, 2002). In that guise, it is a clinical practice to measure creatinine and urea clearance to give information about impairment of renal function. Assault of the hepatotoxin-CCl₄ and related chemicals like chloroform can lead to many physiological aberrations such as steatosis, necrosis, and cirrhosis, etc (Frank, 2002). The *M. lucida* extract-treated groups showed low creatinine concentration and urea indicating both protective and curative capacities of the extracts on the kidney from CCl₄ damage.

5.8.2 Total protein concentration

The total protein among the group of rats administered with daily single doses of 100mg/kg methanol extracts of parts of *M. lucida* for 7 days without any CCl₄ injection showed no significant difference from the normal control group. Also, the normal control group did not differ in total protein concentration from the groups administered with daily single doses of 100mg/kg of methanolic root and bark extracts of *M. lucida* for 7 days after the single dose of 0.60ml/kg of CCl₄ injection, but significantly lower than the 'CCl₄ only' group. It was an indication that the extracts were capable of restoring the protein statuses of the rats groups that were pre-injected with CCl₄ before the administration of extracts for 7days. A similar trend was observed with the rat groups that were administered with daily single doses of 100mg/kg of methanolic extracts of different parts of *M. lucida* for 7 days prior to the single dose injections of CCl₄. There was significant increase in total protein concentration except in the groups that were administered with daily single doses of 100mg/kg of methanolic fruit and bark extracts of *M. lucida* for 7 days prior to single dose of 0.60ml/kg of CCl₄ injection. The fact that total protein concentration among the 'CCl₄ only' group was significantly low, indicated the assuaging effects of the methanol extracts of the leaf and root of *M. lucida*.

5.9 CYTOTOXIC EFFECT OF EXTRACTS

5.9.1 Hatchability effect of extracts on *Artemia* cysts (brine shrimp eggs)

The methanol extracts of parts of *M. lucida* were studied using the brine shrimp hatchability assay. The LC₅₀ values obtained after the analysis showed that all the parts were variedly toxic to the brine shrimp. The fruit extract was most toxic to the *Artemia* cysts (eggs), at (LC₅₀ =

180mg/L) followed by the root, then the leaf and bark extracts at LC₅₀ values of 190, 230 and 400mg/L respectively. In a similar assay carried out on the extracts of 26 different parts of some 19 Thai medicinal plants, 13 of the extracts were non-toxic to the shrimps (LD₅₀ > 1000mg/L) (Noppamas *et al.*, 2005). The most toxic of all the extracts experimented was the stem bark of *Acronychia pedunculata* followed by the root extract of *Ellipeiopsis ferruginea* var. *cherrevensis* and *Trigonostemon reidioides* Craib (LD₅₀ = 1 and 6 mg/L, respectively).

In brine shrimp hatchability assay, the extent of inhibition of the extract might likely be due to the toxic compounds present in the extracts, possessing ovicidal and larvicidal properties (Aseer *et al.*, 2009). Cytotoxic property of a plant material is due to the presence of compounds possessing anti-tumor properties (Ara *et al.*, 1999), pesticidal activity (Sanchez *et al.*, 1993), antiviral, antiplasmodial, antifilarial, and antimalarial activities (Solis *et al.*, 1993).

5.9.2 Lethality effect of extracts on *Artemia* nauplii (brine shrimps)

Methanol extracts of parts of *M. lucida* studied using the brine shrimp lethality assay revealed LD₅₀ values (Table 4.11) indicating varied values of the different parts to the nauplii. The leaf extract was most toxic to the nauplii (LD₅₀ = 316.2mg/L) followed by the root, then the bark and fruit extracts at LD₅₀ values of 398.1, 501.2 and 631.0mg/L respectively. In a similar assay carried out on the extracts of 26 different parts of some 19 Thai medicinal plants, 13 of the extracts were non-toxic to the shrimps (LD₅₀ >1000mg/L) (Noppamas *et al.*, 2005). The most toxic of all the extracts experimented was the stem bark of *Acronychia pedunculata* followed by the root extract of *Ellipeiopsis ferruginea* var. *cherrevensis* and *Trigonostemon reidioides* Craib (LD₅₀ = 1 and 6 mg/L, respectively). Ishrak *et al.*, (2000) had examined the cytotoxicity

of seven common essential oils including anise *Pimpinella anisium* L., black cumin *Nigella sativa* L., caraway *Carum carve* L., clove *Syzgium aromaticum* L., Cumin *Cuminum cyminum* L., fennel *Foeniculum graveolens* Mill, and rosemary *Rosmarinum officinalis* L. The oils of clove and rosemary showed strong cytotoxicity activity against the nauplii of brine shrimps at 4.2 and 5.0 mg/L respectively.

In a brine shrimp lethality assay carried out by Aseer *et al.*, (2009), active fraction of *L. brandenii* showed prominent result, with an LD₅₀ value of 93mg/L obtained. The extent lethality of the extract might likely be due to the toxic compounds present in the extracts possessing strong cytotoxicity properties (Aseer *et al.*, 2009). Similar to the hatchability assay, the cytotoxic property of a plant material is due to the presence of compounds possessing anti-tumor properties (Ara *et al.*, 1999), pesticidal activity (Sanchez *et al.*, 1993), antiviral, antiparasmodial, antifilarial, and antimalarial activities (Solis *et al.*, 1993).

5.10 Genotoxic effect of extracts on pUC18 plasmid

Morinda lucida has been used in managing some ailments and known as a very good antioxidant/scavenger of reactive metabolites (Ogunlana, 2008). In a similar stance, polyphenols-rich red onion peel could offer protection to pBR322 plasmid DNA against the damage caused by Fenton's reagent by a mechanism of electron or hydrogen-atom transfer (Prakash *et al.*, 2007a). In this work however, it was observed that the methanol leaf extract offered no protection to the pUC18 plasmid DNA against the damages caused by Fenton's reagent. The loss of DNA protective abilities seemed to have increased with increasing doses of the methanol extract. This was made evident by the presence of nicked circular forms in lanes D to

F. It seemed as though there was a combined damaging effects of the extract plus the Fenton's reagent because, while the plasmids were incubated with the same amount of Fenton's reagent, they were treated with different doses of the methanol leaf extract. The lane F with the highest extract dose (500µg/10µl) showed a very faint or an almost non-visible band while lane D with the least dose (100µg/10µl) showed the most visible bands (the supercoil form and the 'damaged' nicked circular form). Validating the observation is the fact that in lane B where pUC18 plasmid DNA was incubated with the highest dose (500µg/10µl) of *M. lucida* methanol extract only (i.e., without Fenton's reagent), the bands became as visible as in lane D, but not without the damage (made evident by the presence of the nicked circular form).

A different scenario from the leaf was observed with the root, bark and fruit extracts. In the experiments using the methanol extracts of the root, bark and fruit of *M. lucida*, every material and conditions were the same except that in these cases, the root or bark or fruit extract of *M. lucida* were used. There was no genotoxic effect observed with the root or bark or fruit extracts, evidenced by the absence of the nicked circular form and/or, the linear strand breaks. However, it was observed that the bands became less visible with increasing dose of the methanol extracts of *M. lucida*. Observed also was that in lanes B (figures 4.2, 4.3 & 4.4) where the highest dose (500µg/10µl) of only the extracts were added to the pUC18 plasmid DNA (without Fenton's reagent), there were no noticeable bands; an evidence that the entire DNA was damaged in each case, similar to the event that must have taken place in lanes G where pUC18 plasmid DNA and Fenton's reagent only were incubated together. It may thus be opined that the root, bark and fruit extracts of *M. lucida* may have conferred protection against the damaging effect of Fenton's reagent at a lower dose but genotoxic at higher doses. Furthermore, the DNA protection capabilities of the *M. lucida* root, bark and fruit extracts at the lowest dose of

100µg/10µl (lane D) used were similar to that of quercetin standard (lane C) at a dose of 200µg/10µl.

Parts of *M. lucida* have been acknowledged as excellent antioxidants and good scavengers of reactive metabolites such as OH[·], O₂^{·-}, and ROO[·] (Ogunlana *et al.*, 2008; Flescher *et al.*, 2011). Polyphenols rich red onion peel could protect pBR322 plasmid DNA against the damage caused by free radicals by a mechanism of electron or hydrogen-atom transfer (Prakash *et al.*, 2007a). In this experiment, it was possible to examine the oxidative DNA damage or the protective properties of the leaf, stem bark, root and fruit extracts against the OH[·]-induced damage on pUC18 plasmid DNA by using in vitro method of Lee *et al.* (2002). As observed in figure 4.1, the methanol leaf extract of the *M. lucida* was unable to protect the pUC18 plasmid DNA from oxidative damage.

A similar work reported by Singh *et al.* (2009) showed that after incubation of pUC18 plasmid DNA with Fenton's reagent for 45 min, there was a resultant cleavage of the supercoil to yield an open circular and linear forms of plasmid DNA, indicating that OH[·] generated from iron-mediated decomposition of H₂O₂ produced both single-strand and double-strand DNA breaks. Gutteridge (1984) reported that H₂O₂ and [·]O₂⁻ are potentially cytotoxic; most of the oxidative damage in biological systems is caused by the OH[·], which is generated by the reaction between H₂O₂ and [·]O₂⁻ in the presence of redox-active metals. This type of oxidative damage can be reduced in the presence of standard antioxidants, which converts H₂O₂ into water and oxygen. In the present experiment, addition of the root, bark and fruit at the lowest dose (100ug) to DNA and Fenton's reagent mixture induced the significant reduction in the formation of open circular and linear forms and increased supercoiled or native form of plasmid DNA.

Also, Singh *et al.* (2009) showed that the aqueous fruit and seed extracts of *Moringa oleifera* at concentrations of between (10–25ug/ml) and trolox (10–25uM), showed partial and complete protection of supercoiled DNA, and mitigated the oxidative stress produced by Fenton-like reaction-mediated OH[·]. Recently, studies have shown that redox-active metals in solution could bind to phenolic antioxidant compounds and form complexes, and consequently prevent the reduction of redox-active metal ions with H₂O₂ (Lee *et al.*, 2002; Wei *et al.*, 2006).

The *M. lucida* methanol root, stem bark and fruit extracts used in this experiment, like the *Moringa oleifera* extracts used by Singh *et al.* (2009), might prevent the reaction of Fe³⁺ with H₂O₂. On the other hand, natural polyphenols can influence the free radicals mediated oxidation of DNA through simple mechanism including quenching of reactive oxygen species by donating hydrogen atom or electron. This type of action might diminish the reduction potential of Fe³⁺ leading to the inhibition of Fenton-like reaction, and might also directly scavenge OH[·] and therefore protecting the supercoiled plasmid DNA from OH[·]-dependent strand breaks.

5.11 Effects of extracts against CCl₄ on histopathology of liver and kidney tissues of rats

The leaf and root extracts of *M. lucida* were unable to effectively cure or protect the liver tissues from necrotic damages and vacuolation due to CCl₄ administration, as damages were similar or almost akin to those observed in the ‘CCl₄ only’ group. Good curative and protective effects were presented by the bark extracts as the histopathological slides showed hepatocytes

architecture that were just like the ‘normal control’ group. Close to the bark was the fruit which however, showed some level of hepatoprotective abilities rather than hepatocurative as presented by the slides. Kidney tissue slides showed lack of nephroprotective or curative abilities of the leaf, root, and fruit extracts of *M. lucida*. Although, the fruit extract presented some nephroprotective effects, the best protective and curative effects were displayed by the tissue slides of the bark extracts of *M. lucida*.

5.12 Effects of extracts against CCl₄ on haematological parameters of rats

The RBC counts among all the different groups were not significantly different, including between the normal control and ‘CCl₄ only’ group. However, there was a slight (not statistically significant) increase in RBC count observed among the groups that received CCl₄ prior to any of the methanolic extracts of parts of *M. lucida* once daily for 7 days, and those that received root extract and fruit extract only for 7 days. Among the groups that were pre-treated on a daily basis for 7 days with the extracts before the injection of a single dose of CCl₄, the groups that received leaf or fruit extracts showed significantly higher RBC count than the ‘CCl₄ only’ group. It was noted that increased or decreased RBC count beyond the normal indicates inflammatory disease, renal failure and several complications that may have arisen due to high dose of some drugs. However, the test is not sensitive or specific for other conditions than arteritis and polymyalgia rheumatica (Bedell and Bush, 1985; Sox and Liang, 1986). The PCV values did not indicate any statistically significant difference among all the different treatment groups, including the control, the various ‘extracts only groups’ and the ‘CCl₄ only’ group. The none indication of statistical difference in value among the groups may likely be due to the brevity of the duration of treatment, since the test is clinically indicated in

the diagnosis of polycythemia vera (Beutler, 1991; Donald *et al.*, 1999; Orth VH, *et al.*, 1998; Silver *et al.*, 1997) which is established after a longer period before it is clinically diagnosed by this test. However, a lay assessment of the values obtained (tables 4.24 and 4.25) show that the 'CCl₄ only' group had presented the lowest PCV value. The implication therefore, is that all other groups that received only the extract and those that received extract before CCl₄ or vice-versa had an increase in their PCV, which supports the increase in the levels of haemoglobin (tables 4.24 and 4.25) with administration of the methanolic extracts of *M. lucida* before (or after) CCl₄ injection. This may be due to the presence of haematinic factors in *M. lucida*, such as iron which plays a significant role in iron metabolism that increases the level of PCV and synthesis of haemoglobin (Iweala and Osundiya).

Hemoglobin is the major protein of erythrocytes and transports oxygen from the lungs to peripheral tissues whose decrease indicates several diseases including liver disease (Nicoll *et al.*, 2001). The experiment here showed a statistically lowered Hb count in the 'CCl₄ only' group than all the other groups. This test has depicted both the protective and curative properties of the extracts of *M. lucida* against the damaging effects of CCl₄ injection. However, hypertriglyceridemia and very high white blood cell counts can cause false elevations of Hb (Beutler, 1988).

WBC count is usually decreased during aplastic anemia (decreased production), B12 or folate deficiency (maturation defect), sepsis (decreased survival), and increased during infection, inflammation, hematologic malignancy, leukemia, and lymphoma (Nicoll *et al.*, 2001). The WBC count in this experiment was significantly lower in the 'CCl₄ + *M. lucida* bark extract' group than all other test groups but there were no significant differences between the normal

control and the 'CCl₄ only' group. This is attributable to the fact that in this experiment, none of the disease conditions for which there is a defectable WBC production was involved.

In both experiments where *M. lucida* leaf extracts were administered after or before CCl₄ injection, there was an observed significantly higher lymphocytes value in the 'CCl₄ only' group when compared to the normal control group ($p < 0.05$). According to (Nicoll *et al.*, 2001), increased lymphocytes occur in reaction to drug, beside infections and some other factors. The increased lymphocyte count may be due to the injected CCl₄. But this was checked by the post-administration of the extracts particularly among the groups administered daily for 7 days with *M. lucida* bark and fruit extracts after CCl₄, and the groups that were pre-administered with the extracts daily for 7 days before CCl₄ injection. In either case, apart from the 'CCl₄ + *M. lucida* leaf extract' and 'CCl₄ + *M. lucida* root extract' groups, the lymphocyte values were not significantly different from the normal control (tables 4.24 and 4.25).

Neutrophil concentration may be increased or decreased by drug (Nicoll *et al.*, 2001) administration, depending on the drug type. The significantly lowered neutrophil concentration in the 'CCl₄ only' group was likely attributed to the CCl₄ injection. But the administration of extracts particularly among the groups administered daily for 7 days with *M. lucida* bark and fruit extracts after CCl₄, and the groups that were pre-administered with the extracts daily for 7 days before CCl₄ injection, there was an increase of neutrophil concentrations toward the values obtained for the normal control (Tables 4.24 and 4.25).

Similar to the observed trend in neutrophil concentration is the platelets count. The significantly lowered platelets count in the 'CCl₄ only' group may likely be attributed to the

CCl₄ injection. But the administration of extracts daily for 7 days with *M. lucida* root and fruit extracts after CCl₄, and the pre-administration with the extracts daily for 7 days before CCl₄ injection, brought about an increase in platelets count toward the values obtained for the normal control. Noticeable also was the fact that even among pre- and post- extract administered groups where values were not significantly different from the 'CCl₄ only' group, the platelets counts were higher, indicating the impacts of the *M. lucida* extracts towards restoration or normalization of the physiological aberrations created by CCl₄ (tables 4.24 and 4.25).

The MCV had been touted to remain normal in combined iron and folate deficiency, and is an insensitive test in the evaluation of anaemia (Seward *et al.*, 1990; Bull and Westengard, 1994; Lafferty *et al.*, 1996). As such, using the MCV to make some valid interpretations in this experiment may be quite difficult and unconvincing. This becomes understandably clear with the observed absence of statistical difference between the normal control and 'CCl₄ only' group (Tables 4.24 and 4.25).

5.13 Total flavonoid and total polyphenol contents of different parts of *Morinda lucida*

High contents of total polyphenolic and total flavonoids have always been attributable to antioxidative activity (Asami *et al.*, 2003, Ljiljana *et al.*, 2009), antitumour activities (Kuo 1997, Lahiri-Chatterjee *et al.*, 1999, Ingram *et al.*, 1997, Messina *et al.*, 1994, Barnes *et al.*, 1995) and adjuvant to various therapies (Khoshyomn *et al.*, 2000, Khoshyomn *et al.*, 2002). This research has shown the rich presence of flavonoid and polyphenols in the different parts of *M. lucida* particularly, the methanol bark and leaf extracts. The root and fruit equally contained

appreciable amounts of flavonoid and polyphenols comparable to those reported by various investigators.

In similar experiments, Ljiljana *et al.* (2009) obtained 243.98 ± 2.14 and 81.52 ± 0.24 as total polyphenol and total flavonoids, respectively from the methanolic extracts of *Hieracium pilosella* while the aqueous extracts gave unsignificantly lower values of 239.59 ± 2.03 and 79.13 ± 0.47 , respectively. Also, Pourmorad *et al.* (2009) found the flavonoid/polyphenol contents of the methanolic extracts of *Mellilotus officinalis*, *Adiantum capillus-veneris*, *Plantago major*, *Equisetum maximum*, *Urtica dioica* as $57 \pm 5.41/289.5 \pm 51$, $78.3 \pm 4.5/22.3 \pm 3$, $25.15 \pm 0.18/31 \pm 4$, $36.2 \pm 1.2/54.5 \pm 7$, and $43.3 \pm 0.37/24.1 \pm 1$, respectively.

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

- i. Obtained results presented the highest methanol extract yield from the stem bark of *M. lucida*, while none of the extracts of the different parts showed any form of acute toxicity in rats.
- ii. Group treated with *M. lucida* bark extracts nullified the negative influence of CCl_4 , as body weight changes were not significantly different (statistically), from the normal control group. Also comparable to the normal control group, the *M. lucida* leaf and bark extracts maintained a relatively higher liver/body ratio than the 'CCl₄ only' group. The leaf, root and bark extracts maintained relative kidney/body ratio comparable to the normal control

- during both hepatoprotective and hepatocurative studies, pointing to both the protective and curative abilities of *M. lucida*.
- iii. There were higher activities of the endogeneous antioxidant enzymes: SOD, CAT and GPx, and lower TBARs concentration by the extracts of different parts of *Morinda lucida*, compared to the 'CCl₄ only' group with the bark and leaf extracts generally presenting better protection against CCl₄ –induced damages.
 - iv. The methanolic root extract of *M. lucida* showed the highest anti-inflammatory effect with respect to (shorter) time action and (least) dose that elicited maximal anti-inflammatory effect, followed by the leaf then, bark and fruit in the carrageenan induced inflammation in rats. But the nitric oxide assay portrayed a different order, with bark > fruit > root > leaf in anti-inflammatory effect.
 - v. There were reduced levels of ALT and AST, and concentrations of Bilirubin (conjugated, unconjugated and total), triglycerides, LDL, total cholesterol but higher HDL and total protein in the sera of animals that received CCl₄ either before or after 7 days of administration of the various extracts particularly the bark and leaf, than the 'CCl₄ only' group, indicating hepatoprotective/hepatocurative effects of these extracts on the integrity of the liver and associated disorders like hyperlipidemia and hyperbilirubinaemia.
 - vi. Studies indicated that all the different parts of *Morinda lucida* were cytotoxic to *Artemia salina* with the highest hatchability and lethality effects shown by the fruit and leaf extract, respectively.

- vii. The root, bark and fruit extracts of *M. lucida* protected pUC18 plasmid DNA against the damaging effect of Fenton's reagent at the lowest dose of 100ug/10uL, comparable to quercetin standard at a dose of 200ug/uL but genotoxic at higher doses.
- viii. Histologically, the liver and kidney tissues architecture were generally unprotected in the animals that received hepatotoxin (CCl₄) either before or after 7 days of administration of the various extracts of *M. lucida*, except the bark extract which presented the best protection and curation against CCl₄ damage.
- ix. Haematological parameters showed the RBC count, PCV value, among all the different groups were not significantly different except among the groups that were pre-treated on a daily basis with methanolic extracts of the bark, leaf or fruit extracts for 7 days before injection of a single dose of CCl₄ that showed significantly higher RBC, PCV, than the 'CCl₄ only' group. The lowered Hb count in the 'CCl₄ only' group than all the other groups depicted both protective and curative properties of the extracts of *M. lucida* against the damaging effects of CCl₄ injection in those groups. WBC count was significantly lower in the 'CCl₄ + *M. lucida* bark extract' group than normal control. The neutrophil, platelets, lymphocyte, and MCV either presented values that indicated protection of the various extracts against CCl₄ damages or there was no statistical difference between them and the normal control or 'CCl₄ only' group.
- x. The relative flavonoid and total polyphenol contents of *M. lucida* have portrayed the bark as containing the highest amounts, followed by the leaf.

6.2 CONCLUSIONS

The findings made in the course of this research have clearly revealed the following, concerning the methanolic extracts of the different parts of *Morinda lucida* Benth:

- i. The anti-oxidant effects against CCl_4 – induced toxicity in the order: bark > leaf > root $\geq$ fruit.
- ii. The (*in vivo*) anti-inflammatory properties of *M. lucida* were in the order: root > leaf > bark > fruit.
- iii. The hepatoprotective and hepatocurative effects against CCl_4 – induced hepatotoxicity were the order: bark > leaf > fruit > root.
- iv. The cytotoxic properties of *M. lucida* against *Artemia salina* were in the order: fruit > root > leaf > bark (hatchability) and leaf > root > bark > fruit (lethality).
- v. The fruit, leaf and root of *M. lucida* offered no protection to pUC18 plasmid DNA against Fenton's reagent but were rather genotoxic except the bark at lower doses.
- vi. The flavonoid and total polyphenol composition indicated the bark > leaf > root $\geq$ fruit.

6.3 RECOMMENDATIONS

- i. The isolation, structural elucidation and different fractions of especially the most active parts of *M. lucida* should be pushed beyond the present boundaries.
- ii. The DNA fingerprinting of *M. lucida* should be strongly considered in order to protect the quality and characteristic data, in order to forestall possible future loss due to mutation.
- iii. Germplasms of the present *M. lucida* found to possess highly useful properties should be stored.
- iv. Standardization of drugs, medications and other useful products arising from the present and future researches should be considered.
- v. Commercialization of products, protection of intellectual property rights and patenting of useful or vital information/intellectual works emanating from this research and other future researches on *M. lucida* are strongly suggested.

REFERENCES

- Abuja, P. M. and Albertini R. (2001). Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clinica Chimica Acta*, **306**(1-2): 1–17.
- Abbiw, D. K. (1990). Useful plants of Ghana: West African uses of wild and cultivated plants. *Intermediate Technology Publications*, London and Royal Botanic Gardens, Kew, Richmond, United Kingdom. 337pp.
- Adam, S. I. Y., Idris I. A., Abdelgadir E. H., Ahmed R. H. and Kamal E. E. (2011). Evaluation of Hepatoprotective Activity of *Argemone mexicana* Aerial Part extracts on CCl_4 - induced Liver Damage in Wistar Rats. *British Journal of Pharmacology and Toxicology*, **2**(5): 251-256.
- Adesida, G. A. and Adesogan, E. K., (1972). Oruwal, a novel dihydroanthraquinone pigment from *Morinda lucida* Benth. *Journal of the Chemical Society: Chemical Communications* **1**: 405–406.
- Adewunmi, C. O. and Adesogan E. K. (1984). Anthraquinones and oruwacin from *Morinda lucida* as possible agents in fascioliasis and schistosomiasis control. *Fitoterapia*, **55**: 259–63.
- Adomi, O. P. and Umukoro, G. E. (2010). Antibacterial activity of aqueous and ethanol crude extracts of the root barks of *Alstonia boonei* and preliminary phytochemical test of *Morinda lucida*. *Journal of Medicinal Plants Research*. **4**(8): 644-648.
- Aebi, H. (1984). Catalase *in vitro* In: S.P. Colowick, N.O. Kaplan (eds). *Methods in Enzymology*. **105**: 121 – 126.
- Afaf, I., Abuelgasim, N. S. and Mohammed , A. H. (2008). Hepatoprotective effect of *Lepidium sativum* against carbontetrachloride-induced damage in rats. *Research Journal of Animal Veterinary Science*, **3**: 20-23.
- Akerele, O., Heywood, V. and Synge, H. (Eds.) (1991). Conservation of medicinal plants. *Cambridge University Press Ltd.*, Cambridge, UK, pp.362.
- Amador, E. and Wacker, W.E.C. (1962). Serum Glutamic-Oxaloacetic Transaminase activity: A New Modification and an Analytical Assessment of Current Assay Technics. *Clinical Chemistry*, **8**: 343-359.
- Anderson, A. J. (1970): Lysosomal enzyme activity in rats with adjuvant induced arthritis. *Annals of the Rheumatic Diseases*. **29**: 307-313.
- Aniagu, S. O., Olanubi, F. C. B Akumaka, D. D. Ajoku, G.A. Izebe, K. S. Agala, P. Agbani, E.O. Enwerem, N.M. Iheagwara C. K. and Gamaniel, S. (2004). Is *Berlina grandiflora* (Leguminosae) toxic in rats? *Phytomedicine*, **11**: 352 – 360.

- Ara, J., V. Sultana E. S. and Ahmad, Q.R.V.U. (1999). Cytotoxic activity of marine macroalgae on *Artemia salina* (Brine shrimp). *Phytotherapy Research*, **13**: 213-219.
- Arteel, G. E. (2003). Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology*, **124**: 778-790.
- Arulkumaran, K. S., Rajasekaran, A., Ramasamy, A., Jegadee-san, M., Kavimani, S and Somasundaram, A (2009). Cassia rox-burghii seeds protect liver against toxic effects of ethanol and carbontetrachloride in rats. *International Pharmaceutical Technology Research*, **1**(2): 273-246.
- Arulmozhi, D. K., Veeranjanyulu A., Bodhankar, S. L and Arora, S. K. (2005). Pharmacological investigations of *Sapindus trifoliatus* in various *in vitro* and *in vivo* inflammatory models. *Indian Journal of Pharmacology*, **37**: 96-102.
- Asami, D. K., Hong, Y. Barrett, D. M. and Mitchell, A. E., (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Agriculture and Food Chemistry*, **51**(5):1237-1241.
- Aseer, M. S. Sujith, G., Kiran, S., Selvin, J. and Chippu, S. (2009). Cytotoxic Potentials of Red Alga, *Laurencia brandenii* Collected from the Indian Coast. *Global Journal of Pharmacology*, **3**(2): 90-94.
- Aspinall, R. L. and Commarata, P. S. (1969): Effect of prostaglandin E on adjuvant arthritis. *Nature* **224**: 1320-1321.
- Asuzu, I. U. and Chineme, C. N. (1990). Effects of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. *Journal of Ethnopharmacology*, **30**: 307–31.
- Atawodi, S. E (2005). Antioxidant potentials of African medicinal plants. *African Journal of Biotechnology*, **4**(2): 128 - 133.
- Atawodi, S. E., Beate, P., Roswitha. H., Bertold. S., Helmut. B. and Robert W. O. (2007). Content of Polyphenolic Compounds and Antioxidant Capacity Of Nigerian Stimulants: *Cola nitida* Alba, *Cola nitida* Rubra A. Chev and *Cola acuminata* Schott & Endl. *Journal of Agriculture & Food Chemistry*, **55**(24): 9824 - 9828
- Atawodi, S. E., Atawodi, J. C., Pala Y. I. and Dakwo P. (2009). Assessment of the Polyphenol Profile and Antioxidant Properties of Leaves, Stem and Root Barks of *Khaya senegalensis* (Desv.). *Electronic Journal of Biology*, **5**(4):80 – 84.
- Atawodi, S. E, Atawodi, J. C, Pfundstein B., Spiegelhalder B., Bartsch, H. and Owen, R (2011).

- Assessment of the Polyphenol Components and In Vitro Antioxidant Properties of *Syzygium aromaticum* (L.) Merr.& Perry. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **10**(3):1970-1978.
- Atawodi, S. E., Atawodi, J. C., Idakwo G. A., Pfundstein, B., Haubner, R., Wurtele G., Spiegelhalder B., Bartsch H and Owen, R. W (2009). Polyphenol Composition and Antioxidant Potential of *Hibiscus esculentus* L.Fruit Cultivated in Nigeria, *Journal of Medicinal Food*, **12**(6):1316-1320.
- Atawodi, S. E. Atawodi, J. C. Idakwo, P., Pfundstein, B., Haubner R., Wurtele, G., Spiegelhalder, B., Bartsch, H and Owen, RW (2009). Evaluation of the Polyphenol Composition and Antioxidant Activity of African Variety of *Dacryodes edulis* (G.Don) H.J Lam Fruit. *Journal of Medicinal Food*, **12**(6): 1321-1325.
- Atawodi, S. E. (2010). Polyphenol Composition and in vitro Antioxidant Potential of Nigerian *Canarium schweinfurthii* Engl. Oil. *Advances in Biological Research*, **4**(6): 314-322.
- Awe, S. O. and Makinde, J. M., (1998). Evaluation of sensitivity of *Plasmodium falciparum* to *Morinda lucida* leaf extract sample using rabbit in vitro microtest techniques. *Indian Journal of Pharmacology* **30**(1): 51–53.
- Baker, B. R. and Hurlbut, J. A. (1969): Irreversible Enzyme Inhibitors CL111, Proteolytic enzymes XI Inhibition of guineapig complement by substituted Phenoxy acetamides *Journal of Medicinal Chemistry*, **12**: 415-419.
- Barnes, S., Peterson T. G. and Coward L (1995). Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *Journal of Cellular Biochemistry*, **22S**:181-187.
- Bast, A., Haesen G. R. and Doelman, C. J. A. (1991). Oxidants and antioxidants: State of the art of review. *American Journal of Medicine*, **91**(Suppl. 3c): 25435.
- Bedell, S. E and Bush, B. T (1985). Erythrocyte sedimentation rate: from folklore to fact. *American Journal of Medicine*, **78**:1001-9.
- Bergendi, L, Benes L., Durackova, Z. and Ferencik, M. (1999). Chemistry, physiology and pathology of free radicals. *Life Science*. **65**: 1865-1874.
- Beutler, E. (1991). Problems in the diagnosis of the hemoglobinopathies and of polycythemia [editorial]. *Mayo Clinical Procedures*, **66**:102-4.

- Bhagobaty, R. K and Joshi, S. R (2008). DNA damage protective activity of the crude metabolites of endophytic fungi isolated from two ethno-pharmacologically important medicinal plants of the Khasi hills of Meghalaya, India. *Pharmacologyonline* **3**: 882-888.
- Bhat, R., and Hadi, S. M. (1994). DNA breakage by tannic acid and Cu(II): sequence specificity of the reaction and involvement of active oxygen species. *Mutation Research*, **313**(1): 39–48.
- BIOO Scientific Corp. (2009). BIOO Research Products: Total Bilirubin Assay Kit Manual. *MaxDiscovery™* Total Bilirubin Assay Manual – 3460-10. [www.biooscientific.com].
- Bo Y., Yu-xia Z., Fei Y., Hong-lei C., Dong X., Mingqiu L., and Bai-Tang L. (2007). Induction of lung lesions in Wistar rats by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its inhibition by aspirin and phenethyl isothiocyanate. *Biomedical Central Cancer*, **7**:90. Retrieved: December 12, 2010 from [http://www/biomedcentral.com/1471-2407/7/90](http://www.biomedcentral.com/1471-2407/7/90).
- Bogner, Z., Sondra C., Travis D. and Maluniu S. (2010). How to raise brine shrimp. Retrieved: December 12, 2010, from <http://www.wikihow.com/Raise-Brine-Shrimp>.
- Bolton J. L., Trush M. A., Penning T. M., Dryhurst G. and Monks T. J. (2000). Role of quinones in toxicology. *Chemical Research and Toxicology*, **13**: 135-160.
- Boutayeb, A. and Boutayeb, S. (2005). The burden of non communicable diseases in Developing Countries. *International Journal for Equity in Health*, **4**:2.
- Brabec M. J., Bradley C. and Bernstein I. A. (1978). Studies on the antagonism by chloramphenicol of carbon tetrachloride induced damage: Examination of mitochondrial protein synthesis. *American Journal of Toxicology and Applied Pharmacology*, **38**: 157-167.
- Bruckner, J. V., Luthra R. and Kyle, G.M (1984). Influence of time of exposure to carbon tetrachloride on toxic Liver injury. *Annual Review of Chronopharmacology*, **1**: 373-376.
- Bruckner J. V., Mackenzie, W. F and Muralidhara, S. (1986). Oral toxicity of carbon tetrachloride. *Acute, Fundamental and Applied Toxicology*, **6**: 16-34.
- Bruneton, J. (1999). Medicinal Plants. *Pharmacognosy Phytochemistry*, 2nd Edition, Lavoisier Publishing, pp.207-209.
- Bull, B. and Westengard C. J. (1994). Measurement of MCV. *British Journal of Haematology*, **88**: 443 – 444.
- Burkill, H. M. (1997). The useful plants of West Tropical Africa. 2nd Edition. Volume 4, Families M–R. *Royal Botanic Gardens, Kew, Richmond, United Kingdom*. pp.969.
- Burrows, C.J and Muller, J.G. (1998). Oxidative nucleobase modifications leading to strand scission. *Chemical Reviews*, **98**: 1109–1151.

- Chadwick, D.I. and Marsh, J. (1994). Eds. CIBA foundation Symposium 185, *John Wiley and Sons*, Chichister, UK, pp.280.
- Chanda, S. and Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, Vol. **3**(13): 981-996.
- Chen, P. C., Wheeler, D. S., Malhotra, V., Odoms, K., Denenberg, A. G. and Wong, H. R. (2002). A green tea-derived polyphenol, epigallocatechin-3-gallate, inhibits I-kappa B kinase activation and IL-8 gene expression in respiratory epithelium. *Inflammation*, **26**: 233–241.
- Cheyrier, V. (2005). Polyphenols in foods are more complex than often thought. *The American Journal of Clinical Nutrition*, **81**(Suppl): 223S–229S.
- Cho S. Y., Park, J. Y. Park, E. M. Choi, M. S. Lee, M. Y. Joen, S. M. Jang, M. K. Kim, M. J. and Park, Y. B. (2002). Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin –induced diabetic rats by supplementation of dandelion water extract, *Clinica Chimica Acta*, **317**: 109-117.
- Chung, H. S, Chong, L. C., Lee, S. K., Shamon, L. A., Breemen, R. B. V, Mehta, R. G, Farnsworth, N. R, Pezzuto, J. N and Kinghorn, A. D. (1999). Flavonoids constituents of chlorinzan diffused with potential cancer chemo-preventive activity. *Journal of Agriculture and Food Chemistry*, **47**: 35-41.
- Clarissa, G., Klimo K., Heiss, E., Neumann, I., Gamal-Eldeen, A., Knauff, J., Liu, G., Sitthimonchai, S. and Frank, N. (2003). Mechanism-based in vitro screening of potential cancer chemopreventive agents. *Mutation Research* **523-524**: 163-172.
- Clarkson, P. M. and Thompson, H. S. (2000). Antioxidants: what role do they play in physical activity and health? *American Journal of Clinical Nutrition*, **72**:637–46.
- Claudine, M., Augustin, S., Christine, M. Christian, R. and Liliana, J. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**:727–47.
- Correa, H., Alba L., Valenzuela, Luis, F. O. and Carmenza, D. (2009). Anti-inflammatory effects of the gorgonian *Pseudopterogorgia elisabethae* collected at the Islands of Providencia and San Andrés (SW Caribbean). *Journal of Inflammation*. **6**:5. Retrieved November 12, 2011, from <http://www.journal-inflammation.com/content/6/1/5>].
- Cory, M., Glovsky, M. and Whitehouse, M. M., (1972): Taken from Drugs for chronic inflammatory disease by Paulus and Whitehouse: *In Search for New Drugs*. Ed. A. Rubin, pp. 1-114 N.Y. Dekker.

- Crunkhorn, P. and Wills, A., (1971): Cutaneous reaction to intradermal prostaglandins. *British Journal of Pharmacology*, **41**: 49-56.
- Crunkhorn, P. and Meacock, S. E. R. (1971). Mediators of the inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology*, **42**:392-402.
- Dandona, P., Thusu, K., Cook, S., Snyder, B., Makowski, J., Armstrong, D. and Nicotera, T. (1996). Oxidative damage to DNA in diabetes mellitus. *Lancet*, **347**: 444-445.
- Dar, M. S., Ikram, M., and Fakouhi, T. (1976). Pharmacology of *Quercus infectoria*. *Journal of Pharmaceutical Sciences*, **65**(12): 1791–1794.
- Dawkins, M. J. (1963). Carbon tetrachloride poisoning in the liver of the new-born rat. *Journal of Pathology and Bacteriology*, **85**(1): 189 - 196.
- Denko, C. W. (1974): Effect of prostaglandins in urate crystal inflammation. *Pharmacology* **12**: 331-339.
- Devi, U. P, Ganasoundari, A., Vrinda, B., Srinivasan K. K, Unni K. M. K., (2000). Radiation protection by the *Ocimum* flavanoids orientin & vicenin- Mechanism of Action. *Radiation Research*, **154**: 455 – 460.
- Dhingra M., Nain Parminder, Nain Jaspreet, Malik Manisha (2011). Hepatotoxicity vs hepatoprotective agents – A Pharmacological review. *International Research Journal of Pharmacy*, **2**(3): 31 -37.
- Diallo, D., Marston, A., Terreaux, C., Toure, Y., Paulsen, B.S., Hostettmann, K. (2001). Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. *Phytotherapy*, **15**(5):401-406.
- DiRosa, M., Giroud, J. P., Willoughby, D. A. (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology*, **104**:15-29.
- Domínguez-Ortiz, A., Migue, O. Muñoz-Muñiz, R. V., García-Rodríguez, M., Vázquez-Hernández, J., Gallegos-Estudillo, J. and Samuel, C. (2010). Bol Latinoam Caribe. *Planta Medica Aromaticum*, **9**(1): 13 – 19.
- Donald, M., Gary, L., Lankford, J.A., Widness, L, F., Burmeister, D. K, and Ronald. G. S. (1999). Measurement of circulating red cell volume using biotin-labeled red cells: validation against ⁵¹Cr-labeled red cells. *Transfusion*, **39**(2): 149–155.

- Ebiloma, G. U. O. J. and Aminu. R. O. (2011). Suppressive, Curative and Prophylactic Potentials of *Morinda lucida* (Benth) against Erythrocytic Stage of Mice Infective Chloroquine Sensitive Plasmodium berghei NK-65. *British Journal of Applied Science and Technology*. **1**(3): 131-140.
- Ebringer, A. and Mackay, I. R. (1969): Pemphigus vulgaris successfully treated with cyclophosphamide. *Annals of Internal Medicine*, **71**: 125-127.
- Eidi, A., Eidi M. and Esmaeili, E. (2006). Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-diabetic rats. *Phytomedicine*, **13** (9-10): 624-629.
- Elhag, R. A. M., Badw, S. M. A. Eli, Bakhiet A. O. and Galal, M. (2011). Hepatoprotective activity of *Solanum nigrum* extracts on chemically induced liver damage in rats. *Journal of Veterinary Medicine and Animal Health*, **3**(4): 45-50.
- Evans, P. and Halliwell, B. (1999). In Ototoxicity: Basic Science and Clinical Application. *New York Academy of Science*, N. York, **884**: 19-40.
- Ferreira, S. H., Moncada, S. and Vane, J. R. (1971): Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature New Biology*. **240**: 200-203.
- Fleischer, T. C., Sarkodie, J.A. Komlaga G., Kuffour G., Dickson, R. A. and Mensa, M. L. K. (2011). Hypoglycaemic and Antioxidant Activities of the Stem Bark of *Morinda Lucida* Benth in Streptozotocin –Induced Diabetic Rats. *Pharmacognosy Communications*, **1**(2): 23 – 29.
- François, M. N, Amadou, D. and Rachid, S. (2010). Chemical composition and biological activities of *Ficus capensis* leaves extracts. *Journal of Natural Products*, **3**: 147-160.
- Frank, C. L. (2002). *Lu's Basic Toxicology*. Taylor & Francis, 11 New Fetter lane, London. Fourth Edition. pp. 38-97.
- Friedberg, E. C., Walker, G. C. and Siede, W. (1995). *DNA repair and mutagenesis*. Washington DC: ASM Press, 1–47.
- Gao, J. J, Igalashi, K. and Nukina, M. (1999). Radical scavenging activity of phenylpropanoid glycosides in *Caryopteris incana*. *Bioscience, Biotechnology and Biochemistry*, **63**: 983-988.
- Gaur, K., Nema, R. K., Kori, M. L, Sharma, C. S. and Singh, V. (2008). Anti-inflammatory and analgesic activity of *Balanites aegyptiaca* in experimental animal models. *International Journal of Green Pharmacy*, **2**: 214-7.
- Gebhardt, R. and Beck, H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids*, **31**(12): 1269-1276.

- Geetha, K. R. and Vasudevan D.M. (2004). Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum*: In vivo and in vitro studies. *Life Science*, **76**: 21-28.
- Glenn, E. M., Rohloff, N., Bowman, B. and Lyster, S. (1972): Anti-inflammatory and Pro-inflammatory effects of certain prostaglandins. *Arthritis and Rheumatism*, **15**: 110.
- Gond, N.Y. and Khadabadi S.S. (2008). Hepatoprotective activity of *Ficus carica* leaf extract on rifampicin induced hepatic damage in rats, *Indian Journal of Pharmaceutical Sciences*, **70**:365-67.
- Gonzalez-Avila, M. M., Arriaga-Alba, M., Garza, de la. Carmen H. P., M. del Dominguez-Ortiz, M. A., Fattel-Fazenda S. and Villa-Trevino, S. (2003). Antigenotoxic, antimutagenic and ROS scavenging activities of a *Rhoeo discolor* ethanolic crude extract. *Toxicology in Vitro* **17**, pp. 77-83.
- Goodman, L. and Gilman, A. (1970): The pharmacological basis of Therapeutics. Fourth edition. *The Macmillan Co.* N.Y. pp.314-347.
- Goyal, V., Chetal, S. and Nainawatee, S.S. (1986). Alterations in *Rhizobium trifolii* catalase under water stress. *Journal of Folia Microbiologica*, **31**: 164 – 166.
- Guidi, I., Galimberti, D., Lonati, S., Novembrino, C., Bamonti, F., Tiriticco, M, Fenoglio, C, Venturelli, E., Baron, P. and Bresolin, N. (2006). Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology and Aging* **27**: 262-269.
- Gupta, A. K, Chitme, H., Dass, S. K. and Misra, N. (2006) Antioxidant activity of *Chamomile recutita capitula* methanolic extracts against *CCl₄*-induced liver injury in rats *Journal of Pharmacology and Toxicology*.**1**(b):101- 107.
- Gutteridge, J. M., (1984). Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid-reactive material from deoxy-sugars, nucleosides and benzoate. *Journal of Biochemistry*, **224** (3), 761-767.
- Guyton, K. Z., Kensler T. W. and Posner, G. H. (2001). Cancer chemoprevention using natural vitamin D and synthetic analogs. *Annual Review of Pharmacology & Toxicology*, **41**: 421-42.
- Gyamfi, M. A., Yonamine, M. and Aniya, Y. (1999). "Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries." *General Pharmacology*, (32): 661 – 667.
- Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974). The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, **294**(22): 7130-7139.

- Halliwell, B. (1994). Free radicals, antioxidants and human disease: Curiosity, cause or consequence? *Lancet*, **344**: 721-724.
- Halliwell, B. (1994). How to characterize an antioxidant: an update. *Biochemical Society Symposia*, **61**: 73-101.
- Halliwell, B. and Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**: 1-14.
- Handa, S. S. (2003). Text Book of Pharmacognosy, IInd, Vallabh Prakashan, Delhi, 202-211.
- Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, **55**: 481-504.
- Higgs, G. A., and Youlten, L. F. J. (1972): Prostaglandin production in rabbit peritoneal polymorphonuclear leukocytes in vitro. *British Journal of Pharmacology*, **44**: 320p.
- Horton, E. W. (1963): Action of prostaglandin E I on tissue which respond to bradykinin. *Nature (Lond.)*, **20**: 982-893.
- Hsiao, G., Lin, Y. H, Chou, D. S., Lin, W. C. and Sheu, J. R. (2003). The Protective effects of PMC against chronic carbon tetrachloride – induced hepatotoxicity in vivo. *Journal of Pharmacology*, **4**: 9-10.
- Hyun, D. H., Hernandez, J. O, Mattson, M. P., and de Cabo, R. (2006). The plasma membrane redox system in aging. *Aging Research Reviews*, **5**: 209-220.
- Iga, T., Sugiyama, Y., Yokota, M., Tomono, Y., Ansazu, S. and Hanano, M. (1977). Pharmacokinetic aspects of sulfobromophthalein transport in chronically carbon tetrachloride intoxicated rats. *Biochemistry and Pharmacology*, **26**: 1867-1873.
- Ignarro, L. J. (1974): Regulation of lysosomal enzyme secretion-Role in inflammation. *Agents and Action* **4**, 241-258.
- Ingram, D., Sanders, K., Kolybaba, M. and Lopez, D., (1997). Case control study of phyto-oestrogens and breast cancer. *Lancet*, **350**: 990-994.
- Ito, N., Fukushima S., Hagiwara A., Shibata M. and Ogiso T. (1983). Carcinogenicity of butylated hydroxyanisole in F344 rats. *Journal of National Cancer Institute*, **70**: 343-347.
- Jaeschke, H., Gores, G. J., Cederbaum, A. I., Hinson, J. A., Pessayre, D. and Lemasters, J. J. (2002). Mechanism of hepatotoxicity. *Toxicological Science*, **65** (2): 166 – 176.
- Jahan, B. I., Obayed, M. U., Azizur, M. R., Ashfaqur K. R., Kumar A. P. and Choudhuri M. S. K. (2009). “Effect of Garbha Cintamani Rasa”, an Ayurvedic Formulation on Lipid Profile, Liver

- Function and Kidney Function Parameters of Rat Plasma after Chronic Administration. *European Journal of Scientific Research*, **32 (1)**: 25-32.
- Jainu, M. and Shyamala, D. C. S. (2005). In vitro and in vivo evaluation of free radical scavenging potential of *Cissus quadrangularis*. *African Journal of Biomedical Research*, **8**: 95-99.
- Jalil, S., Bozhanka, M., Rilka, T., Maya, M., Helmut, D., Muhammad, I. C., and Atta-ur R. (2003). In vitro Anti-inflammatory effect of *Carthamus lanatus* L. Z. *Naturforsch.* **58(c)**: 830 – 832.
- Jang, D. and Murrell, G. (1998). Nitric oxide in arthritis. *Free Radical Biology and Medicine*, **24**:1511-1519.
- Jaspreet, S., Geeta, D., Parminder, N. and Kanika, A. (2011). Liver toxicity and hepatoprotective herbs. *International Journal of Pharmaceutical Science Review and Research*, **9(1)**: 116 – 121.
- Jensen, J. E., Stainberg, S. E., Freese, P. and Marino, E. (2004). Liver function tests. *Journal of Digestive Disorders*, **6**: 1-3.
- Kaley, G. and Weiner, R. (1971): A potential mediator of the inflammatory response. *Annals of New York Academy of Scienc*, **180**: 338-350.
- Kaley, G. and Weiner, R. (1971a): Effect of prostaglandin E 1 on leukocyte migration. *Nature New Biology*. **234**: 114-115.
- Kapur, V., Pillai K.K., Hussain S.Z. and Balani D.K. (1994). Hepatoprotective activity of Jigrine on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. *Indian Journal of Pharmacology*, **26**: 35 – 40.
- Karize Uy and Fritsky Lauren (2003). what-is-cytotoxicity? [Assessed on 16th June 2012 at: <http://www.wisegeek.com/what-is-cytotoxicity.htm>].
- Kaur, G., Athar, M., and Alam, M. S. (2008). Quercus infectoria galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages. *Chemico-Biological Interactions*, **171(3)**: 272–282.
- Kaur, G., Hamid, H., Ali, A., Alam, M. S., and Athar, M. (2004). Antiinflammatory evaluation of alcoholic extract of galls of Quercus infectoria. *Journal of Ethnopharmacology*, **90(2–3)**: 285–292.
- Kenrad, E. N. and Williams, C. F. (2012). Early History of infectious diseases. [Assessed on 23/11/2012 at: www.jblearning.com/samples/.../28799_CH01_001_022.pdf]
- Khoshyomn, S., Manske, G.C., Lew, S.M., Wald, S.L. and Penar, P.L. (2000). Synergistic action of genistein and cisplatin on growth inhibition and cytotoxicity of human medulloblastoma cells. *Pediatric Neurosurgery*, **33**:123-131.

- Khoshyomn, S., Nathan D., Manske G. C., Osler T. M. and Penar P. L. (2002). Synergistic effect of genistein and BCNU on growth inhibition and cytotoxicity of glioblastoma cells. *Journal of Neurooncology*, **57**:193-200.
- Khuri, R. F., and Cohen, V. (2004). Molecularly targeted approaches to the chemoprevention of lung cancer. *Clinical Cancer Research*. **10**: 4249s - 4253s.
- Kinghorn, A. D., Su B. N., Jang, D. S., Chang, L. C., Gu, J. Q., Carcache-Blanco, I. J., Pawlus, A. D., Lee, S. K., Park, E. J., Cuendet, M., Gills, J. J., Bhat, H. S., Meta Greenwood, I., Song, L. L., Jang, M. and Pezzuto, J. M. (2004). Natural inhibitors of carcinogenesis. *Planta Medica*, **70**(8): 691-705.
- Kinnula, V. L. and Crapo, J. D. (2004). Superoxide dismutases in malignant cells and human tumors. *Free Radical Biology and Medicine*. **36**: 718-744.
- Kokate, C. K., and Gokhle S. B (2007). *Pharmacognosy*, 39, Nirali Prakashan, Pune, **(156-157)**: 249-250.
- Konaté, K., Alain, S., Roland, M., Ahmed, Y. C., Martin, K., Aline. L., Maroufath. L., Jeanne, M. and Odile, G. N. (2010). Polyphenol Contents, Antioxidant and Anti-Inflammatory Activities of Six Malvaceae Species Traditionally used to treat Hepatitis B in Burkina Faso. *European Journal of Scientific Research*. **44**(4): 570-580.
- Koulibaly M., Kabba I. S., Cisse A., Diallo S. B., Diallo M. B., Keita N., Camara N. D., Diallo M. S., Sylla B. S. and Parkin D. M. (1997). Cancer incidence in Conakry, Guinea: first results from the cancer registry. *International Journal of Cancer*, **70**: 39-45.
- Koumaglo, K., Gbeassor, M., Nikabu, O., de Souza, C. and Werner, W. (1992). Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. *Planta Medica*, **58**: 533-4.
- Krishna, M. G., Pallavi, E., Ravi, K.B., Ramesh, M. and Venkatesh, S. (2007). Hepatoprotective activity of *Ficus carica* (Linn) leaf extract against carbon tetrachloride-induced hepato-toxicity in rats. *DARU*, **15**(3): 162-167.
- K'upeli, E., Alev, T. and Erdem, Y. (2006). Anti-inflammatory and anti-nociceptive activities of *Seseli L.* species (Apiaceae) growing in Turkey. *Journal of Ethnopharmacology* (104): 310-314.
- Kulonen, E. (1970): *Chemistry and Biology of Intracellular Matrix* (Balazs, E. A., Ed.), **3**: 1811-1820.
- Kumar, A., Rajbir, K. and Saroj, A. (2010). Free radical scavenging potential of some Indian medicinal plants. *Journal of Medicinal Plants Research*, Vol. **4**(19): 2034-2042.

- Kumar, S., Vipin, T., Sanjeev, S., Ajay, S., Pravesh, K. and Anil, S. (2012). A Review on Hepatoprotective Activity of Medicinal Plants. *International Journal of Advanced Research in Pharmaceutical and BioSciences*, **1**(1): 31 – 38.
- Kumar, S., Dinesh, K., and Prakash, O. M. (2008). Evaluation of antioxidant potential, phenolic and flavonoid contents of *Hibiscus tiliaceus* flowers. *EJEAFChe*, **7**(4): 2863-2871.
- Kuo, S. M. (1997). Dietary flavonoid and cancer prevention: evidence and potential mechanism. *Critical Reviews in Oncogenesis*, **8**: 47-69.
- Krishnan, R. and Maru, G. B. (2004) Inhibitory effect(s) of polymeric black tea polyphenol fractions on the formation of [3H]-B (a) P-derived DNA adducts. *Journal of Agriculture and Food Chemistry*, **52**(13): 4261 -4269.
- Lafferty, J. D., Crowther M. A., Ali M. A. and Levine M. (1996). The evaluation of various mathematical RBC indices & their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. *American Journal of Clinical Pathology*, 106:201-205.
- Lahiri-Chatterjee, M., Katiyar, S. K., Mohan R. R. and Agarwal R. (1999). A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. *Cancer Research*, 59:622-632.
- Lal, A. S. A., Balakrishna M. P. and Sadasivan P. K.. (2007). Screening of hepatoprotective effect of a herbal mixture against *CCl₄* induced hepatotoxicity in Swiss albino mice. *Journal of Environmental Biology*, **28**(2): 201-207.
- Lal ,V. K., Amit, K., Prashant, K. and Kuldeep, S. Y. (2010). Screening of Leaves and Roots of *Eclipta alba* for Hepatoprotective Activity. *Archives of Applied Science Research*, **2** (1): 86-94.
- Landbo, A. K . and Meyer, A. S. (2001). Ascorbic acid improves the antioxidant activity of European grape juices by improving the juices' ability to inhibit lipid peroxidation of human LDL in vitro. *International Journal of Food Science and Technology*, **36**: 727–735.
- Lawal, H. O., Etatuvie, S. O. and Fawehinmi, A. B. (2012). Ethnomedicinal and Pharmacological properties of *Morinda lucida*. *Journal of Natural Products*, **5**: 93-99.
- Lee, J.C., Kim, H.R., Kim, J. and Jang, Y.S. (2002). Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. *Journal of Agriculture and Food Chemistry*, **50**: 6490–6496.
- Lee, K.T., Sohn, I.C., Kim, D. H., Choi, J. W., Kwon, H and Park, H. J. (2000). Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their antioxidant activity in vitro. *Archives of Pharmaceutical Research*, **23**: 461-466.

- Lesschaeve, I and Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition*, **81**:330–335.
- Ling, J. J., Maryati M., Asmah, R. and Mohd, F. A. B. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*). *Journal of Medicinal Plants Research*, **4**(1): 027 – 032.
- Lipkin, R. (1995). Secondary plant metabolitis. *Science News*, **14**: 8-9.
- Lipsky, P. (1999). The clinical potential of COX-2 specific inhibitors. *American Journal of Medicine*, **106**: 51-57.
- Ljiljana, S., Mihajlo, S., Vesna, N., Ljubiša, N., Dušica, R., Jasna, Č. and Vesna, T. (2009). Antioxidant Activity and Total Phenolic and Flavonoid Contents of *Hieracium pilosella* L. Extracts. *Sensors* **9**, pp. 5702-5714.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, **54**: 275 – 287.
- Lowry, O.H., Rosebrough, N. J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265-275.
- Madani, H., Talebolhosseini, M., Asgary, S. and Nader, G.H. (2008). Hepatoprotective activity of *Silybum marianum* and *Ci-chorium intybus* against thioacetamide in rat. *Pakistan Journal of Nutrition*, **7**(1): 172-176.
- Madhumita, R., Dona, S., Sutapa, M., Susmita, P. I and Bhattacharya, R. K. (2008). Protective effect of dietary phytochemicals against arsenite induced genotoxicity in mammalian V79 cells. *Indian Journal of Experimental Biology*; **46**: 690-697.
- Maduka, H. C. and Okoye, Z. S. (2002). The effect of *Sacoglottis gabonensis* stem bark, a Nigerian alcoholic beverage additive, on the natural antioxidant defences during 2,4-diphenyl hydrazine-induced membrane peroxidation *in vivo*. *Vascular Pharmacology*. **39**(1-2): 21-31.
- Malhotra, S., Singh, A. and Munjal, G. (2001). Hepatotoxic potential of commonly used herbal products. *Gastroenterology Today*, **2**(5): 110-111.
- Manach, C., Scalbert, A., Morand, C., Re´me sy, C. and Jime´nez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**:727–747.

- Manach, C., Williamson, G., Morand, C., Scalbert, A. and Re' me'sy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, **81**(Suppl), 230S–242S.
- Mantri, P. and Witiak, D. T. (1994). Inhibition of cyclooxygenase and 5-lipoxygenase. *Current Medicinal Chemistry*, **1**: 328-355.
- Maria, C. M., Ana, P. M., Milene, G., Jorge, P., Antonio, P.C. and Virgí'lio, R. (2002). Antimalarial activity of medicinal plants used in traditional medicine in S. Tome' and Principe islands. *Journal of Ethnopharmacology*, **81** (2002) 23 – 29.
- Mathew, S. and Abraham, T.E (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chemistry and Toxicology*. **44**: 198-206.
- Mbarek, L. A., Mouse, H. A., Elabbadi, N., Bensalah, M., Gamouh, A., Aboufatima, R., Benharref, A., Chait, A., Kamal, M., Dalal, A. and Zyad, A. (2007). Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. *Brazilian Journal of Medical and Biological Research*, **40**: 839-847.
- Meena, B., Ezhilan, R. A., Rajesh, R., Hussain, K. S., Ganesan, B. and Anandan, R. (2008). Antihepatotoxic potential of *Sargassum polycystum* (Phaeophyceae) on antioxidant defense status in D-galactosamine-induced hepatitis in rats. *African Journal of Biochemical Research*, **2**(2):051-055.
- Messina, M.J., Persky, V., Setchell, K.D.R. and Barnes, S. (1994). Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutrition and Cancer*, **21**:113-131.
- Mike, O. S., Amusa, N., Salmot, A., Raji-Esan, O. E., Chukwuma, C. and Ayanbamiji, A. T. (2010). Ethnobotanical Survey of Anti-Cancer Plants in Ogun State, Nigeria. *Annals of Biological Research*, **1** (4): 261-273.
- Minhua, L., Bo, Y., Yuxia, Z., Honglei, C., Dong, X., Mingqiu, L. and Fei, Y. (2009). Proteomic Analysis of Serum in Lung Cancer Induced by 3-Methylcholanthrene. *Journal of Biomedicine and Biotechnology*. **2009**, ID: 397910.
- Mironova, M. A., Klein, R. L., Virella, G. T and Lopes-Virella, M. F. (2000). Anti-modified LDL antibodies, LDL-containing immune complexes and susceptibility of LDL to in vitro oxidation in patients with type -2 diabetes. *Diabetes*, **49**:1033-1049.
- Mishra, B. B., Gautam, S., and Sharma, A. (2006). Microbial decontamination of tea (*Camellia sinensis*) by gamma radiation. *Journal of Food Science*, **71**: (6), M151 – M156.

- Mohamed, H. A., El-Sayed, I. H. and Moawad, M. (2010). Protective effect of *Nigella sativa* seeds against dimethylaminoazobenzene (DAB) induced liver carcinogenesis. *Nature and Science*, **8**(6): 80-87.
- Mohamed, S. T. S., Madhusudhana C. C., Ramkanth, V. S. T., Rajan, K., Mahesh, K. and Gauthaman, K. (2010). Hepatoprotective Herbs – A Review. *International Journal of Research in Pharmaceutical Sciences*, **1**(1): 1-5.
- Mohammed, A. A, Mahmood, A. A, Salmah, I. and Zahra, A. A. (2011). Hepatoprotective Effects of *Orthosiphon stamineus* extract on Thioacetamide-Induced Liver Cirrhosis in Rats. *Evidence-Based Complementary and Alternative Medicine*, Article ID 103039, pp. 1 - 6.
- Mourelle, M., Ameziua, J. L. and Hong, E. (1987). Effects of riprostil and colchicines on carbon tetrachloride acute liver damage in rats: Relationship with Plasma Membrane Lipids. *Prostaglandins*, **33**: 869-877.
- Murray, R. K., Daryl, K. G., Peter, A. M. and Victor, W. R. (2000). Harper's Illustrated Biochemistry. Lange Medical Books/McGraw-Hill; 26th Edition, pp.88.
- Murugaian, P., Ramamurthy, V. and Karmegam, N. (2008). Hepato-protective activity of *Wedelia calendulacea* L. against acute hepatotoxicity in rats. *Research Journal of Agriculture & Biological Science*, **4**(6): 685-687.
- Naik, S. R., and Sheth, U. K. (1974a) Effect of Glycine, aspartic acid and glutamic acid on carrageenin oedema and cotton pellet granuloma in rats (*Unpublished*).
- Naik, S. R. and Sheth, U. K. (1976). Inflammatory process and screening methods for anti-inflammatory agents-A Review. *Journal of Postgraduate Medicine*, **22**:5-21.
- Nanasombat, S. and Teckchuen, N. (2009). Antimicrobial, antioxidant and anticancer activities of Thai local Vegetables *Journal of Medicinal Plants Research*, **3**(5):443 – 449.
- Nassr-Allah, A. A., Ahmed, M., Aboul-Enein, K., Aboul-Enein, M., David, A. L., Alan, C. and Hany, A. E. (2009). *Journal of Medicinal Plants Research* .**3**(10): 799-808.
- NHANES (2002). Lab Protocol for NHANES 2001-2002 data.
- Nicoll, D., Stephen, J. M., Michael, P., Tony, M. C. and William, M. D. (2001). *Pocket guide to Diagnostic tests* (3rd ed.), Lange books, The McGraw-Hill Companies, New York.
- Noppamas, S., Vanna, M. and Wongsatit, C. (2005). Acaricidal activity of Thai medicinal plants. *Thai Journal of Phytopharmacy*, **12**(1): 1 – 7.
- Nwakaego, E. N. (2012). In vitro anti-trypanosomal activity of *Morinda lucida* leaves. *African Journal of Biotechnology*, **11**(7):1812-1817.

- Nweze, E.I. and Okafor, J.I. (2010). Antifungal activities of a wide range of medicinal plants extracts and essential oils against *Scedosporium apiospermum* isolates. *American-Eurasian Journal of Scientific Research*, **5**(3): 161 – 169.
- Obih, P. O., Makinde, J. M. and Laoye, J. O. (1985). Investigations of various extracts of *Morinda lucida* for antimalarial actions on *Plasmodium berghei berghei* in mice. *African Journal of Medicine and Medical Sciences.*, **14**: 45–9.
- Ogunlana, O. E., Olubanke, O. and Farombi, O. E. (2008). *Morinda Lucida*: Antioxidant and Reducing Activities of Crude Methanolic Stem Bark Extract. *Advances in Natural and Applied Science*, **2**(2): 49-54.
- Okobia, M. N. (2003). Cancer care in sub-saharan Africa – Urgent need for population-based cancer registries. *Ethiopian Journal of Health Development*. **17**(2): 89 – 98.
- Olatunde, F. (2000). Mechanisms for the hepatoprotective action of Kolaviron. Studies on hepatic enzymes, microsomal lipids, and lipid peroxidation in carbon tetrachloride treated rats. *Pharmacology Research*, **42**: 75-80.
- Oliver-Bever, M. (1986). Medicinal Plants in tropical West Africa. Cambridge University Press, Cambridge, pp. 89 – 90.
- Onasanwo, S. A. and Elegbe, R. A. (2006). *African Journal of Biomedical Research*, **9**: 109 – 117.
- Orth, V.H., Rehm, M., Thiel, M., Kreimeier, U., Haller, M., Brechtelsbauer, H. and Finsterer, U. (1998). First clinical implications of perioperative red cell volume measurement with a nonradioactive marker (sodium fluorescein). *Anesthesia and Analgesia*, **87**:1234-1238.
- Osawa, T. and Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agricultural and Biological Chemistry*, **45**: 735-739.
- Ozbek, H., Ozturk, M., Ozturk, A., Ceylan, E. and Yener, Z. (2004). Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. *Eastern Journal of Medicine*, **9** (1): 4 – 6.
- Pallab, M., Dhananjay, H., Uday, B. and Dipak, K. M. (2009). Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (L.) Corr., *Indian Journal of Experimental Biology*, **47**: 849 – 861.
- Passos, D. C. S., Ferreira, H. D. Vieira, I. L. F. B. Nunes, W. B., Felício, L. P., Silva, E. M., Vale C. R., Duarte, S. R., Silva, E. S. and Carvalho, G. S. (2010). Modulatory effect of *Palicourea*

- coriacea* (Rubiaceae) against damage induced by doxorubicin in somatic cells of *Drosophila melanogaster*. *Genetic and Molecular Research*, **9**(2): 1153-1162.
- Park, Z. J. and Pezzuto, J. M. (2002). Botanicals in cancer chemoprevention. *Cancer and Metastasis Reviews*, **21**: 231-255.
- Paulus, H. E. and Whitehouse, M. W. (1973). Non-steroid anti-inflammatory agents. *Annual Review of Pharmacology*, 107-125.
- PDR. (1998). PDR for herbal medicines. First edition. Montvale, New Jersey, 1177-1178.
- Pepato, M. T., Baviera, A. M., Vendramini, R. C., Perez, M. P., Kettelhut, I. C. and Brunetti, I.L. (2003). *Cissus sicyoides* (Princess wine) in the long term treatment of streptozotocin-diabetic rats. *Biotechnol. Applied Biochemistry*, **37**:15-20.
- Pereira, A., Michael, S., Herren, L., Britt, A. L. and Marlene, M. K. (2010). Initiation/promotion bioassay in rat liver: use of gamma glutamyltranspeptidase-positive foci to indicate carcinogenic activity. *Toxicologic Pathology*, **10**(11). DOI: 10.1177/019262338-201-000-203. Retrieved February 11, 2010 from <http://tpx.sagepub.com/cgi/content/abstract/10/2/11>.
- Perez, G. R.M. and Solis, R.V. (2009). Anti-inflammatory and wound healing potential of *Prosthechea michuacana* in rats. *Phcognosy Magazine*. **5**:219-225.
- Perianayagam, B., James, S. K. and Sharma, K. K. (2006). Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. *Journal of Ethnopharmacology* (**104**): .410–414.
- Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. and Perry, N. S. (1998). Medicinal Plants and Alzheimer's Disease: Integrating Ethnobotanical and Contemporary Scientific Evidence. *Journal of Alternative and Complementary Medicine*, **4**(4):419-428.
- Pfeilschifter, J., Eberhardt, W., Beck, K. F. and Huwiler, A. (2003). Redox signaling in mesangial cells. *Nephron Experimental Nephrology*, (**93**): 23–26.
- Prabhat, K. D., Prasanna, P., Somya, R. P. and Ranjan, S. (2009). Hepatoprotective activity of plant *Argemone mexicana* (linn.) against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. *International Journal of Pharmaceutical Research and Development-Online*, **8**(2): 1 – 20.
- Pritchard, D. J. and Butler, W. H. (1989). Apoptosis; The mechanism of cell death in dimethyl-nitrosamine induced hepatotoxicity. *Journal of Pathology*, **156**: 253-260.
- Prior, R .L., Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Horticultural Science*, **35**: 588-592.

- Proestos, C., Bakogiannis, A., Psarianos, C., Koutinas, A. A., Kanellaki, M. and Komaitis, M. (2005). High performance liquid chromatography analysis of phenolic substances in Greek wines. *Food Control*, **16**: 319–323.
- Prohp, T. P. and Onoagbe, I. O. (2012). Acute toxicity and dose response studies of aqueous and ethanol extracts of *Triplochiton scleroxylon* K. schum, (sterculiaceae). *International Journal of Applied Biology and Pharmaceutical Technology*, **3**(1): 400-409.
- Pushparaj, P., Tan, C. H. and Tan, B. K. H. (2000). Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. *Journal of Ethnopharmacology*, **72**:69-76.
- Qadry, J. S. (2007). *Pharmacognosy*, 10, B.S. Prakashan, Ahmedabad, 156, 195.
- Rackova, L., Oblozinsky, M., Kostalova, D., Kettmann, V. and Bezakova, L. (2007). Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation*, **4**:15.
- Rai, S., Wahile, A., Mukherjee, K., Saha, B. P. and Mukherjee, P. K. (2006). Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. *Journal of Ethnopharmacology* **104**: 322 – 327.
- Raj, D. S., Vennila, J. J., Aiyavu, C. and Panneerselvam, K. (2009). The hepatoprotective effect of alcoholic extract of *Annona squamosa* leaves on experimentally induced liver in-jury in Swiss albino mice. *International Journal of Integrative Biology*, **5**(3): 162-166.
- Raja, S., Nazeer A., K.F.H., Kumar, V., Kakali, M. Bandyopadhyay, A. and Mukherjee, P. K. (2007). Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats. *Journal of Ethnopharmacology*, **109**(1): 41 – 47.
- Raji, Y., Udoh, U. S, Oluwadara, O. O., Akinsomisoye, O. S, Awobajo, O. and Adeshoga, K. (2002). Anti-Inflammatory and Analgesic Properties of the Rhizome Extract of *Zingiber officinale*. *African Journal of Biomedical Research*, **5**: 121 – 124.
- Ramakrishna. B. S., Varghese, R., Jayakumar, S., Mathan, M. and Balasubramanian, K.A. (1997). Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. *Journal of Gastroenterology and Hepatology*, **12**: 490-494.
- Rice-Evans, C. (2001). Flavonoid: antioxidants. *Current Medicinal Chemistry*, **8**, 797–809.
- Rice-Evans, C. (2004). Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Radical Biology and Medicine* **36**: 827-828.

- Romero, S., Lasher, S. B., Suberviola, L. S. and Genarruzabeitia, E. (1994). Protective effects of calcium channel blockers in carbon tetrachloride- induced liver toxicity. *Life Science*, **55**: 981-990.
- Ross, R. N., (1999). Atherosclerosis—An Inflammatory Disease. *England Journal of Medicine*, **340**: 115-126.
- Rubin, R. P. (1970): The role of calcium in the release of neurotransmitter substances and hormones. *Pharmacology Review*, **22**, 389.
- Rutao, C. U. I., Wang, B., Zhang, F., Chen, G. and Yin, S., (2003). Suppressive effect of herbal compound 861 on chemical hepatocarcinogenesis in rats. *Chinese Medical Journal*, **116**(3); 378 – 382.
- Sabu, M. C., Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacology*, **81**:155-160.
- Saenjum, C., Chaiyavat, C., Sarinya, K., Sunee, C. and Maitree, S. (2010). Antioxidant activity and protective effects on DNA damage of *Caesalpinia sappan* L. extract. *Journal of Medicinal Plants Research*, **4**(15): 1594-1600.
- Sakamoto, A., Ueda, M. and Morikawa, H. (2002). Arabidopsis glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase. *FEBS Letters*, **515**:20-24.
- Sandhir R., and Gill, K. D. (1999). Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. *Indian Journal of Experimental Biology*, **37**: 762 – 766.
- Santanu, S., Sambit, P., Jagannath, V. P., Uma, S. M. and Ashish, P. (2010). Antioxidant and Anti-Inflammatory Potential of *Pterospermum acerifolium*. *International Journal of Pharmaceutical Sciences Review and Research*, **2**(1): 001.
- Santosh, V., Shreesh, O. and Mohammad, R. (2010). Anti-inflammatory activity of *Aconitum heterophyllum* on cotton pellet-induced granuloma in rats. *Journal of Medicinal Plants Research*, **4**(15): 1566-1569.
- Saraswat. B., Visen, P. K. S. Dayal, R., Agarwal, D. P. and Patnaik, G. K. (1996). Protective action of ursolic acid against chemical induced hepatotoxicity in rats. *Indian Journal of Pharmacology*, **28**: 232 – 239.
- Sas, K., Robotka, H., Toldi, J. and Vecsei, L. (2007). Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. *Journal of Neurological Science*, **257**: 221-239.

- Satoh, K. and Hatayama, I. (2002). Anomalous elevation of glutathione S-transferase P-form (GST-P) in the elementary process of epigenetic initiation of chemical hepatocarcinogenesis in rats. *Carcinogenesis*, **23**(7): 1193 – 1198.
- Scartezzini, P., Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of Ethnopharmacology*, **71** (1-2):23-43.
- Scheck, C., Adrienne, K., Nicole, C. H. and Clark, D. W. (2006). Anticancer activity of extracts derived from the mature roots of *Scutellaria baicalensis* on human malignant brain tumor cells. *BMC Complementary and Alternative Medicine*, **6**:27.
- Scott, R. E. (1970): Effect of prostaglandins, epinephrine and NaF on human leukocyte platelet and liver adenylate cyclase. *Blood*. **35**: 514-518.
- Searle, A. J. and Wilson, R. L. (1980). Glutathione peroxide: Effect of superoxide, hydroxy and bromine free radicals on enzymes activity. *International Journal of Radiant Biology*, **37**: 213-217.
- Serpeloni, J. M., Bisarro, R. M., Rodrigues, J., Campaner, S. L., Vilegas W., Varanda E. A., Dokkedal, A. L. and Cólus, I. M. (2008). In vivo assessment of DNA damage and protective effects of extracts from *Miconia spp.* using the comet assay and micronucleus test. *Mutagenesis*, **23** (6): 501–507.
- Seward, S. J., Safran, C., Marton, K. I. and Robinson, S. H. (1990). Does the mean corpuscular volume help physicians evaluate hospitalized patients with anemia? *Journal of General and Internal Medicine*, **5**: 187-91.
- Sharma, M. K., Khare, A. K. and Feroz, H. (1983). Effect of neem oil on blood glucose levels of normal, hyperglycemic and diabetic animals. *Indian Medical Gazette*, **117**: 380-383.
- Shi, J., Asiaki, K., Ikawa, Y. and K. (2003). Evidence of hepatocyte apoptosis in rat liver after the administration of carbontetrachloride. *Journal of Medicinal Research*, **4**: 1-8.
- Shinkim, M. B. and Anderson, M. N. (1963). Acute toxicities of rotenone and mixed pyrethrins in mammals. *Proceedings of the Society of Experimental and Biological Medicine*, **34**: 135-138.
- Silver, H. M, Seebeck, M. A., Cowett, R. M., Patterson, K. Y. and Veillon, C. C. (1997). Red cell volume determination using a stable isotope of chromium. *Journal of Society of Gynecological Investigation*, **4**(5):254-8.
- Simões, C. M. O., Schenkel, E. P., Gosmann, G. and Mello, J. C. P. (2000). Farmacognosia: da Planta ao Medicamento. 2nd edn. *Universidade Federal de Santa Catarina*, Florianópolis.

- Singh, B. N., Singh, B. R., Singh, R. L., Prakash, D., Dhakarey, R., Upadhyay, G., Singh, H. B. (2009). *Food and Chemical Toxicology* **47**: 1109–1116.
- Singh, U., Jialal, I., (2006). Oxidative stress and atherosclerosis. *Pathophysiology*. **13**: 129-142.
- Smith, J. B. and Wills, A. L. (1971): Aspirin selectively inhibits prostaglandin production in human platelets. *Nature New Biology*, **231**: 235-237.
- Smith, M. A., Rottkamp, C. A., Nunomura, A., Raina, A. K. and Perry, G. (2000). Oxidative stress in Alzheimer's disease. *Biochemistry and Biophysics Acta.*, **1502**: 139-144.
- Smith, R. J. and Ignarro, L. J. (1974): Stimulation-secretion coupling in human Neutrophils: Link between calcium influx and guanosine 3', 5' monophosphate accumulation in lysosomal enzyme secretion. *Pharmacologist*, **16**: 309.
- Smith, W. L. and Lands, W. E. M. (1971): Stimulation and blockade of Prostaglandin biosynthesis. *Journal of Biological Chemistry*, **246**: 6700-6706.
- Solt, D. and Farber, E. (1976). New principle for the analysis of chemical analysis. *Nature*, 263: 701-703.
- Song, I., Lee, Y., Chung, S. and Shim, C. (2003). Multiple alteration of canalicular membrane transport activities in rat with *CCl₄* induced hepatic injury. *Journal of Pharmacology and Toxicology*, **8**: 506-571.
- Soria, J., Kim, E., Fayette, J., Lantuejoul, S., Deutsch, E. and Hong, W. (2003). Chemoprevention of lung cancer. *The Lancet Oncology*, **4** (11): 659-669.
- Sox, H. C. and Liang, M. H. (1986). The erythrocyte sedimentation rate: guidelines for rational use. *Annals of Internal Medicine*, **104**: 515-523.
- Szabó, C., Ischiropoulos, H. and Radi, R., (2007). Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nature Reviews Drug Discovery*, **6**: 662-680.
- Tabassum, I., Siddiqui, Z. N. and Rizvi, S. J. (2009). Protective effect of *Ocimum sanctum* on lipid peroxidation, nucleic acids and protein against restraint stress in male albino rats. *Biology and Medicine*, **1** (2): 42-53.
- Tanko, A., Yaro, A. H., Yerima, A. I., Isa, M., Saleh, M. I. A. and Mohammed, A. (2007). Toxicological and hypoglycaemic studies on the leaves of *Cissampelos mucronata* (Menispermaceae) on blood glucose levels of streptozotocin-induced diabetic wistar rats. *Journal of Medicinal Plants Research*, **1** (5): 113 -116.
- Tomlinson, R. W., Ringold, H. J., Quershi, M. C. and Forchielli, E. (1972): Relationship between inhibition of prostaglandin synthesis and drug efficacy: Support for the current theory on mode

- of action aspirin-like drugs. *Biochemistry and Biophysical Research Communications*, **46**: 552-559.
- Touyz, R. M. (2004). Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension. What is the clinical significance? *Hypertension*, **44**: 248.
- Trager, W., Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*: **193**: 673-S.
- Trease and Evans (2007). *Pharmacognosy*, **10**, Elsevier Publishers, New Delhi, 414-418.
- Trush, M. A., Kensler, T.W. (1991). An overview of the relationship between oxidative stress and chemical carcinogenesis. *Free Radical Biology and Medicine*, **10**:201–9.
- Tsao, A. S., Kim, E. S. and Hong, W. K. (2004). Chemoprevention of cancer. *CA Cancer Journal for Clinicians*.**54**:150-180.
- Upston, J. M., Kritharides, L., Stocker, R. (2003). The role of vitamin E in atherosclerosis. *Progress in Lipid Research*. **42**: 405-422.
- Usuh, I. F., Akpan, E. J., Etim, E. O. and Farombi, E. O. (2005). Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa* L. *Pakistan Journal of Nutrition*, **4**(3): 135-141.
- Vadivu, R., Krithika, A., Biplab, C., Dedeepya, P., Shoeb, N. and Lakshmi, K.S. (2008). Evaluation of hepatoprotective activity of the fruits of *Coccinia grandis* Linn. *Journal of Health Research*, **1**(3): 163-168.
- Vaidya, A. B. (1996). *Picrorhiza kurroa* (Kutaki) Royle ex Benth as a hepatoprotective agent. *Experimental & clinical studies*, **42**(4): 105-108.
- Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. (2006). Free radical metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, **160**: 1–40.
- Vane, J. R. (1971): Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. *Nature New Biology*. **231**: 232-235.
- Verma, S., Shreesh, O. and Mohammad, R. (2010). Anti-inflammatory activity of *Aconitum heterophyllum* on cotton pellet-induced granuloma in rats. *Journal of Medicinal Plants Research*, **4**(15): 1566-1569.
- Vinegar, R., Schreiber, W. and Hugo, R. (1969). Biphasic development of carrageenan in rats. *Journal of Pharmacology and Experimental Therapeutics*, **166**: 96-103.
- Vuillaume, M., (1987). Reduced oxygen species, mutation, induction and cancer initiation. *Mutation Research*, **186**:43–72.

- Wan-Ibrahim, W. I., Sidik, K.S. and Kuppasam, U.R. (2010). A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chemistry* **122**: 1139–1144.
- Wei, Q.-Y., Zhou, B., Cai, Y.-J., Yang, L., Liu, Z.-L. 2006. Synergistic effect of green tea polyphenols with trolox on free radical-induced oxidative DNA damage. *Food Chemistry*, **96**: 90–95.
- Weissman, G., Spilberg, I. and Krakauer, K. (1969): Arthritis induced in rabbits by lysates of granulocyte lysosomes. *Arthritis and Rheumatism*, **12**: 103-110.
- Wettstern, M., Gerol, W. and Hausinger, D. (1990). Hypoxia and CCl_4 - induced liver injury, but not acidosis, impair metabolism cysteinyl. *Hepatology*, **11**: 866-873.
- Williams, G.M., Iatropoulos, M.J. and Whysner, J. (1999). Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food Chemistry and Toxicology*, **37**: 1027-1038.
- Willoughby, D. A. (1971): Aspirin-like drugs and prostaglandins. *Lancet* **2**. 542.
- Wills, A. L. (1969): Release of histamine, kinin and prostaglandins during carrageenin induced inflammation of the rat. In prostaglandins, peptides and amines. *CP-Montagazza and Horton*, (Eds.) pp. 31-38, Academic Press, London.
- Wiseman, H. and Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. *Biochemical Journal*, **313**: 17-29.
- Wittig, J., Herderich, M., Graefe, E. U. and Veit, M. (2001). Identification of quercetin glucuronides in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications*, **753**: 237–243.
- Witz, G. (1991). Active oxygen species as factors in multistage carcinogenesis. *Proceedings of the Society of Experimental Biology and Medicine*. **198**: 675– 682.
- Woodwin, A. M. and Weineke, A. A. (1963): The accumulation of calcium by the polymorphonuclear leukocyte treated with staphylococcal leucocidin and its significance in the extrusion of protein. *Biochemical Journal*, **87**: 567-572.
- Wu, L. T., Chu, C. C., Chung, J. G., Chen, C. H., Hse, L. S. and Liu, J. K. (2004). Effects of tannic acid and its related compounds on food mutagens or hydrogen peroxideinduced DNA strands breaks in human lymphocytes. *Mutation Research*, **556** (1–2): 75–82.

- Yim, M. B, Chock, P. B. and Stardtman, E. R. (1990). Copper-zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. *Proceedings of the National Academy of Science*. USA., **87**: 5006 – 10.
- Yoga, L. L., Sasidharan Zuraini S. Z., Suryani S., Shirley L., Sangetha, S. and Davaselvia, M. (2007). Antimicrobial activities and toxicity of crude extract of the *Psophocarpus tetragonolobus* Pods. *African Journal of Complementary and Alternative Medicine*, **4**(1): 59 – 63.
- Zimmerman, S. W., Norback, D.H. and Powers, K. (1983): Carbon tetrachloride nephrotoxicity in rats with reduced renal mass. *Archives of Pathology and Laboratory Medicine*, **107**: 264-269.
- Zimudzi, C. & Cardon, D., (2012). *Morinda lucida* Benth. Record from Protabase. PROTA (Plant Resources of Tropical Africa/Ressources végétales de l’Afrique tropicale), Wageningen, Netherlands. <[http://database.prota.org/ search. htm](http://database.prota.org/search.htm)>. Accessed 8 June 2012.

APPENDIX I

Table 1: Percentage extract yields of different parts *Morinda lucida* by methanolic soxhlet extraction:

S/N	<i>Morinda lucida</i> Plant part	Wt. of Starting Material (g)	Wt. of Crucible	Wt. of Crucible + Extract	Wt. of Extract	Percentage yield
1	Fruit powder (i)	20	54.64	58.48	3.84	19.20
2	Fruit powder (ii)	10	60.48	62.24	1.76	17.60
3	Fruit powder (iii)	20	43.50	47.53	4.03	20.15
4	Fruit powder (iv)	10	46.70	48.49	1.79	17.90
5	Fruit powder (v)	10	56.97	58.85	1.88	18.80
	TOTAL	70			13.3	19.00
6	Leave powder (i)	20	60.50	63.98	3.48	17.40
7	Leave powder (ii)	20	60.51	64.26	3.75	18.75
8	Leave powder (iii)	10	48.98	50.32	1.34	13.40
	TOTAL	50			8.57	17.14
9	Bark powder (i)	20	60.49	64.78	4.29	21.45
10	Bark powder (ii)	25	60.50	65.45	4.95	19.80
11	Bark powder (iii)	20	60.50	64.87	4.37	21.85
	TOTAL	65			13.61	20.94
12	Root powder (i)	10	44.20	45.36	1.16	11.60
13	Root powder (ii)	20	52.63	55.05	2.42	12.10
14	Root powder (iii)	20	60.50	63.31	2.81	14.05
15	Root powder (iv)	20	51.69	54.53	2.84	14.20
	TOTAL	70			9.23	13.19

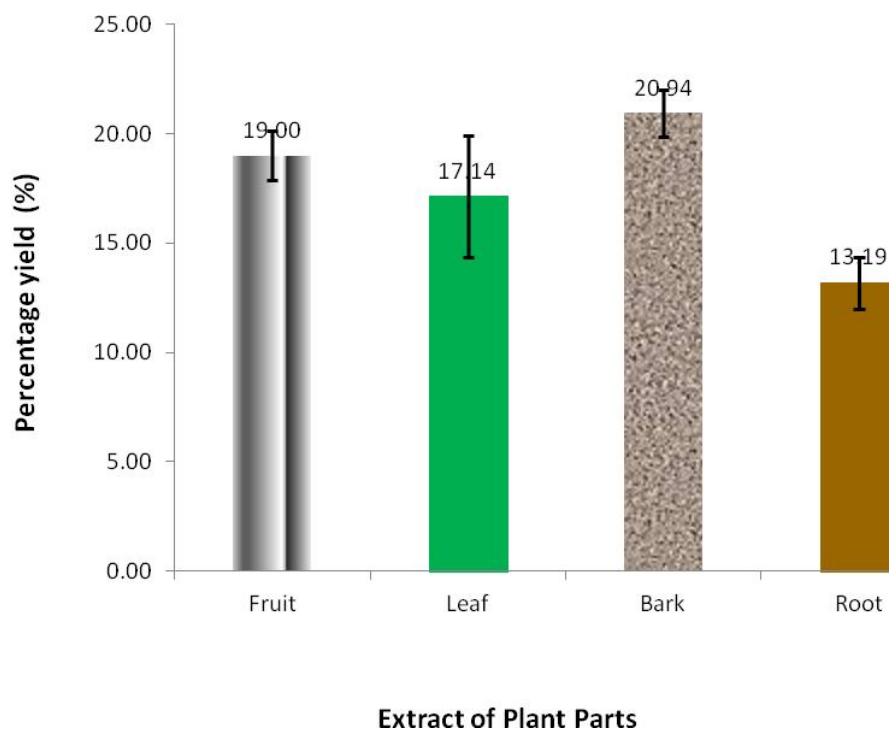


Figure 1: Average yield (%) of extracts of parts of *Morinda lucida* following three times extraction with methanol (3 x 5hr)s by Soxhlet

LD₅₀ (Stage 1)

Table 2: LD₅₀ determination of methanolic extracts of parts of *Morinda lucida* in rats after single oral administration of 10, 100 and 1000mg/kg body weight (Stage 1)

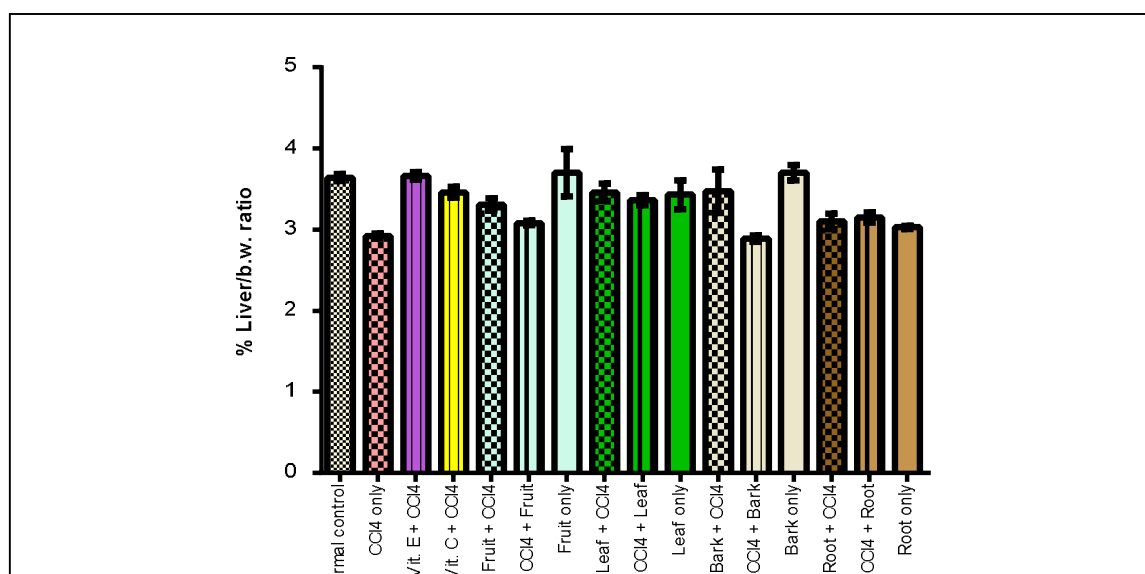
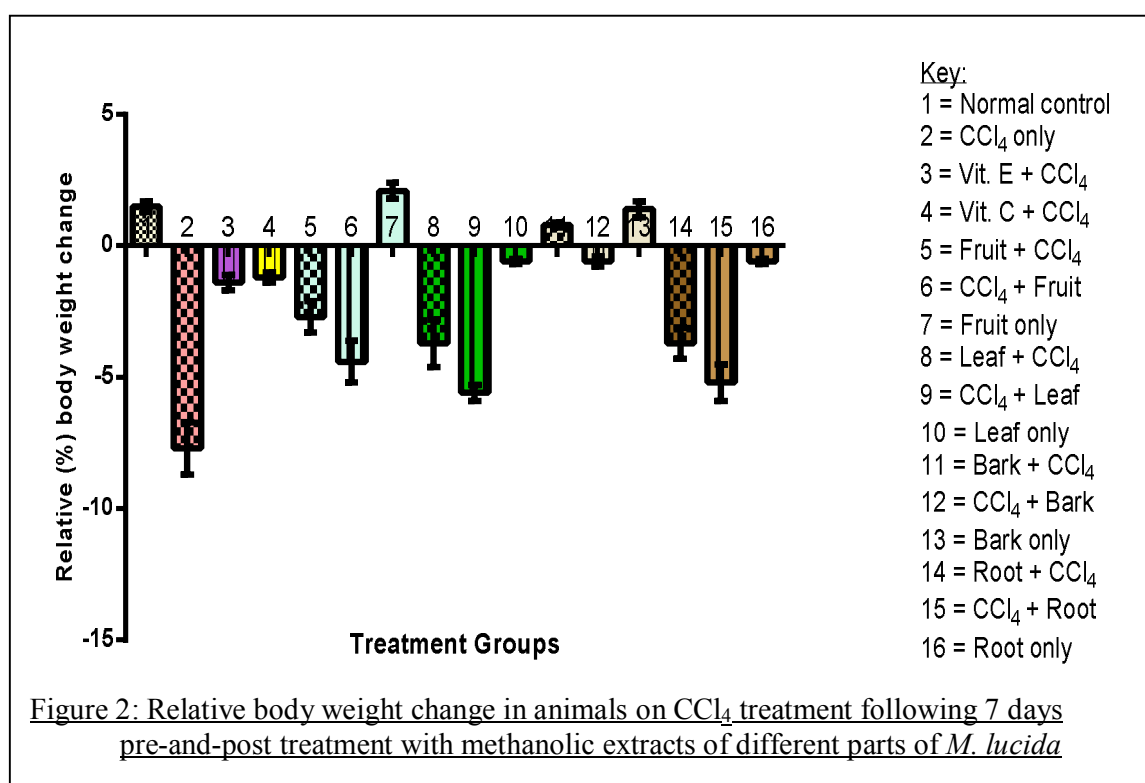
Plant part	Dose (mg/kg b.w.)	No. of rats/ test group	No. of death	No. of Survival	Mortality ratio
Fruit	10	3	0	3	0/3
Leaf	10	3	0	3	0/3
Stem bark	10	3	0	3	0/3
Root	10	3	0	3	0/3
Fruit	100	3	0	3	0/3
Leaf	100	3	0	3	0/3
Stem bark	100	3	0	3	0/3
Root	100	3	0	3	0/3
Fruit	1000	3	0	3	0/3
Leaf	1000	3	0	3	0/3
Stem bark	1000	3	0	3	0/3
Root	1000	3	0	3	0/3

LD₅₀ (Stage 2)

Table 3: LD₅₀ determination of methanol extracts of parts of *Morinda lucida* in rats after single oral administration of 1,600, 2,900 and 5,000mg/kg body weight (Stage 2).

Plant part	Dose (mg/kg b.w.)	No. of rats/ test group	No. of death	No. of Survival	Mortality ratio
Fruit	1,600	1	0	1	0/1
Leaf	1,600	1	0	1	0/1
Stem bark	1,600	1	0	1	0/1
Root	1,600	1	0	1	0/1
Fruit	2,900	1	0	1	0/1
Leaf	2,900	1	0	1	0/1
Stem bark	2,900	1	0	1	0/1
Root	2,900	1	0	1	0/1
Fruit	5,000	1	0	1	0/1
Leaf	5,000	1	0	1	0/1
Stem bark	5,000	1	0	1	0/1
Root	5,000	1	0	1	0/1

RELATIVE CHANGES IN BODY AND ORGAN WEIGHTS DURING EXPERIMENT



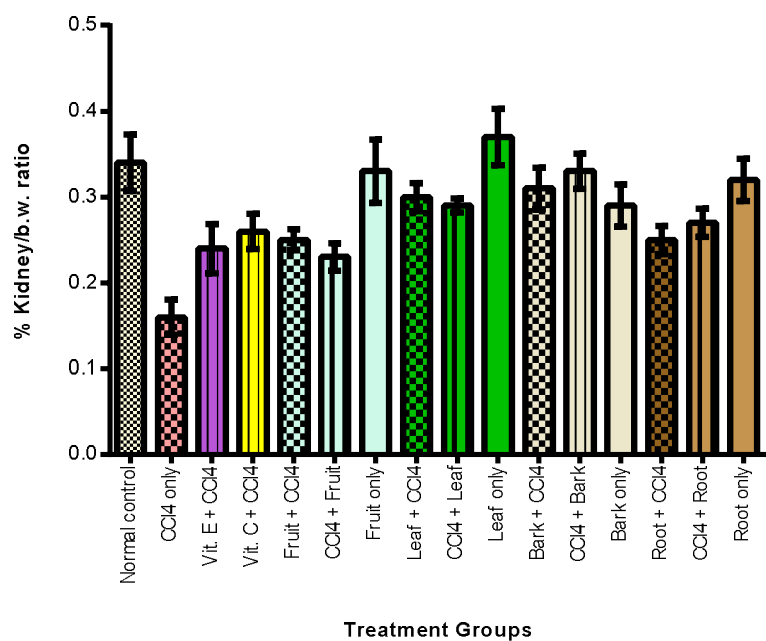
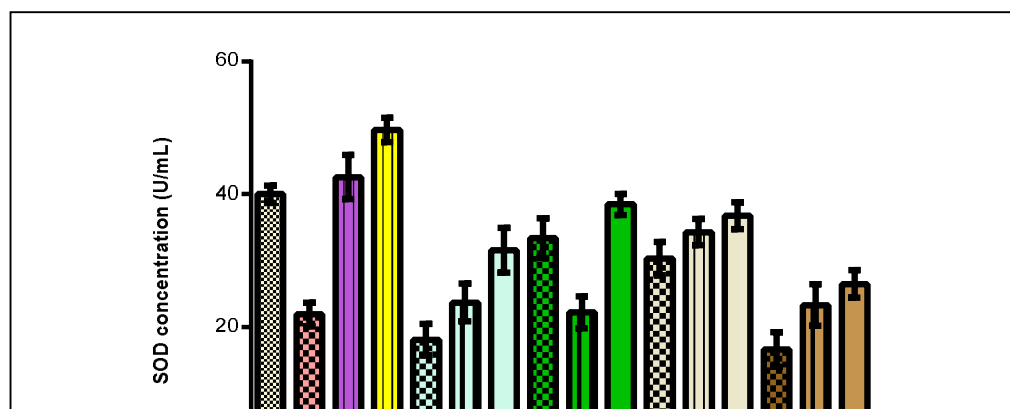
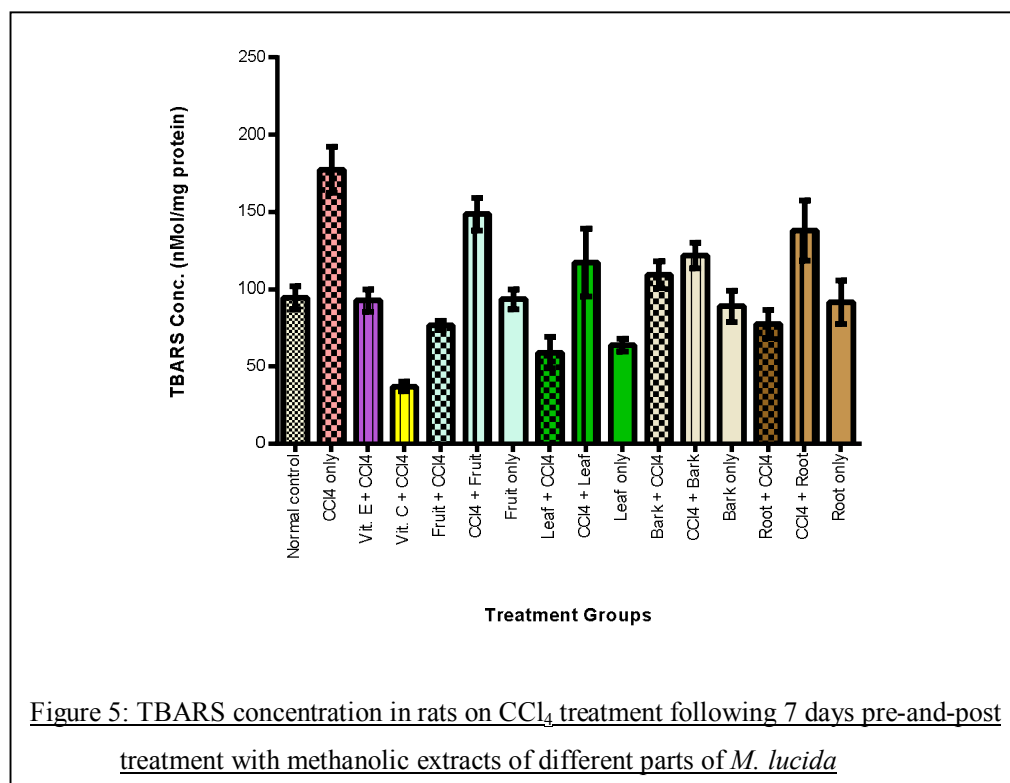


Figure 4: Relative kidney to body ratio in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

I. GRAPHS OF ANTI-OXIDATION EXPERIMENTS:



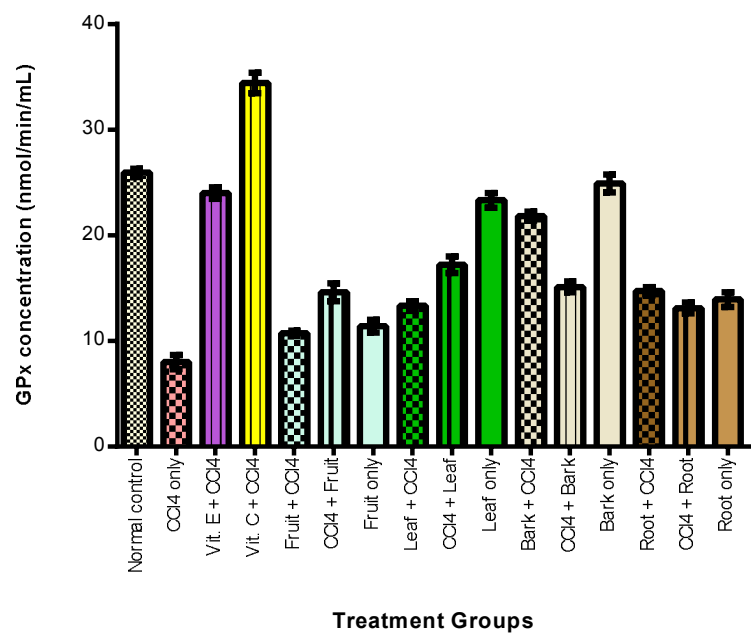
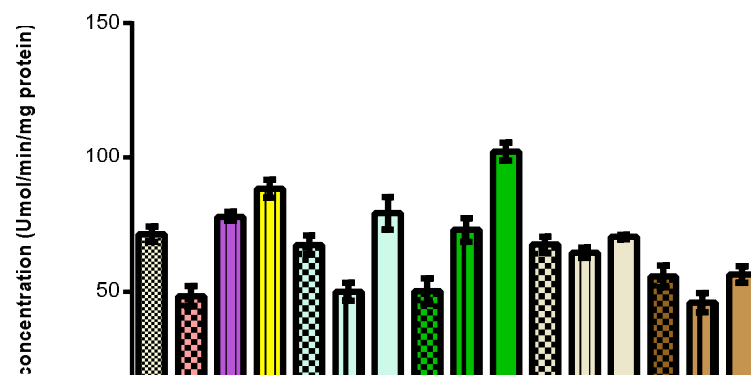


Figure 7: GPx activity in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*



II. GRAPHS OF ANTI-INFLAMMATORY EXPERIMENT:

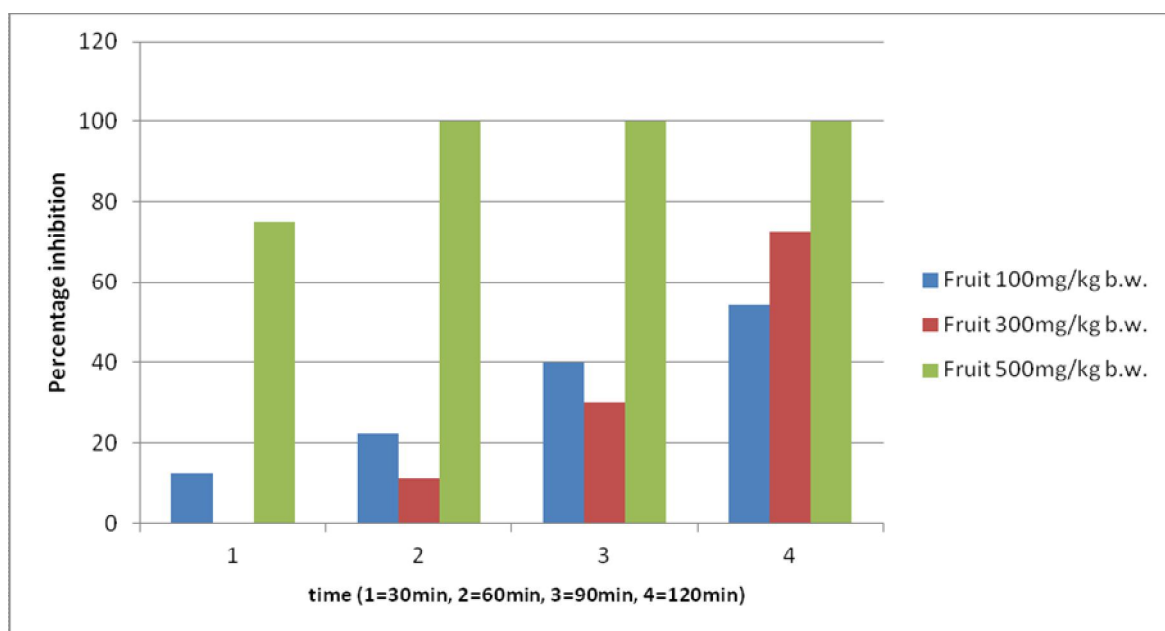


Figure 9: Percentage inhibition of inflammation of methanol fruit extracts of *Morinda lucida* on carrageenan induced rats paw oedema at different doses

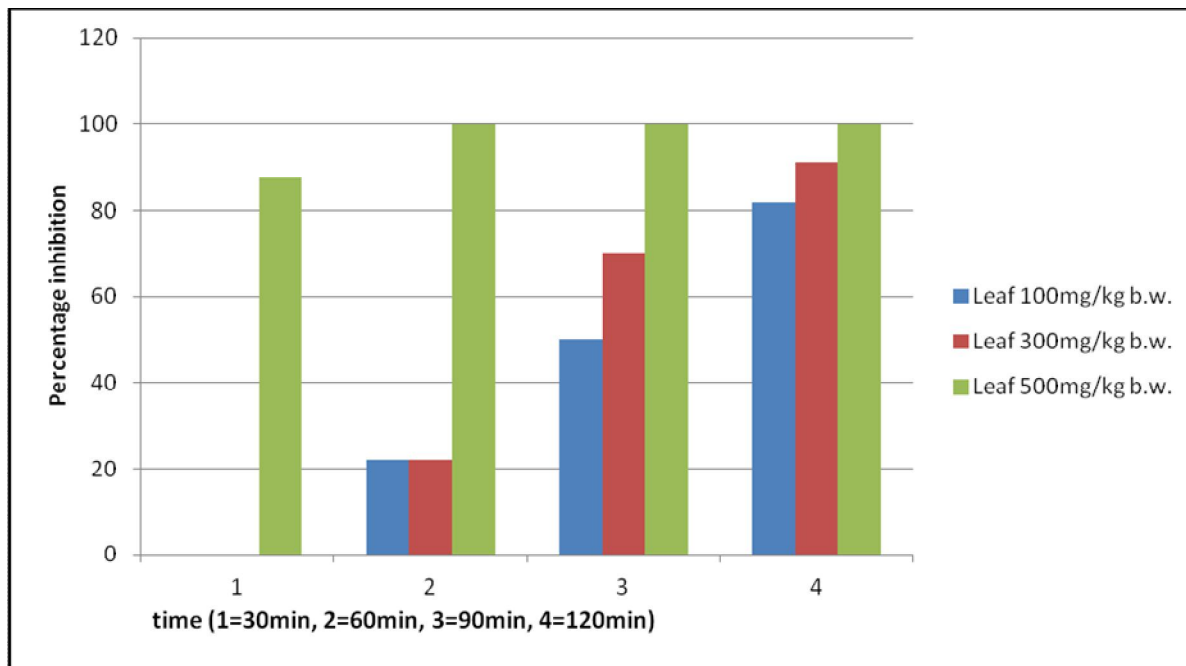


Figure 10: Percentage inhibition of inflammation of methanol leaf extracts of *Morinda lucida* on carrageenan induced rats paw oedema at different doses.

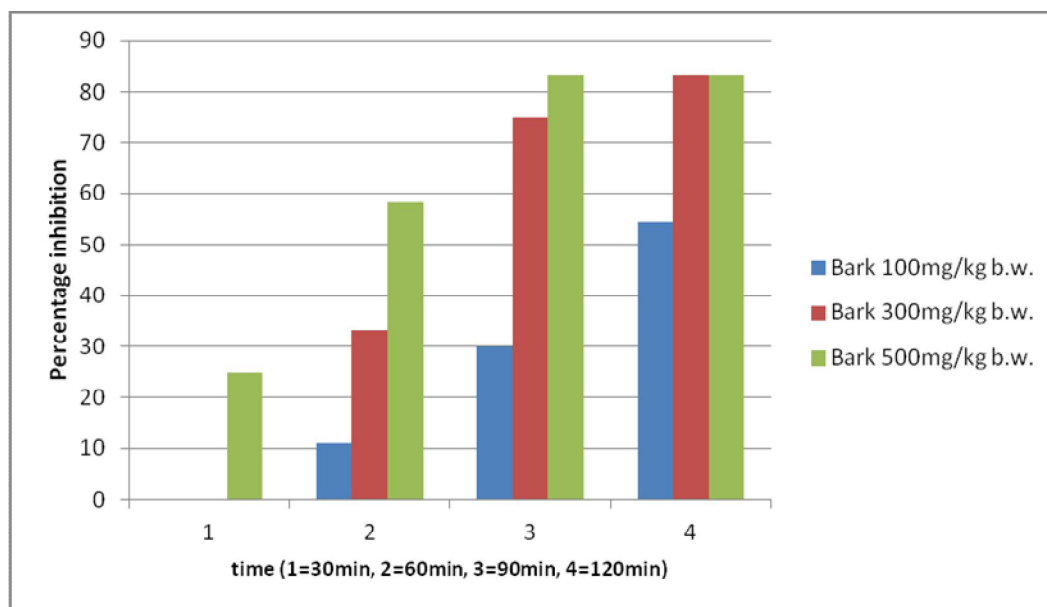


Figure 11: Percentage inhibition of inflammation of methanol bark extracts of *Morinda lucida* on carrageenan induced rats paw oedema at different doses.

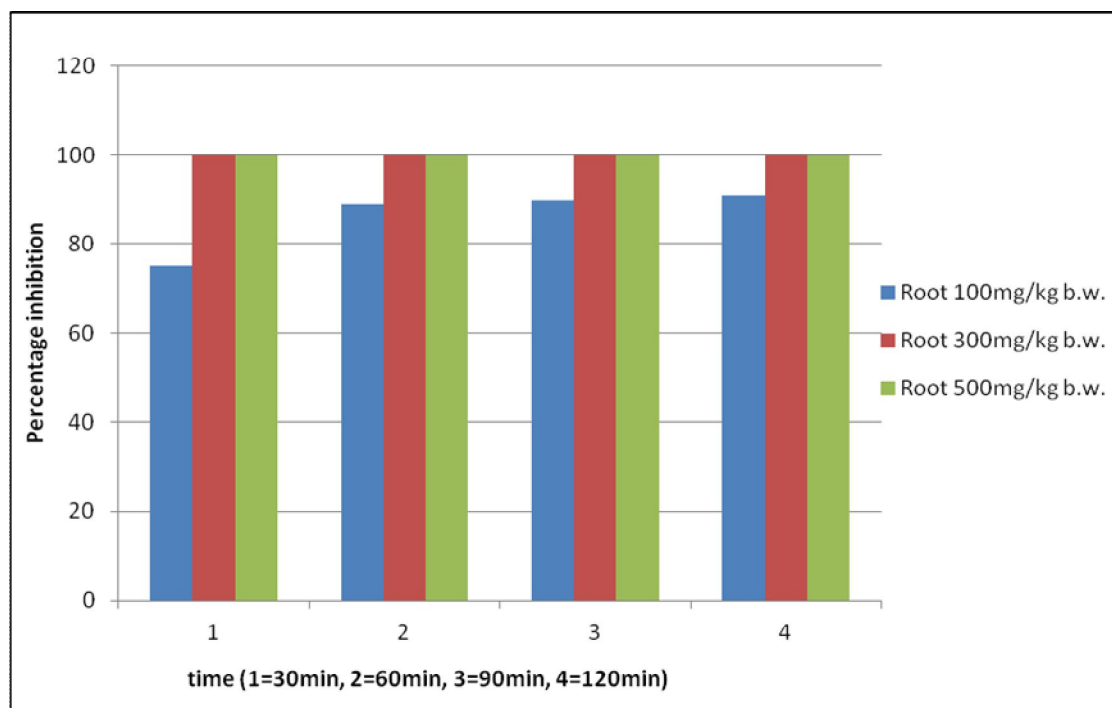


Figure 12: Percentage inhibition of inflammation of methanol root extracts of *Morinda lucida* on carrageenan induced rats paw oedema at different doses.

Table 4: Effect of *M. lucida* MeOH extracts on Carrageenan induced rat paw oedema

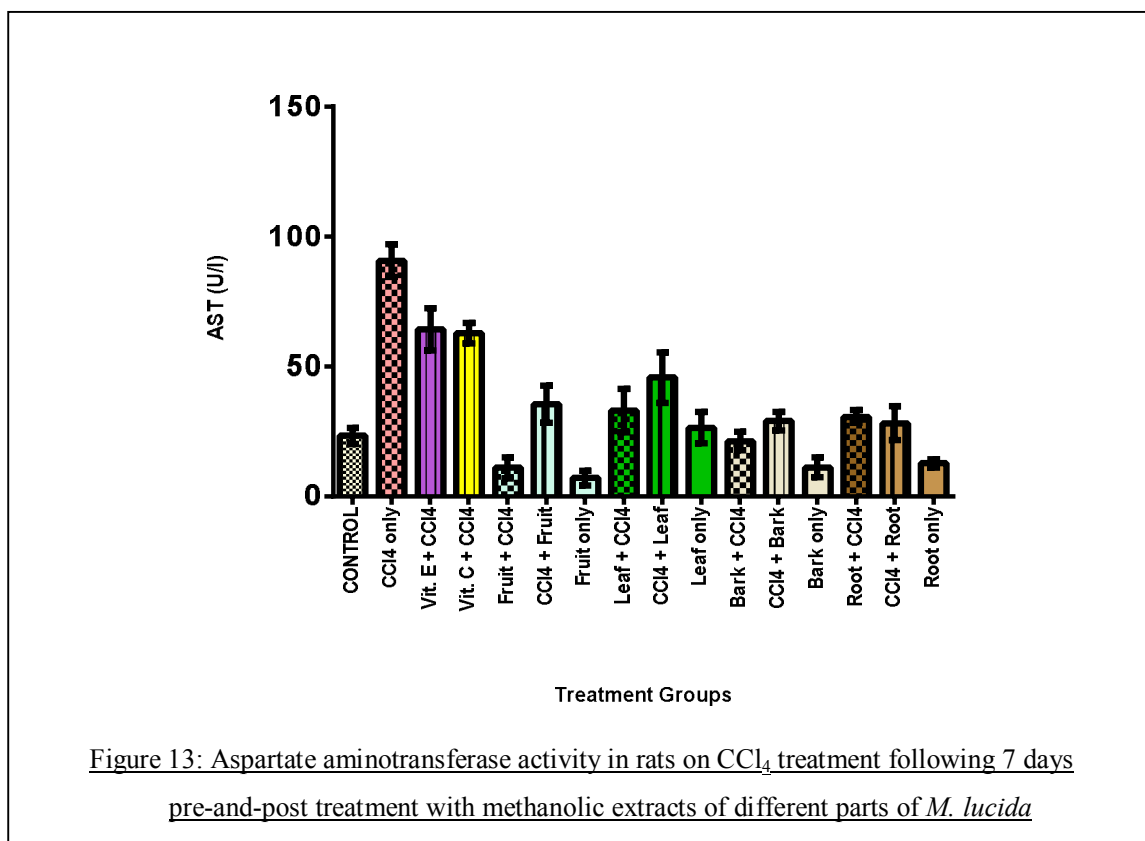
Part	Dose (mg/Kg b.w)	%age inhibition at time (t)			
		t=30min	t=60min	t=90min	t=120min
Fruit	100.0	12.5 ± 0.12 ^b	22.2 ± 0.08 ^b	40.0 ± 0.05 ^{ab}	54.5 ± 0.08 ^b
	300.0	0.0 ± 0.13 ^b	11.1 ± 0.14 ^b	30.0 ± 0.15 ^b	72.7 ± 0.16 ^{ab}
	500.0	75.0 ± 0.18 ^{ab}	100.0 ± 0.18 ^a	100.0 ± 0.18 ^a	100.0 ± 0.18 ^a
Leave	100.0	0.0 ± 0.08 ^b	22.2 ± 0.06 ^b	50.0 ± 0.08 ^b	81.8 ± 0.10 ^a
	300.0	0.0 ± 0.08 ^b	22.2 ± 0.08 ^b	70.0 ± 0.05 ^a	90.9 ± 0.04 ^a
	500.0	87.5 ± 0.11 ^a	100.0 ± 0.13 ^a	100.0 ± 0.13	100.0 ± 0.13 ^a
Bark	100.0	0.0 ± 0.13 ^b	11.1 ± 0.14 ^b	30.0 ± 0.10 ^{ab}	54.5 ± 0.13 ^{ab}

	300.0	0.0 ± 0.14^b	33.3 ± 0.09^{ab}	75.0 ± 0.10^a	83.3 ± 0.10^a
	500.0	25.0 ± 0.04^{ab}	58.3 ± 0.10^a	83.3 ± 0.06^a	83.3 ± 0.06^a
	100.0	75.0 ± 0.09^a	88.9 ± 0.12^a	90.0 ± 0.12	90.9 ± 0.12^a
Root	300.0	100.0 ± 0.13^a	100.0 ± 0.13^a	100.0 ± 0.13^a	100.0 ± 0.13^a
	500.0	100.0 ± 0.08^a	100.0 ± 0.08^a	100.0 ± 0.08	100.0 ± 0.08^a
(Diclofenac Na)	5.0	$0.4 \pm 0.12a$	$1.0 \pm 0.15a$	20.0 ± 0.12^b	45.5 ± 0.15^{ab}

Values obtained were means of groups made of six (6) rats each.

Values with same alphabets down the column are not significantly different ($p < 0.05$).

I. GRAPHS OF HEPATOPROTECTIVE EXPERIMENTS:



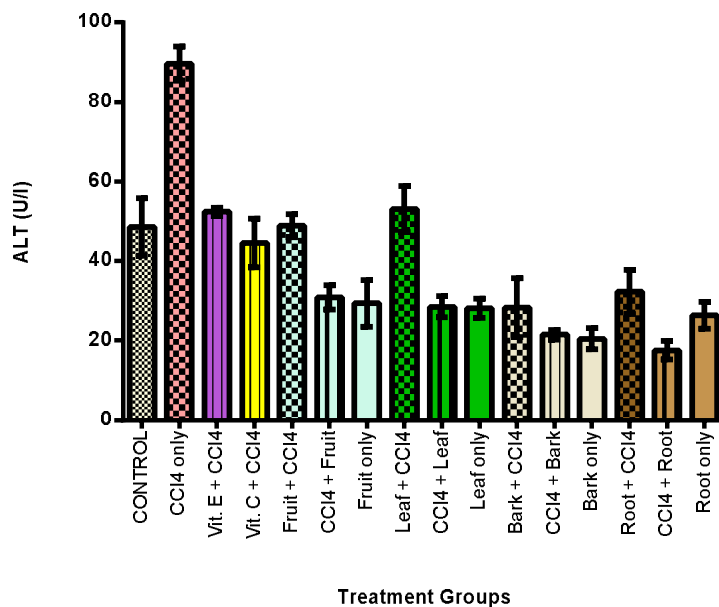


Figure 14: Alanine aminotransferase activity in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

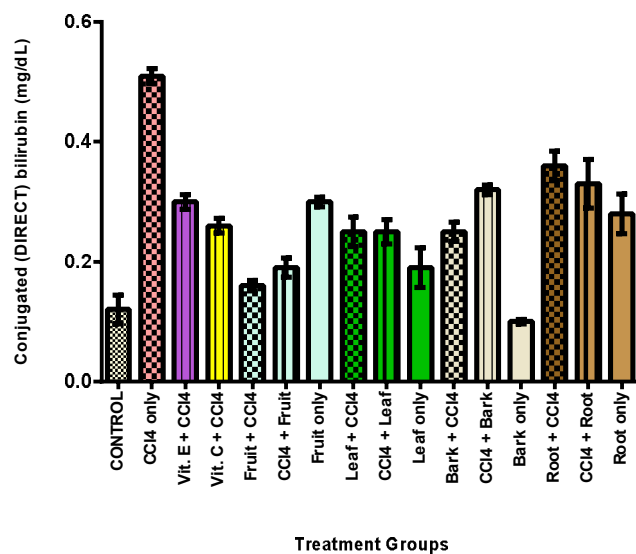


Figure 15: Bilirubin (direct) concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

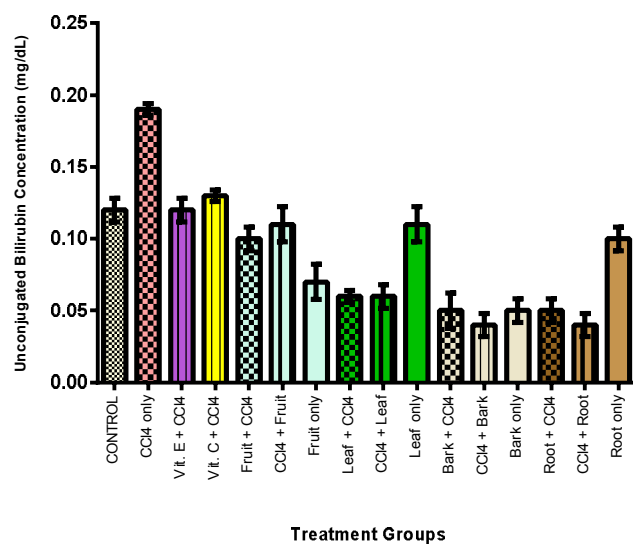


Figure 16: Bilirubin (indirect) concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

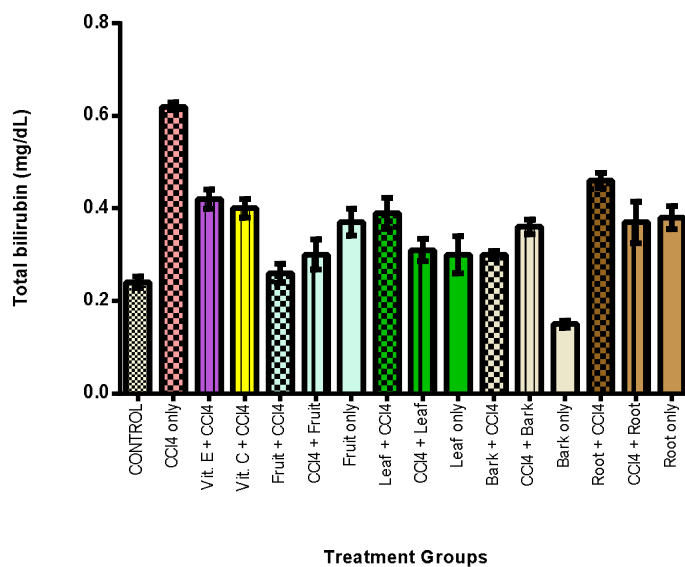


Figure 17: Total bilirubin concentration in rats on CCl₄ treatment following 7 days

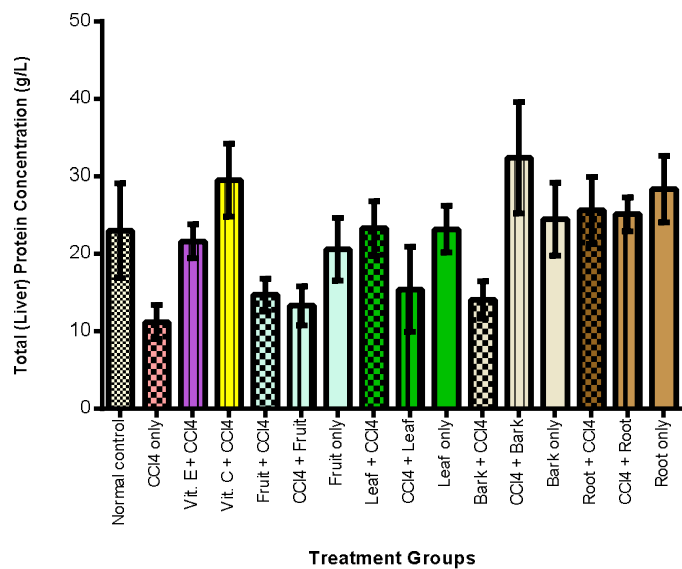
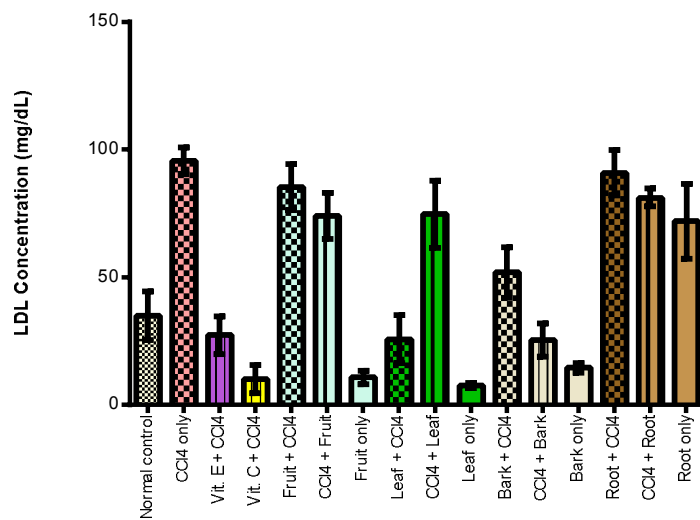


Figure 18: Total (liver) protein concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

GRAPHS OF LIPID PROFILE EXPERIMENTS:



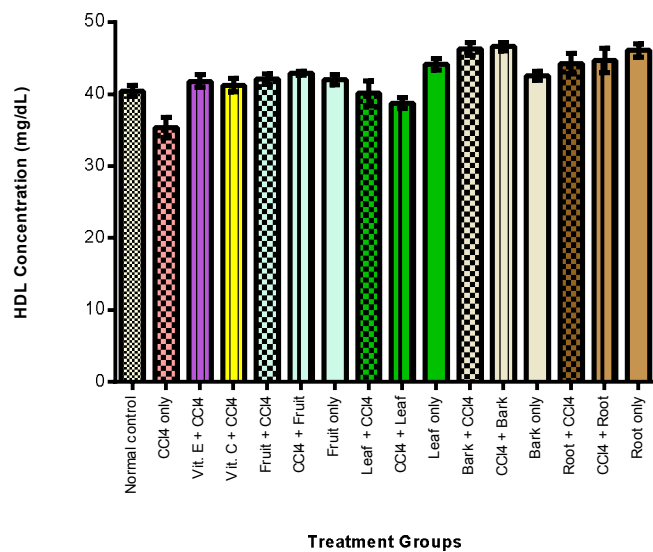
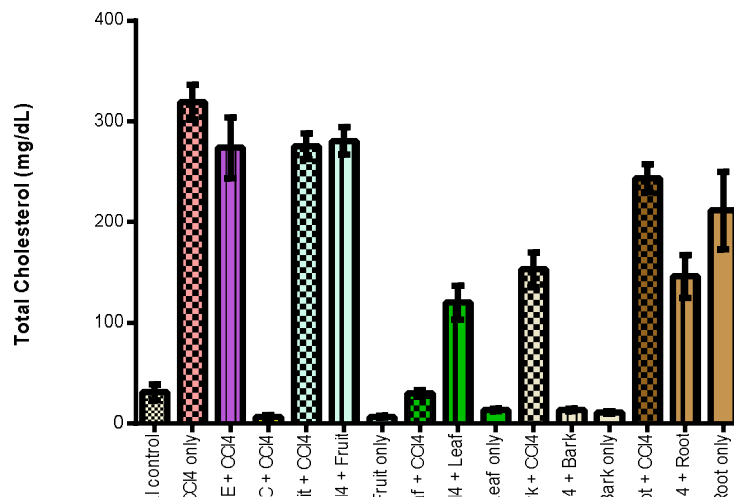


Figure 20: HDL concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*



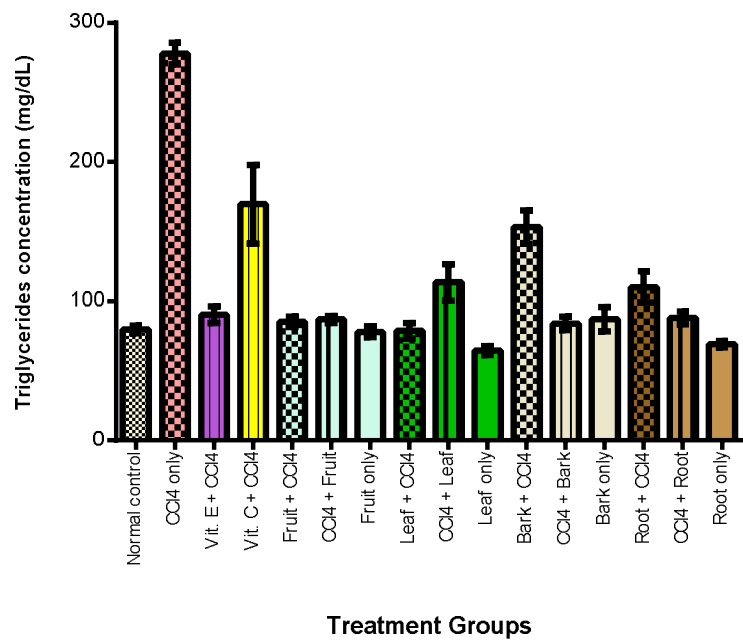
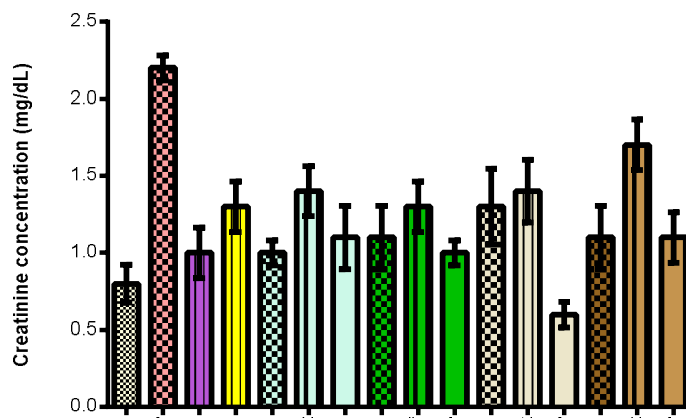


Figure 22: Triglycerides concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

GRAPHS OF KIDNEY FUNCTION EXPERIMENTS:



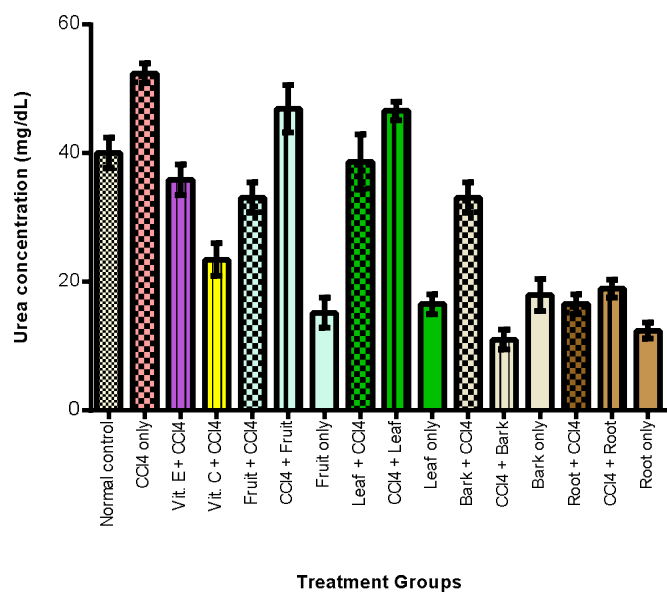


Figure 24: Urea concentration in rats on CCl₄ treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

I. GRAPHS OF CYTOTOXICITY EXPERIMENTS:

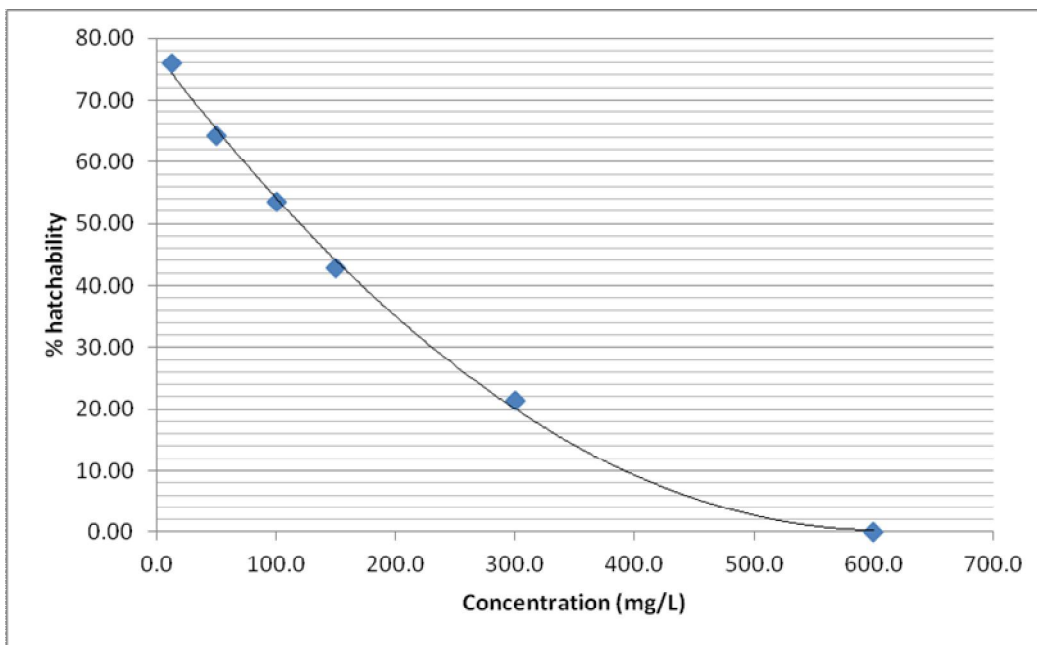


Figure 25: Inhibitory effect of different concentrations of methanolic fruit extracts of *Morinda lucida* on hatchability of *Artemia salina* cysts

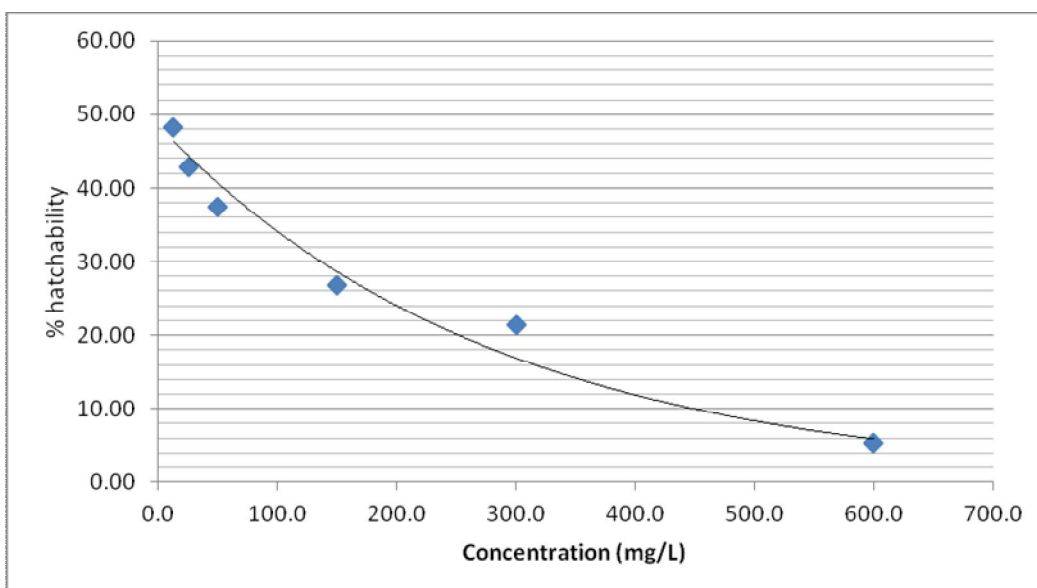


Figure 26: Inhibitory effect of different concentrations of methanolic leaf extracts of *Morinda lucida* on hatchability of *Artemia salina* cysts.

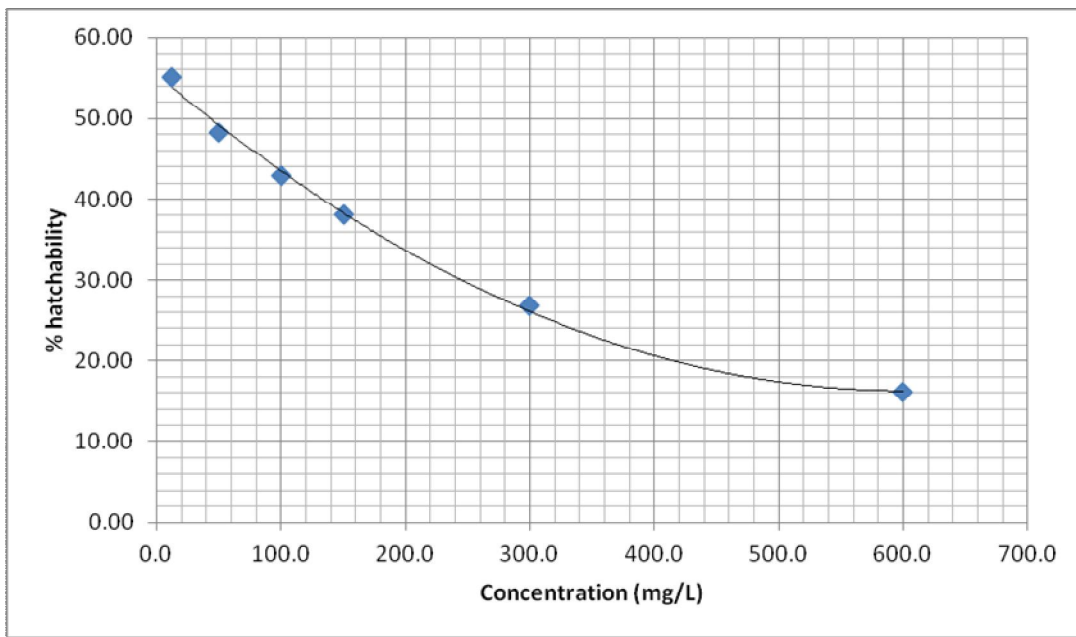


Figure 27: Inhibitory effect of different concentrations of methanolic bark extracts of *Morinda lucida* on hatchability of *Artemia salina* cysts

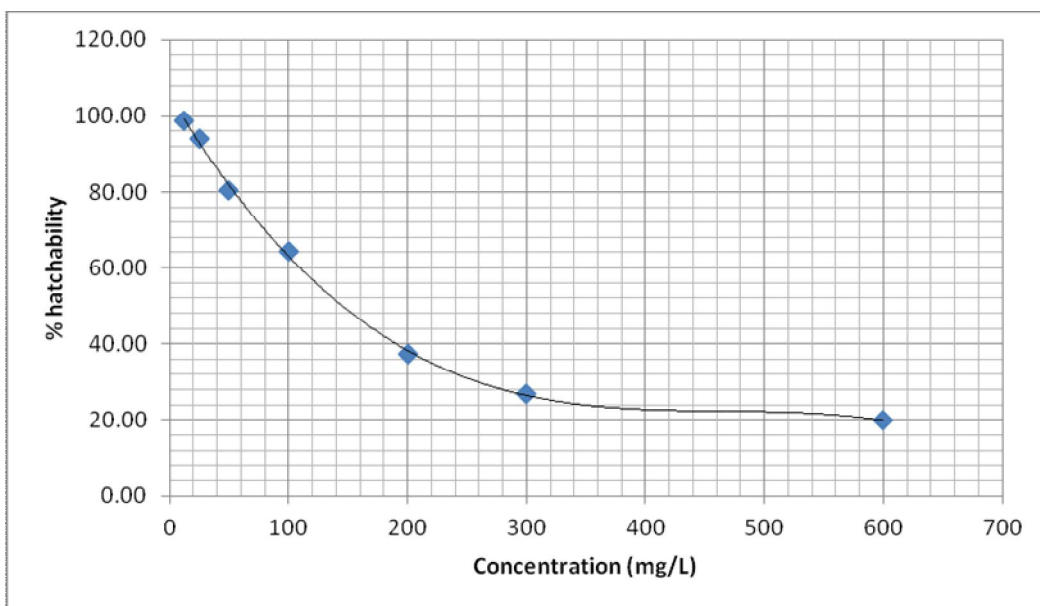


Figure 28: Inhibitory effect of different concentrations of methanolic root extracts of *Morinda lucida* on hatchability of *Artemia salina* cysts

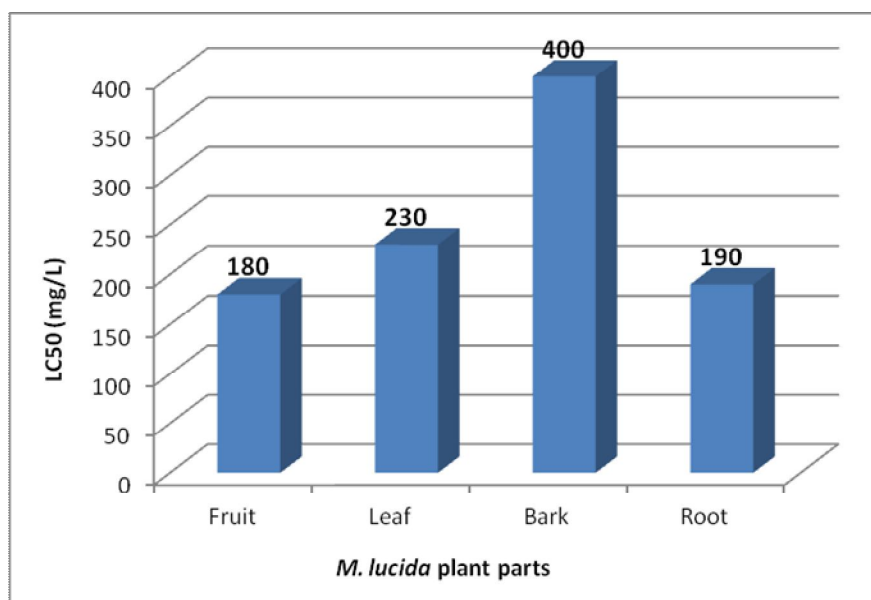


Figure 29: The hatchability LC₅₀ values of methanolic extracts of the different parts of *Morinda lucida*

Table 5: Probit calculation table for *Morinda lucida* fruit extracts on brine shrimps

Conc (mg/L)	Start No. of nauplii	No. of dead nauplii	Log10 Conc.	% Mortality	Corrected % Mortality	Probit
0	10	3	-	30	0	-
150	10	4	2.18	40	14	3.92
450	10	5	2.65	50	29	4.45
750	10	6	2.88	60	43	4.82

mp lethality test:

I. B
r
i
n
e
s
h
r
i

1050	10	7	3.02	70	57	5.18
1350	10	9	3.13	90	86	6.08

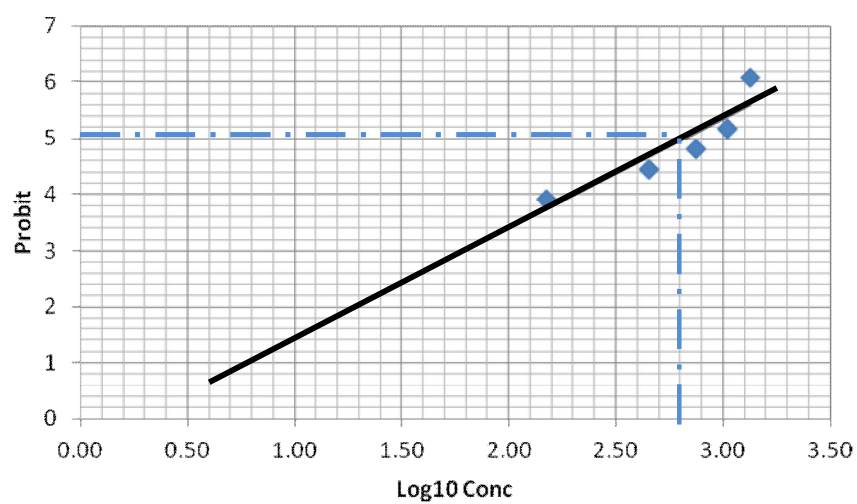


Figure 30: Graph for determination of LC_{50} for *Morinda lucida* fruit extract

$x = 2.80$; $LC_{50} = \text{Antilog } 2.80 = 631.0\text{mg/L}$

Table 6: Probit calculation table for *Morinda lucida* leave extracts on brine shrimps

Conc. (mg/L)	Start No. of nauplii	No. of dead nauplii	Log10 Conc.	% Mortality	Corrected % Mortality	Probit
0	10	0	-	0	0	-
150	10	2	2.18	20	20	4.16
450	10	5	2.65	50	50	5.00
750	10	9	2.88	90	90	6.28
1050	10	9	3.02	90	90	6.28

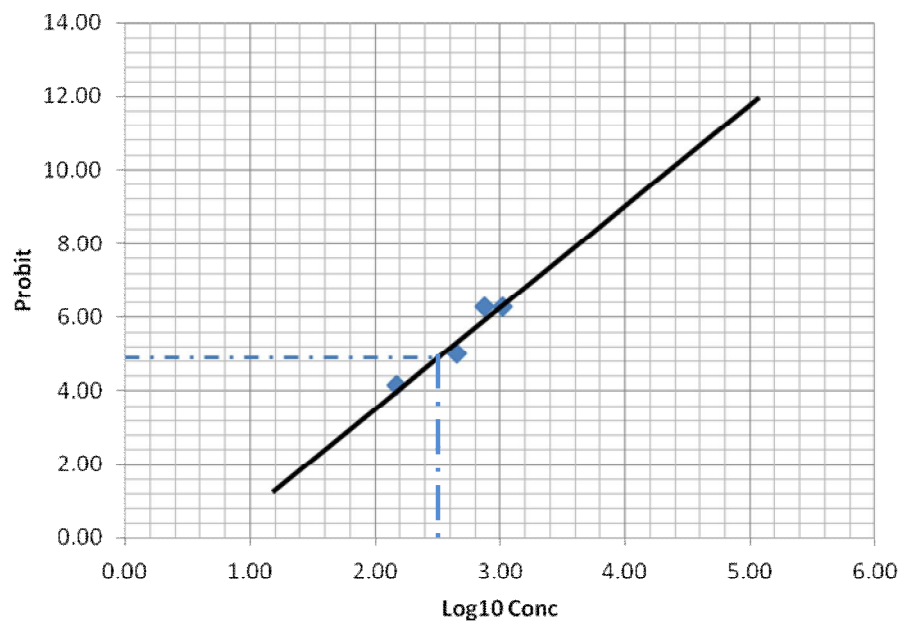


Figure 31: Graph for determination of LC₅₀ for *Morinda lucida* leave extract
 $x = 2.50$; LC₅₀ = Antilog 2.50 = 316.2mg/L

Table 7: Probit calculation table for *Morinda lucida* bark extracts on brine shrimps

Conc (mg/L)	Start No. of nauplii	No. of dead nauplii	Log10 Conc.	% Mortality	Corrected % Mortality	Probit
0	10	1	-	10	0	-
150	10	6	2.18	60	56	5.15
450	10	5	2.65	50	44	4.85
750	10	8	2.88	80	78	5.77
1050	10	9	3.02	90	89	6.23
1350	10	10	3.13	100	100	-

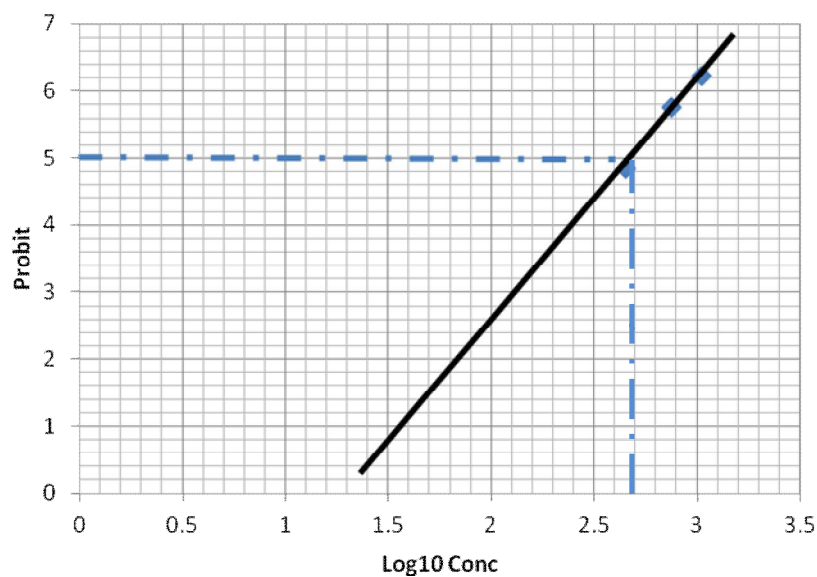


Figure 32: Graph for determination of LC₅₀ for *Morinda lucida* bark extract

x = 2.70; LC50 = Antilog 2.70 = 501.2mg/L

Table 8: Probit calculation table for *Morinda lucida* root extracts on brine shrimps

Conc. (mg/L)	Start No. of nauplii	No. of dead nauplii	Log10 Conc.	% Mortality	Corrected % Mortality	Probit
0	10	2	-	20	0	-
150	10	3	2.18	30	13	3.87
450	10	7	2.65	70	63	5.33
750	10	8	2.88	80	75	5.67
1050	10	8	3.02	80	75	5.67
1350	10	9	3.13	90	88	6.18

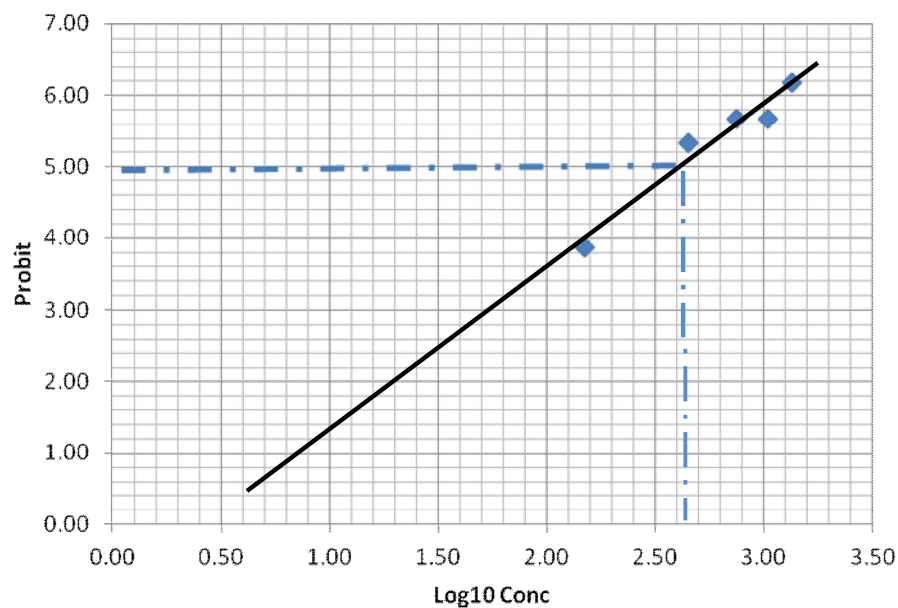


Figure 33: Graph for determination of LC_{50} for *Morinda lucida* root extract
 $x = 2.60$; $LC_{50} = \text{Antilog } 2.60 = 398.1\text{mg/L}$

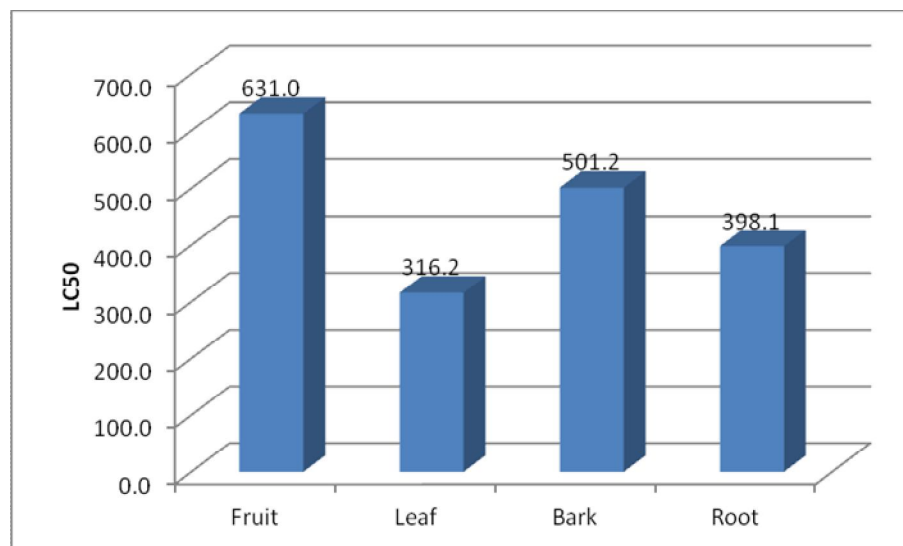


Figure 34: The lethality LC_{50} values of methanolic extracts of
different parts of *Morinda lucida*

GRAPHS OF HAEMATOLOGICAL EXPERIMENTS:

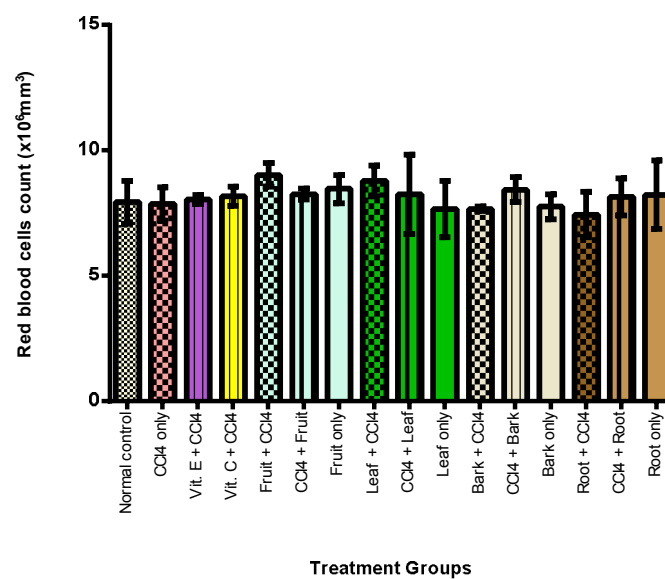


Figure 35: Red blood cell count in rats on CCl_4 treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*.

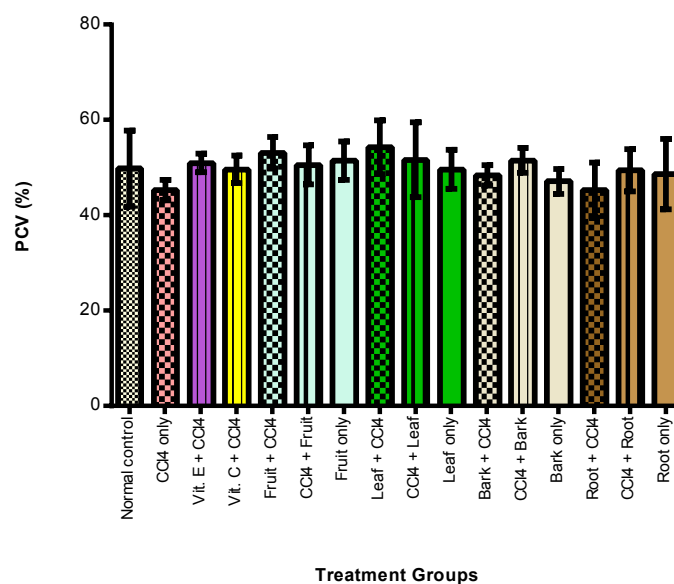


Figure 36: Packed cell volume in rats on CCl_4 treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*.

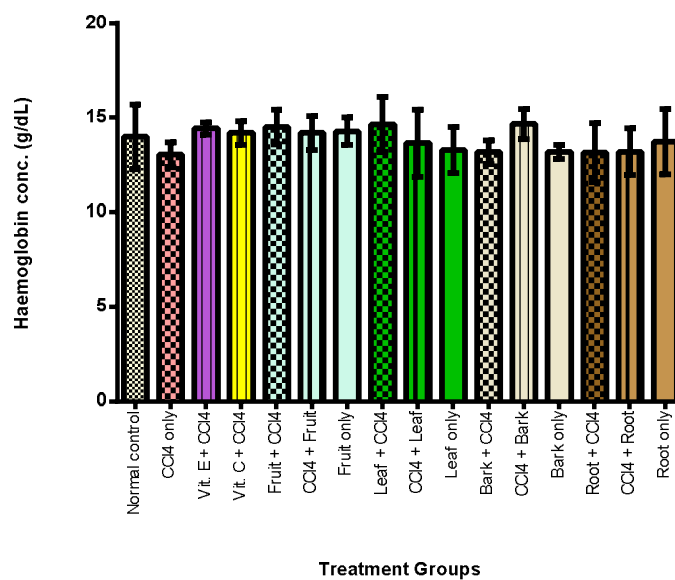


Figure 37: Haemoglobin concentration in rats on CCl_4 treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

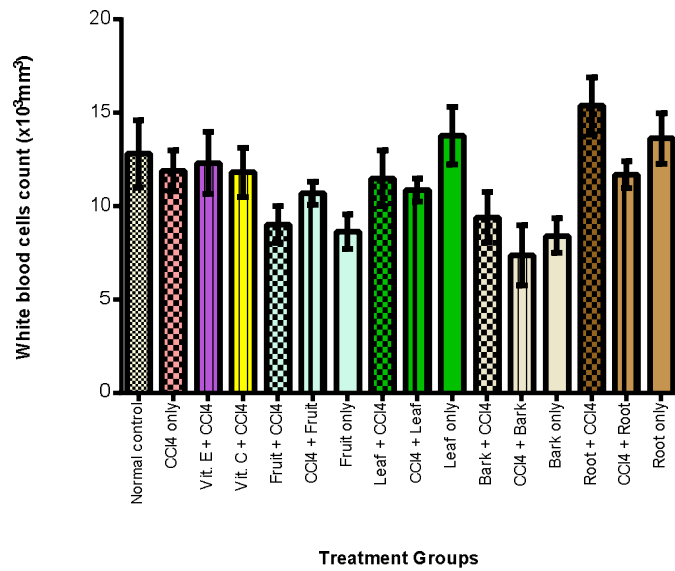


Figure 38: WBC concentration in rats on CCl_4 treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

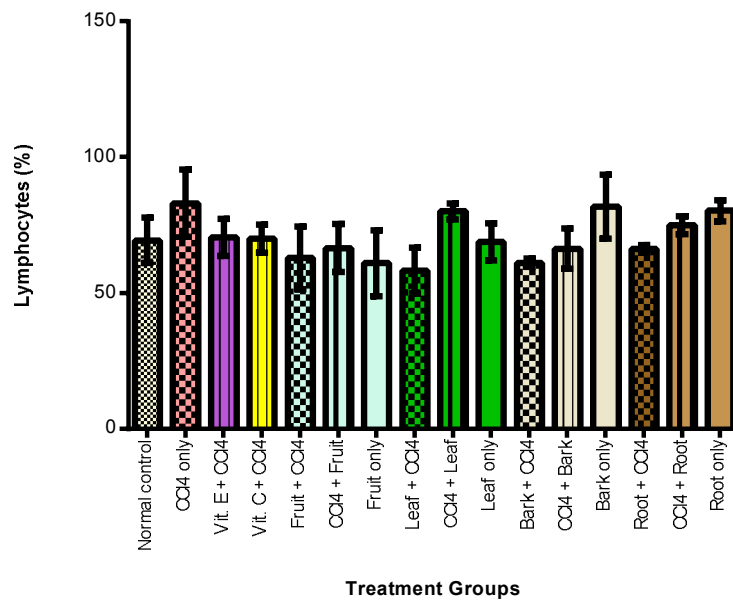


Figure 39: Lymphocytes concentration in rats on CCl_4 treatment following 7 days

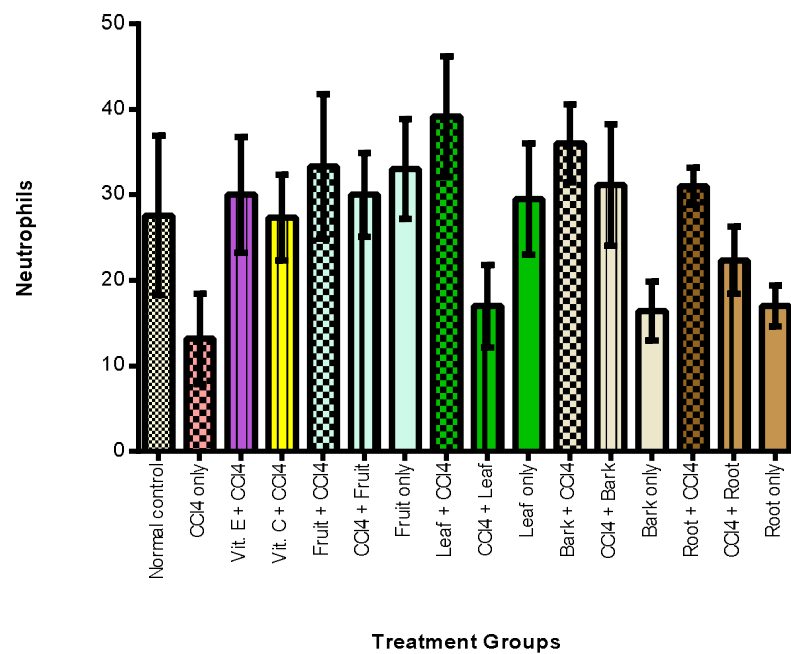


Figure 40: Neutrophil concentration in rats on CCl_4 treatment following 7 days pre-and-post treatment with methanolic extracts of different parts of *M. lucida*

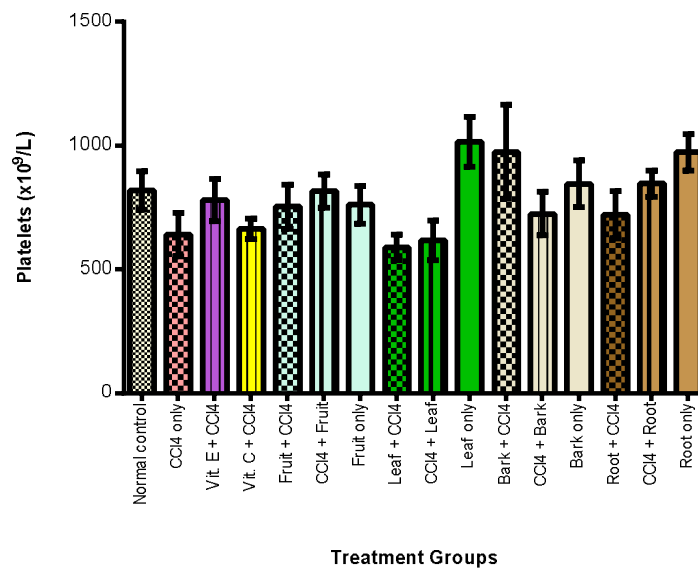
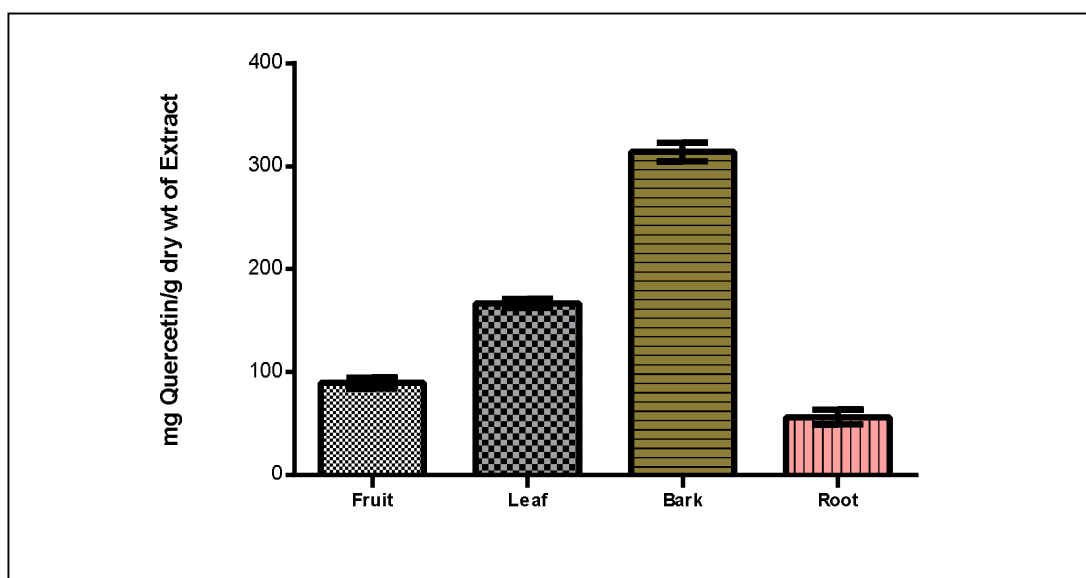
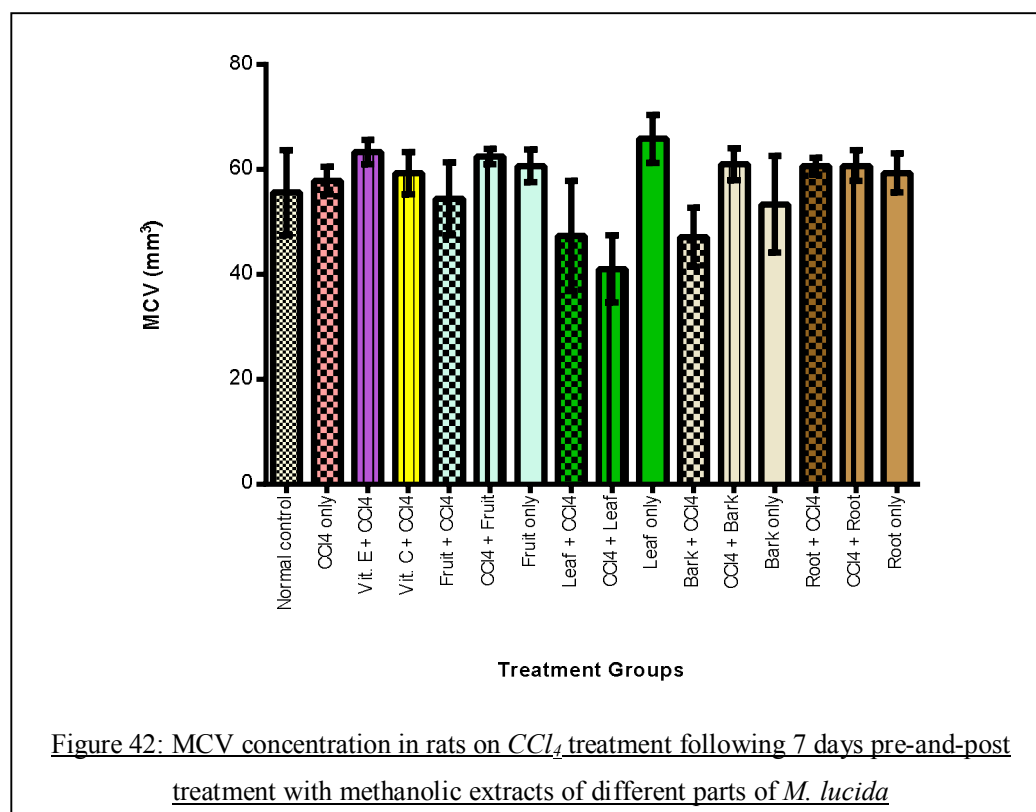
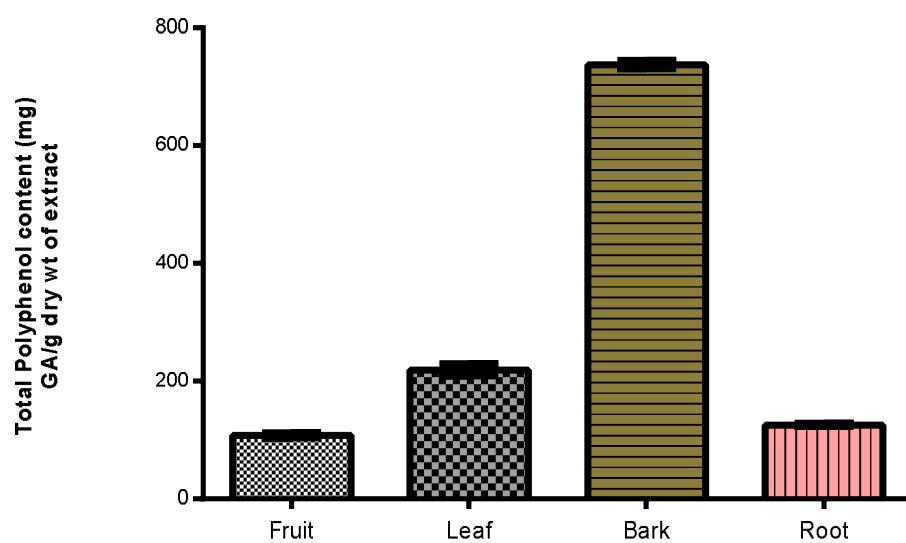


Figure 41: Platelets concentration in rats on CCl_4 treatment following 7 days pre-and-post





Morinda lucida

Figure 44: Total polyphenol content of methanolic extracts of different parts of *M. lucida*

APPENDIX II

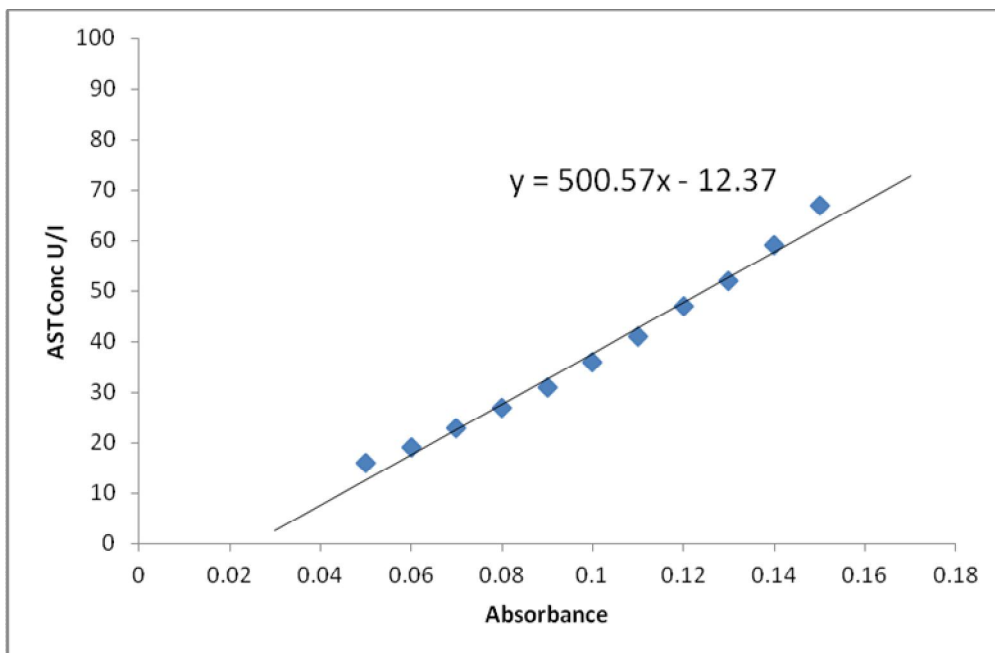


Figure 45: Aspartate aminotransferase (AST) standard curve

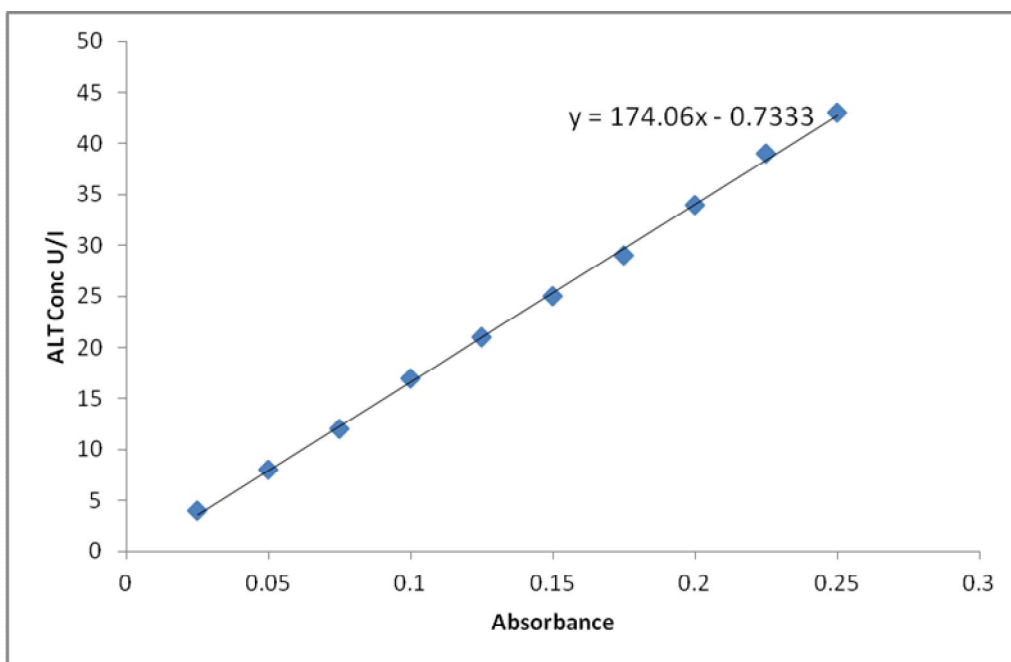


Figure 46: Alanine aminotransferase (ALT) standard curve

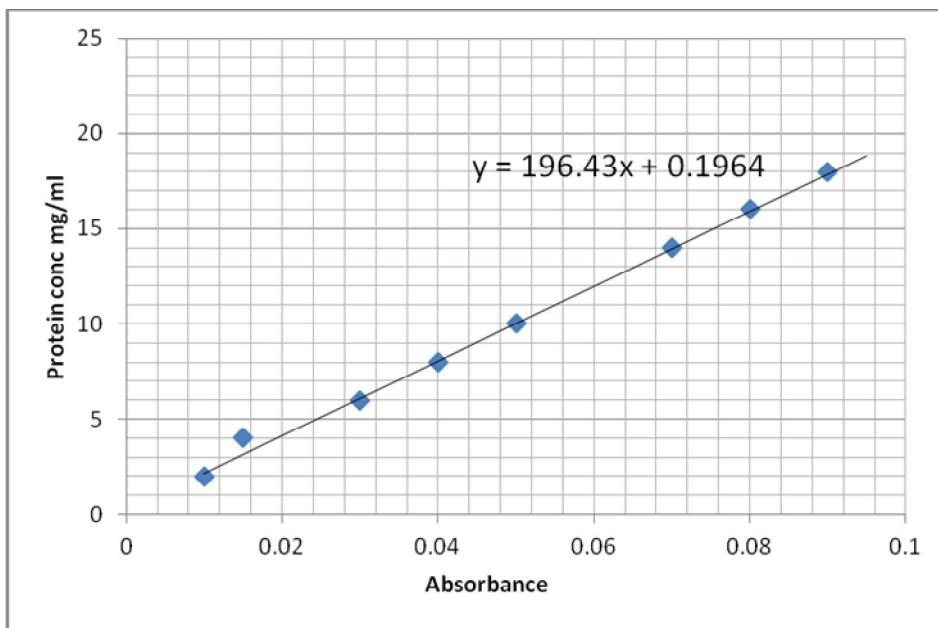


Figure 47: Protein (Albumin) Standard Curve

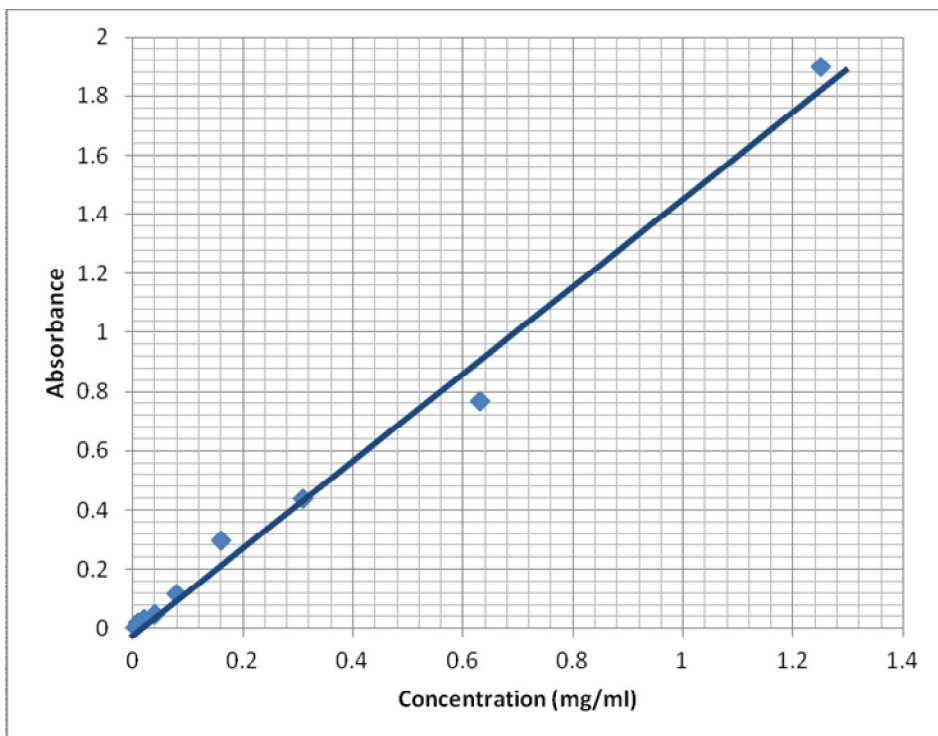


Figure 48: Quercetin Standard Curve

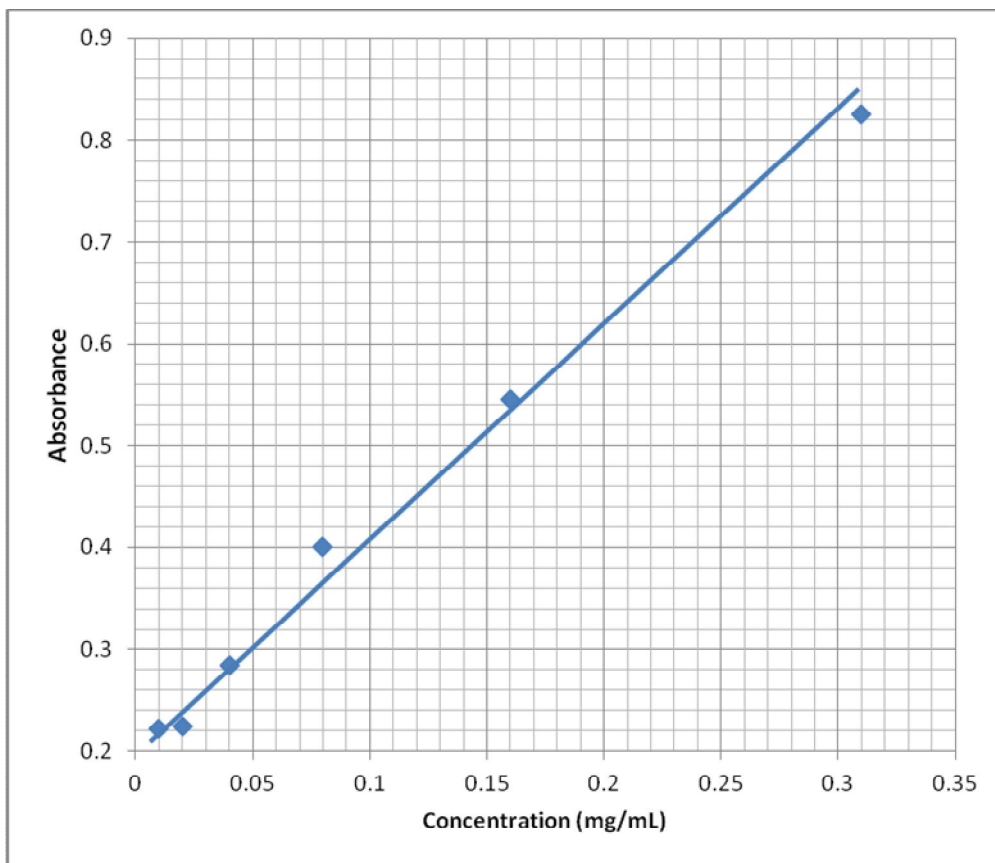


Figure 49: Gallate Standard Curve

APPENDIX III

Table 9: REAGENTS/CHEMICALS

SN	Item (REAGENTS/KITS)
1	Tetrachloromethane (CCl ₄)
2	Carrageenan
3	Vitamin E (α -tocopherol)
4	Indomethacin (I7378-5G)
5	Petroleum ether (60-80°C)
6	Methanol
7	TBARS assay kit
8	Superoxide dismutase assay kit (K335-100)
9	Catalase assay kit (K773-100)
10	Nitric oxide assay kit (K262-200)
11	Glutathione peroxidase assay kit (K762-100)
12	TG assay kit
13	TC assay kit
14	HDL assay kit
15	Total protein assay kit
16	ALT (GPT) assay kit
17	AST (GOT) assay kit
18	Bilirubin (Total and Conjugated) assay kit
19	Agarose Gel Electrophoresis kits
20	Creatinine assay kit
21	Blood urea nitrogen assay kit
22	Creatine phosphokinase (CPK) assay kit
23	Aluminium chloride (AlCl ₃)
24	Quercetin
25	Gallic acid
26	Phosphomolybdic acid
27	Phosphotungstic acid
28	Corn oil
29	Histopathology
30	Haematology

SN	Item (MATERIALS)	Source
1	Rat cages	Wooden cageS

Table 10: ADDITIONAL MATERIALS AND SOURCE

2	Rodent regular feed	Vital Feeds Mini-depot, Mararaba, Abuja
3	Test tubes	Bijo Chemicals Ltd, Kaduna
4	Portable freezer (for sample storage)	-
5	Power generating set	-
6	Microplate reader	NIPRD, Idu, Abuja
7	Multi-channel auto pipette	NIPRD, Idu, Abuja
8	Dissection kits	Bijo Chemicals Ltd, Kaduna
9	Centrifuge	Department of Biochemistry, A.B.U. Zaria
10	Rotary Evaporator	Biochemistry Department, Bingham University
11	Water bath	Biochemistry Department, Bingham University
12	1 ml Injection needles and syringes	Cele Tex Pharmacy, Unity Roundabout, Keffi
13	Air pump (4 outlets)	Kingdom fisheries and Aquarium, Lagos
14	Air pump (12 outlets)	Kingdom fisheries and Aquarium, Lagos
15	Tubings for air pump	Kingdom fisheries and Aquarium, Lagos
16	Tubings for soxhelet extractor	Rasbar Chemicals, Zaria.
17	1.5 litre tanks for brine shrimps	Fabrication work
18	Sea salt	Sa'ad Stores, Garki-Abuja
19	Air stones	Kingdom fisheries and Aquarium, Lagos
20	Light bulbs	Electrical Stores, by Turaki House, Keffi
21	Electricity Cables	Electrical Stores, by Turaki House, Keffi
22	Electrical bulb fittings	Electrical Stores, by Turaki House, Keffi
23	Fueling	For generator
24	Magnifying Hand lens	Bijo Chemicals Ltd, Kaduna
25	Haemocytometer (Nauber chamber)	Bijo Chemicals Ltd, Kaduna
26	Microscope slides	Bijo Chemicals Ltd, Kaduna

S/N	Item (ANIMALS/ORGANISMS)
1	Albino Wistar rats
2	Brine shrimps (Artemia cyst)

Table 11: Logistics

S/N	Item
1	Communication
2	Transportation
3	Guides, PR & Supporters