EVALUATION OF THE EFFECTS OF *VERNONIA AMYGDALINA* AND *TELFAIRIA OCCIDENTALIS* LEAVES SUPPLEMENTED DIET ON N-METHYL-N-NITROSOUREA (NMU) ADMINISTERED MALE WISTAR RATS

BY

OCHAI EMMANUEL ODEH

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

DECEMBER, 2014

EVALUATION OF THE EFFECTS OF *VERNONIA AMYGDALINA* AND *TELFAIRIA OCCIDENTALIS* LEAVES SUPPLEMENTED DIET ON N-METHYL-N-NITROSOUREA (MNU) ADMINISTERED MALE WISTAR RATS

BY

Ochai Emmanuel ODEH, BSc. BIOCHEMISTRY (ABU, ZARIA) 2005

MSC/SCIEN/5355/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD

OF A

MASTER DEGREE IN BIOCHEMISTRY.

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

DECEMBER, 2014

DECLARATION

I hereby declare that the work in this Thesis titled "**Evaluation of the Effects of *Vernonia amygdalina* and *Telfairia occidentalis* Leaves Supplemented Diet on N-Methyl-N-Nitrosourea (MNU) Administered Male Wistar Rats**" was performed by me in the Department of Biochemistry, Ahmadu Bello University Zaria under the supervision of Professor S. E Atawodi, FAS and Dr. K.M Anigo. 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.

ODEH, EMMANUEL OCHAI _____ _____

MSc./Scien /5355/2011-2012 Signature Date

CERTIFICATION

This Thesis titled "**EVALUATION OF THE EFFECTS OF *VERNONIA AMYGDALINA* AND *TELFAIRIA OCCIDENTALIS* LEAVES SUPPLEMENTED DIET ON N-METHYL-N-NITROSOUREA (MNU) ADMINISTERED MALE WISTAR RATS**" by Ochai Emmanuel ODEH**,** meets the regulations governing the award of the degree of Masters in Biochemistry of the Department of Biochemistry, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Professor S.E Atawodi, FAS _____ _____
Chairman, Supervisory Committee Signature Date

Dr. K.M Anigo _____ _____ _____

Member, Supervisory Committee Signature
Date

Professor I. A Umar _____ _____

Head of Department Signature
Date

Professor A. Z Hassan _____ _____
Dean, School of Postgraduate Studies Signature Date

ACKNOWLEDGEMENT

A work of this kind cannot be claimed to be independently achieved. The journey through my Master's degree training was not without challenges, but The LORD GOD who gave me the privilege to be admitted into this program stationed people at every junction to help me through several stages.

First, I would like to acknowledge my Supervisor Professor S.E Atawodi . I should say that I am privileged to have received this training under one of the distinguished professors I have ever known. My exposure to hard work, his efficiency, strong and impressive personality has shown me that challenges are not to be feared but can be conquered. As a father figure, being always available to listen to his students, his instructions, sensitivity and advice helped me in perplexing moments. He personally ensured this work is given the facilitation it needed. Thank you, sir. And to Dr. Kola Anigo my co-supervisor whose advice helped me pass a critical stage of my work when I wondered how I would ever be able to make it through, but God used him. Thank you, sir.

My heart-felt gratitude also goes to The Head of Department for creating the enabling environment for this research by allowing access to needed facilities.

Professor E. Onyike of the Department of Biochemistry, through her sensitive heart, stood by me in prayer and all the way. Her interventions took care of my needs when I least expected. Ma, you were heaven sent. God has used you to touch my life, at such a time as this, in an unforgettable way. Thank you. My profound appreciation also goes to all postgraduate lecturers who taught me during this programme. Their contributions remain indelible even beyond the class room. Thank you for giving and not sparing.

I must thank the technical staff of the Department of Biochemistry and Pharmacology who were very helpful in assisting me in my laboratory work. The Chief Technologist, Department of Biochemistry, Mr. Stephen Jock, understood the peculiarity of my work and used his office to assist. Mr. Apeh Yakubu who being in charge of the Postgraduate Laboratory was inconvenienced by the nature of my work but however still offered technical support that my work required. Also, I am grateful to Mallam Aliyu of the Postgraduate Laborartory, Department of Biochemistry, Alhaji Yau'u, Mr. Salisu, Aliyu and all other technical staff of the Animal Housing Facility, Department of Pharmacology, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria, for their understanding and guidance. Thanks also to Mrs. Kemi Iyun of the Department of Chemistry who assisted me with some needed reagents.

Thanks to Professor A. Oyewale for providing accommodation, and to Mr Idowu Bankole for the laptop that facilitated the completion of my Thesis. I wish to also acknowledge my colleagues Iyabo Bawalla, Ojo Oluwadamilare and Onyeka Stephen, who worked closely with me, Mr. Habila Yusuf, for his humility and kindness, Rahinat Yakubu, Joseph Ameh,

Hauwa Salisu, Habiba Kasimu, William Chintem, Binda Andongma, Theresa Gana, Joy Ugbede, Isaac Eelojo, Femi Adepoju and a host of others, too numerous to mention, for their understanding and support..

Family members also stood by me to encourage and support me. A very big thank you goes to my biological Parents, Mr. and Mrs. Emmanuel Odeh for their support and prayers, Uncle Godwin Odeh for his financial contribution, Adakole Odeh a dependable brother for his support, Inalegwu Odeh and the rest of my siblings and family members for their understanding and support. Dauda Yahaya, Mr. and Mrs. Toryila, Caleb Oke, Josiah Haruna, Yakubu Dodo, David Paul, Yabo Stephen Dauda, Emmanuel Amutse Yerima, Mallam Ibrahim, Justin Thomas were the closest to the family I had in Zaria even when I was far from home. Thank you for the various contributions you made to my welfare. May God reward you all.

My entire story will not be complete without the Post graduate Fellowship, Ahmadu Bello University Main campus Zaria. Too numerous to mention for lack of space are close acquaintances who did not hesitate to take their places in my life when it mattered most. Thanks to anonymous contributors who quietly took my burdens in prayer and giving gifts. The one who sees in secret will reward you all openly.

Finally to all dedicated Scientists world over for their outstanding contributions to making life better for mankind, those whose works also served as references to my work, thank you all.

DEDICATION

This work is dedicated to God Almighty and to anyone living with any form of cancer. May their hope be kept alive in God for a quick breakthrough in cancer research.

TABLE OF CONTENTS

TITLE **PAGE**

Title Page……..…….i

Declaration…….……ii

Certification…….…….....iii

Acknowledgements……...…iv

Dedication…….……vi

Table of Contents……..…….……..vii

List of Tables……..……xiii

List of Figures……...…….....xiv

List of Plates…….....…xv

List of Appendices……..……..….....xvi

List of Abbreviation……..……..……...xvii

Abstract……..…….….xix

CHAPTER ONE

1.0 INTRODUCTION…….….1

1.1Statement of Research Problem…….……4

1.2 Justification for Research……..…….....4

1.3 Aims and Objectives……...……..…….….5

1.31 General Aim……..…..…5

1.3.2 Specific Objectives…….…….…….….5

1.4 Research Hypothesis…….…….....…6

CHAPTER TWO

2.0 LITERATURE REVIEW……..…7

2.1 *Vernonia amygdalina*……...……..……..7

2.1.1 General description of *Vernonia amygdalina*……..…..7

2.1.2 Uses of *Vernonia amygdalina*……..……...…8

2.1.3 Phytochemical composition of *Vernonia amygdalina*……..……...10

2.2. *Telfairia occidentalis* ……..…….….10

2.2.1 General description of *Telfairia occidentalis*…….…….10

2.2.2 Uses of *Telfairia occidentalis*…….……..……...….11

2.2.3 Phytochemical composition of *Teairia occidentalis* ……...13

2.3 Cancer: Biology and Various Forms……..……..……..…….…13

2.4 Early History of Cancer Research…….……..14

2.5 Experimental Cancer Research……..……15

2.6 Carcinogens…….…..……....15

2.7 Nitroso Compounds……..……...…….17

2.7.1 N-Methyl-N-Nitrosourea……...……..……17

2.7.2 Mechanism of Action of Nitroso Compounds……..……...……18

2.8 Chemoprevention as a Novel Strategy in the Approach of Cancer Containment…….19

2.9 Nutrition and Cancer Problems…….…….…….19

2.10 Colon Cancer…….…..20

2.10.1 The Colon……..…….....…….……...20

2.10.2 Carcinogenesis of Colon Carcinoma ……..……..….22

2.10.3 Histologic Characteristics of Colon Carcinoma……...…..….22

2.11 Colon Cancer and Dietary Implications……...…..…….…….22

2.11.1 Dietary factors: Cancer Promoters……...……...…….…….….23

2.11.2 Dietary factors: Anti-Promoters……..…….…..….23

2.11.3 Other Etiological Factors of Colon Cancer…….……..…24.

2.11.4 Diagnosis Colon Cancer…….……..…..…24

2.11.5 Biomarkers in Colorectal Cancer…….…….……....25

2.12 Nutritional Manipulation as a Chemoprevention of Cancer…….25

CHAPTER THREE

3.0 MATERIALS AND METHODS……..…….…….……27

3.1 Materials……...…….…….....……27

3.1.1 Chemical and Reagents…….……..27

3.1.2 Equipment and Apparatus…….……...27

3.1.3 Plants collection…..……...….……28

3.2 Methods……..……..…….....…….……28

3.2.1 Sample collection and preparation……....…….... ……..……28

3.2.2 Experimental diet ……..…….……...…...……..…..…28

3.2.3 Experimental animals/grouping…….…..…..……...……28

3.2.4 MNU exposure and feeding protocols…..…….……..……29

3.2.5 Record of body weights……..…..……30

3.2.6 Record of feed intake……...……..…….…30

3.2.7 Tissue collection……...30

3.2.8 Histopatological analysis……...……..31

3.2.9 Analysis of carcinoembryonic antigen (CEA) ……..…….31

3.2.10 Analysis of endogenous antioxidants and
lipid Peroxidation…….…….....32

3.2.11 Determination of hematological parameters…….…...34

3.2.12Statistical analysis……34

3.2.13 Experimental design……35

CHAPTER FOUR

4.0 RESULT……..36

4.1 Carcinogenic Indices……..……36

4.1.1 CEA levels in sera of animals……..……..……..……36

4.1.2 Histopathological Result…….……39

4.3 Activities of Antioxidant Enzymes…….……...…….……..……42

4.3.1 Catalase Activity in Liver…….…….……...…….…….……44

4.3.2 Catalase Activity in Kidney…...……..…..……..44

4.3.3 Superoxide Dismutase (SOD) Activity In liver……..…..……..…….……...…..46

4.3.4 Superoxide Dismutase (SOD) Activity in kidney…….……..……....…...48

4.4 Lipid Peroxidation Index……..50

4.4.1 Thiobarbituric Reactive Substances (TBARS)
levels in liver……...……....…….…..……..…….……50

4.4.2 Thiobarbituric Reactive Substances (TBARS)
levelsin kidney……..…….…….....…..……..……53

4.5 Total Protein……...……..……..55

4.5.1 Total Protein Concentration in liver……...…….…….……..…55

4.5.2 Total Protein Concentration in kidney……..……...…….……57

4.7 Hematological Parameters…….…….....59

CHAPTER FIVE

5.0 DISCUSSION……..……..….66

CHAPTER SIX

6.0 CONLUSION AND RECOMMENDATION…….……74

6.1 Summary……..…….....……74

6.2 Conclusion……...…….……..…76

6.3 Recommendation……..……...……..…78

REFERENCES……...…...……79

APPENDICIES……...…….……89

LIST OF TABLES

Table **Title** **Page**

4.1 Some hematological parameters of animals fed with diets supplemented With various levels of *Vernonia amygdalina* and *Telfairia occidentalis*……..64

4.2 Some hematological parameters of animals fed with diets supplemented With various levels of *Vernonia amygdalina* and *Telfairia occidentalis* Cont'd…….65

LIST OF FIGURES

Figure	Title	Page
2.3	Chemical Structure of N-Methyl-N-Nitrosourea	17
3.0	Experimental design	35
4.2	CEA concentration in serum of animals fed with diets of varying levels of supplementation of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	38
4.2	Catalase activity in liver of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	43
4.3	Catalase activity in kidney of animals fed with diets supplemented with varying levels of with *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	45
4.4	Superoxide dismutase activity in liver of animals fed with diet Supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	47
4.5	Superoxide dismutase activity in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	49
4.6	TBARS Levels in liver of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	52
4.7	TBARS Levels in kidney of animals fed with diets supplementated with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	54
4.8	Total Protein concentration in liver of subjects fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	56
4.9	Total Protein levels in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU	58

LIST OF PLATES

PLATE	TITLE	PAGE
Plate I	*Vernonia amygdalina* shrub showing leaves…….…….9	
Plate II	*Telfairia occidentalis* plant showing fresh leaves and flower …….……12	
Plate III	Schematic diagram of the human colon……..…….……...21	
Plate IV	Internal view of animal (exposed to MNU and fed with 5% Vernonia amygdalina supplemented diet) bearing an extra-colonic tumour...……..…….....…….....……37	
Plate V	Photomicrographs of cross-section of colon of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and intra-rectally exposed to MNU…..….……..….40	
Plate VI	Photomicrographs of cross-section of colon of animals fed with diets supplemented with varying levels of *Telfairia occidentalis* and intra-rectally exposed to MNU…….…..……..41	

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
1.0	Carcinoembryonic antigen concentration in serum of animals fed with diets of varying levels of supplementation of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……..……..89	
2.0	Standard curve for CEA determination……..……...…90	
3.0	Catalase activity in liver and kidney of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……..…91	
4.0	Standard curve for determining catalase activity……..……...92	
5.0	Superoxide dismutase activity in liver and kidney of animals fed with diet Supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU…….93	
6.0	TBARS Levels in liver and kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……....94	
7.0	Total Protein concentration in liver of subjects fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……..95	
8.0	Total Protein levels in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……...…...…..96	
9.0	Standard curve for Total protein determination……...…….97	
10.0	Some Hematological parameters of animals fed with diets supplemented With various levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU……..…….……98	
11.0	Some Hematological parameters of animals fed with diets supplemented With various levels of *Vernonia amygdalina* and *Telfairia occidentalis* Cont'd. ……..……..……..……99	

LIST OF ABBREVIATIONS AND SYMBOLS

%: Percent

^{o}C: Degree Centigrade

CAT:Catalase

CEA: Carcinoembroynic antigen

EDTA: Ethylene diamine tetra acetate

ELISA: Enzyme Linked Immusorbent Assay

HCT: Hematocrit

Hep2G: Human hepatocellular carcinoma cell line

HGB: Hemoglobin

IC_{50}: Inhibitory Concentration

KRAS: Kristen Rat Sarcoma

LYM: Lymphocyte

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

mg: Milligram

ml: Milliliter

MPV: Mean Platelet Volume

NLR: Neutrophil- Lymphocyte Ratio

MNU: N-Methyl- N-Nitrosourea

NS: Normal saline

PI: Phosphatidyl Inositol

PLT: Platelet

PSA: Prostate Specific Antigen

r.p.m: Revolution per minute

RBC: Red blood cell

ROS: Reactive Oxygen Species

SOD:Superoxide dismutase

TBA: Thiobarbituric acid

TBARS: Thiobarbituric Reactive Substances

TO: *Telfaria occidentallis*

UVB: Ultraviolet-B

VA: *Vernonia amygdalina*

WBC: White blood cells

ABSTRACT

This study investigated and compared the effects of diets supplemented with 2.5%, 5% and 10% *Vernonia amygdalina* and *Telfairia occidentalis* leaves on male Wistar rats intra-rectally exposed to N-Methyl-N-Nitrosourea (MNU). Animals were distributed equally into ten groups. Test animals were fed with supplemented diet and exposed to MNU, while un-exposed animals and fed with normal diet or supplemented diet were designated control groups. Animals were fed with respective supplemented diets for six weeks prior to intra-rectal exposure to MNU or normal saline, as the case may be, for ten weeks. Histopatholgical conditions of various organs and hematological parameters as well as carcinoembryonic antigen (CEA) and some endogenous antioxidants' levels were determined. Groups exposed to MNU showed elevated levels in CEA compared with normal control group. Groups fed *Vernonia amygdalina* and *Telfairia occidentalis* supplemented diets showed a corresponding decrease in CEA levels with increasing supplementation with 5% supplementation with *Vernonia amygdalina* having the overall highest lowering effect. Histological condition of colon showed that intensity of necrosis increased as supplementation increased. Increasing supplementation with *Telfairia occidentalis* stimulated liver and kidney catalase significantly (p□0.05) but an inhibitory effect on liver Superoxide dismutase (SOD). Elevated liver SOD activity at 5% supplementation with *Vernonia amygdalina* was observed. 2.5% *Vernonia amygdalina* and *Telfairia occidentalis* significantly (p□0.05) decreased animals' liver TBARS while there was a significant (p□0.05) lowering effect at higher *Vernonia amygdalina* supplementation (5% and 10%) in kidney of animal. Hematological finding showed evident evasive attempts by immune system of MNU-exposed animals fed with 2.5% and 5% *Vernonia amygdalina*

and 2.5% and 10% *Telfairia occidentalis*. Elevated level of antioxidant enzymes in MNU controls apparently supports animals' adaptive response theory to oxidative stress to a potent carcinogen such as MNU. The foregoing proposes *Vernonia amygdalina* and *Telfairia occidentalis* as potential dietary prophylactic agents, though within therapeutic doses.

CHAPTER ONE

1.0 INTRODUCTION

Daily exposure to different varieties of xenobiotic substances, mostly of dietary origin, have been established to possess cancer initiating or promoting ability. Among the various forms of gastrointestinal malignancy colon cancer is the commonest. A variety of studies have shown that colon cancers stem from nutritional traditions (Michael, 2014) and many foods, beverages and their components influence the risk of developing colon cancer (Doyle, 2007). Epidemiological studies have suggested a positive association between dietary fat and colon cancer. Colon cancer is a major cause of morbidity and mortality throughout the world (Evropi, et al, 2007).

Colon cancer continues to be one of the predominant form of cancer in the world and it is the third most common form of cancer in the world. An estimated 1.24 million cases were diagnosed with colorectal cancer in 2008 accounting for 10% of total cancer cases. Also in 2008, out of a total of 241 cases of colon carcinoma reported in Nigeria, 144 cases (60%) were males and 96 cases (40%) were females (Ibrahim et al, 2011). Colorectal cancer incidence and mortality rates are highest in African American men and women and Incidence rates among non-Hispanic blacks per 100,000 people living in the United States were 63.8 for men and 47.6 for women. Mortality in 2013 were 29.4 for men and 19.4 for women. It was predicted that an estimated 26,270 men and 24,040 women will die from colorectal cancer in 2014. Incidence rates was about 25% higher and mortality rates 50% higher than those in whites. Incidence and mortality rates among other major racial/ethnic groups are lower than those among whites. (American cancer Society, 2014)

This was estimated to be responsible for 694,000 deaths in 2012 making it the fourth most common cause of cancer death worldwide. The incidence of colorectal carcinoma has been on the increase in the developing countries, including Nigeria. This is as a result of change in diet and adoption of western lifestyle. The surge in the incidence reported in the last 5 years calls for a pragmatic action in its control (Ibrahim et al, 2011).

Early history of cancer research dates as far back as recorded developments in biology. Each stage of development in biology have contributed to a better explanation of some puzzling phenomena discovered by people doing cancer research (Cairns, 1978). Much of the previous knowledge of human cancer was derived from epidemiological studies. One approach to a more detailed analysis of the apparent process of carcinogenesis has been to develop experimental cancer condition in animals. Another way has been to study action of known carcinogens upon simple organisms like bacteria. Out of this has come a simple way of testing various agents for their probable carcinogenicity. (Cairns, 1978).

A notable event in the history of cancer research was the discovery by Lacassagne in 1932 that male mice cancer of the breast might follow the injection of oestrogenic hormones. This became the starting point of new researches undertaken chiefly by Cramer and Horning directed to measure the influence of hormones, both in the initiation and control of neoplasia (Cuthbert, 1964).

At present, the prospects for finding a quick solution to the cancer problem seem rather slight for most varieties of cancer. Prevention seems to be a feasible alternative to cure.

This is only possible if individuals choose to eat diets rich in fibre and antioxidants which are known to have cancer preventive abilities.

A family history of adenomatous polyposis or advancing age remains the greatest risk factors favoring the development of Colon cancer. Adenomatous polyps are tumours of malignant or benign neoplastic epithelium that are tubular and papilliary, and which may be predunculated attached by a narrow base to a long stalk, or sessile, attached by a broad base with little or no stalk. (Sleisenger et al, 1998).

Growing attention is being focused on food compoents with potential cancer inhibiting effects, in the hope of identifying chemo preventive diets or dietary supplements for human use. Among potentially chemo preventive food components, polyphenolic flavonoids, characterized by hydroxylated aromatic rings that are ubiquitously present in foods of plant origin, have long been recognized to possess anti-carcinogenic properties (Mutoh et al, 2000, Atawodi, 2011; Asuku et al. 2012).

Polyphenols present in food have been demonstrated to decrease various types of experimental carcinogenesis (Atawodi et al, 2010). In recent years, identification of effective chemo preventive polyphenols in diets or dietary supplements for human use is of much interest. Treatments with such polyphenols result in cell cycle arrest, thereby reducing the growth and proliferation of cancerous cells through apoptosis or programmed cell death. (Clement, 2013)

The evidence for a protective effect of greater vegetable and fruit consumption and wine is consistent for cancers of the stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas, and colon.

A list of possible protective elements in fruits and vegetables includes: dithiolthiones, isothiocyanates, indole-32-carbinol, allium compounds, isoflavones, protease inhibitors, saponins, phytosterols, inositol hexaphosphate, vitamin C, D-limonene, lutein, folic acid, beta carotene (and other carotenoids), lycopene, selenium, vitamin E, flavonoids, and dietary fiber.

1.1 Statement of Research Problem

i. Although infrequent in developing countries relative to developed nations of world, increasing incidence of colon cancer with high morbidity and mortality especially among blacks gives a cause for concern.

ii. Multimodal treatment strategies involving surgery, chemotherapy and radiotherapy have been explored to improve survival. However, chemotherapy is becoming an unpopular alternative due to the resistance of tumour cells to anticancer drugs besides its attendant undesirable cytotoxicity (Liu, et al 2014).

iii. Colonoscopies have always had a bad reputation for being an unpleasant, painful embarrassing physical diagnostic procedure that patients have had to undergo, (Lucio et al, 2014).

iv. Cost of cancer treatment continues to be a burden to governments and sufferers, especially in developing countries.

1.2 Justification for Research

Cancer chemoprevention is a rapidly evolving field that increases our understanding of the biology of carcinogenesis and also an effective strategy in the management of cancer (Steward and Brown, 2013).

In recent years growing attention is being focused on food components with potential cancer inhibiting effects, in the hope of identifying chemo preventive diets or dietary supplements for human use. More research also needs to be carried out to support or clarify existing claims (conflicting or corroborating) on how different diets influence the risk of colon cancer. Also the search for alternative, but yet cheaper and less traumatic therapeutic approaches, to treating and mitigating the incidence of colon cancer are still being explored. *Vernonia amygdalina* and *Telfairia occidentalis* are widely consumed as food especially by most West Africans. If proven to be efficacious, these plants can be easily adopted and therapeutically exploited as components of diets as these plants are easily cultivated and are available all year.

1.3 Aim and Objectives

1.3.1 General aim

The aim of this work is to investigate the effects of *Vernonia amygdalina* and *Telfairia occidentalis* leaves on male Wistar rats intra-rectally exposed to N-Methyl-N-Nitrosourea.

1.3.2 Specific objectives

i. To determine the relationship between levels of endogenous antioxidants and state of colon in animals exposed to N-Methyl-N-Nitrosourea (MNU).

ii. To determine the effect of respective diet supplementation and MNU on hematological parameters.

iii. To compare the chemopreventive effects of *Vernonia amygdalina* and *Telfairia occidentalis* leaves supplemented diet on colon of Wistar rats exposed to N-Methyl-N-Nitrosourea

1.4 RESEARCH HYPOTHESIS: NULL HYPOTHESIS

It was initially assumed that *Vernonia amygdalina* and *Telfairia occidentalis* has no effect on colon exposed to N-Methyl-N-Nitrosourea

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Vernonia amygdalina* Del.

2.1.1 General description of *Vernonia amygdalina*

Vernonia amygdalina is a member of the Asteraceae family. It is a shrub or small tree that can reach up to 10 m high with trunk diameter reaching up to 40cm. The bark is grey to brown, smooth, becoming fissured and when young is densely pubescent. The leaves are alternate, simple, with petiole measuring 0.2 to 4 cm long. The leaf blade is ovate-elliptical to lanceolate and measures 4 – 15 cm by 1 – 4 cm, cuneate or rounded at base, shortly acuminated at the apex, margin minutely toothed to coarsely serrate, finely pubescent but often glabrescent. The inflorescence is in the form of a head, arranged in terminal, compound, umbel-like cymes. Head stalk is a measure of about 1 cm long, pubescent. The flowers are bisexual, regular, 5-merous, strongly extended out from the involucres which are cylindrical to broadly ellipsoid. The fruit is a 10-ribbed achene measuring 1.5 – 3.5 mm long, pubescent and glandular, brawn to black, crowned by a much longer pappus bristles (Grubben, 2004). It thrives well in tropical Africa and found distributed in savannah and forest-margins, often forming thickets, from Guinea to Western Cameroons and Fernando Po.

Though commonly called bitter leaf in Nigeria, it is known as Chusar doki in Hausa, òlúgbù in Igbo, ewúrò in Yoruba and àtídòt in Ibibio. In Berom it is called etulùp, óríwò in Edo and kaɗkaɗɗe in Fula-Fulfulde.

Vernonia amygdalina has a very bitter taste which can be reduced by proper boiling, or in the young leaves by soaking in several changes of water.

2.1.2 Uses of *Vernonia amygdalina*

Vernonia amygdalina is often cultivated for its utility. Its leaves or products are items known for their commercial, medicinal and dietary value.

The leaves are taken in Nigeria as an appetizer and digestive tonic (Singha, 1965,). The Hausa of northern Nigeria prepare a special food called fatefate or mayemaye from the leaves with butter and condiments. Also, feed containing the leaves are given to horses as tonic.

When it comes to herbal therapy, it is reported to be of immense importance because of its potency to cure various diseases. Research have shown that regular consumption of *Vernonia amygdalina* could cure diverse ailment such as cancer, liver damage, drug resistant microbial infections, constipation, fever, high blood pressure and inhibits breast cancer (Clement et al , 2013). Others include arthritis, stroke, diabetes, skin infection stomach ache, pneumonia, insomnia, loss of memory. It is also known to promote safe child birth and useful in reducing body weight.



PLATE I: *Vernonia amygdalina* shrub showing leaves (Source:www. nigerianfoodtv.com)

2.1.3 Phytochemical constituents of *Vernonia amygdalina*

Several plant species of West African origin have been screened to identify classes of phyto- chemicals contained in them. Among the various classes of phytochemicals, flavonoids and Phenols were the most abundant. Leaves of *Vernonia amygdalina* have been investigated and found to have Vernonioiside B and Myricetin which are majorly flavonolic compounds. A quantitative analysis also gave a total phenolic content of about 3.01mg/g of dry matter (Gordian et al, 2007). Other phytochemicals also identified include vernodaline; vernomygdin; vernodalol; vernolide; hydroxyvernolide; tannins; vitamin C; luteolin; luteolin 7-O-b-glucoronide; leteolin 7-O-b-glucoside; vernonioside D and E; vernolepin; vernonioside A1 – A4; vernonioside B1 – B3; vernodalinol; alpha-muurolol; 1,5-dicaffeoyl-quinic acid, dicaffeoyl-quinic acid, chlorogenic acid and luteolin-7-O-glucoside. (Mueller and Mechler 2005, Kupchan et al, 1969; Laekeman et al , 1983; Koshimizu et al, 1994; Luo et al, 2011; Ogunbinu et al, 2009)

2.2 *Telfairia occidentalis* hook f.

2.2.1 General description of *Telfairia occidentalis*

This is a dioecious drought tolerant perennial tropical vine grown in West Africa. It can attain up to l000ft (305meters) in length and its leaves are pedately 3-5 foliolate with shortly stalked leaflets and branched tendrils. The plant's fruit is a large fluted gourd reported to be 16-105 centimeters in length, and an average of 9 centimeters in diameter, in an orange coloured pulp. (Okoli and and Mgbeogu, 1983). *Telfairia occidentalis* flowers

grow in sets of five, with creamy-white and dark red petals, contrasting with the light green colour of the fruit when young, and yellow when ripe. (Okoli, and Mgbeogu, 1983). Dioecious flowering is most common in the fluted gourd, with very few documented cases of monoecious flowering. It is popularly known as *ugu* in igbo, *iroko* in Yoruba, and *ubong* in Efik.

2.2.2 Uses of *Telfairia occidentalis*

Telfairia occidentalis is a tropical vine grown in West Africa for its edible vegetable leaf and seeds. Apart from being used as food, it is reported to have medicinal applications. Lately, the popularity has spanned across other ethnic groups, thereby taking the medicinal and commercial value of the vegetable to a higher level.

Leaf extract pure or mixed with eggs, is drunk to treat anaemia. The infusion of the leaves is given as blood tonic to help convalescents and also help in treating malaria and loss of appetite. (Okoli and Mgbeogu,1983)

Apart from its highly balanced nutritive contents, seed extracts have been reported to have hypoglycemic as well as hypolipidemic effects on experimental rats (Eseyin, et al, 2007; Adaramoye et al, 2007). The leaf extracts on the other hand had demonstrated antimicrobial activities on *Salmonella typhi, Salmonella fecalis* and *Escherichia coli* suggesting that it has antibiotic potential. (Oyewole and Abalaka, 2012).



PLATE II: *Telfairia occidentalis* plant showing fresh leaves and flower (Source: TopTropicals, http://toptropicals.com/pics/garden/09/h3/P4250571.jpg)

2.2.3 Phytochemical composition of *Telfairia occidentalis* Hook F.

Previous quantitative phytochemical analysis have shown the presence of Alkaloids, tanins, flavonoids and phenols with an average total phenolic content of 2.5mg/g dry matter. The main flavonoid found to be abundant is Isorhamnetin which is a Biflavonyl (Gordian, et al, 2007)

2.3 Cancer: Biology and Various Forms

Cancer is a complicated disease that arises from an interplay of several biochemical lesions. Though very variable in its presentation, development and outcome from one patient to the other, it is often characterized by indefinite neoplastic growth. Symptoms range from immunologic complications to weight loss. The under lying mechanism of disease stems from genetic aberrations of susceptibility genes of either or both somatic and germinal cells. The major genes susceptible to aberrations are regulatory in nature and they include: the oncogenes and tumour suppressor genes (anti-oncogenes). Oncogenes are mutated or activated proto-oncogenes. They are virus-associated genes possessed by potential cancer patients or by individuals with tumour cells. Presence of mutated proto-oncogenes are largely responsible for the initiation stage of carcinogenesis. They have cell transforming capability and can confer multiple and complex phenotypic alterations. For example, a mutating proto-oncogene called Kristen Rat Sarcoma (KRAS), turns a small protein located just inside the cell membrane into an amplifier of cell growth signalling. When the KRAS gene is mutant, the corresponding protein behaves like a switch locked in the “on” position generating a permanent cell division signal. This leads to an over expression of

critical gene products such as proteins needed for cell growth. Mutant KRAS are common in many cancers, such as colorectal cancers (in 30–40% of the cases) or adenocarcinomas of the lung (in 20–30% of the cases). Tumour suppressor genes found in normal cells are responsible for the negative growth regulation by activating apoptic signals. However when the regulatory region of this gene becomes mutated, apoptic signals are lost thereby permitting an indefinite cell growth. This level of dysregulation is responsible for the progression stage of carcinogenesis in the presence of promoters such as phorbol esters, lipid metabolites and carcinogenic stimuli, leading to an accelerated development of a heterogeneous population of mass of cells. (Trosko et al 1995; UNSCEAR 1993).

The type of cancer developed depends on the part of the body where initiation began and the stage of carcinogenesis. Parts of the body often commonly exposed to promoters include epithelial tissues. All carcinomas are of epithelial origin. Some cases of epithelial origin include the bladder, mammary gland, reproductive, respiratory and urinary tracts as well as skin and gastro intestinal tracts.

2.4 Early History of Cancer Research

Biology and cancer have developed together. Invariably at each stage, the characteristics of cancer cells have been ascribed to some defect in whatever branch of biology happens at the time to be fashionable and exciting. Today, molecular genetics appears to be demystifying carcinogenesis giving credit to earlier and useful fundamental discoveries in the field cell biology (Cairns, 1978).

One of the great landmarks in the history of cancer research was the work of Kennaway and his colleagues at the Chester Beatty Research Institute, which culminated in 1930 in the

preparation of the first chemically pure carcinogens. The discovery and synthesis of chemical substances which will produce cancer had a profound influence on the course of cancer research. It demonstrated for the first time that malignant tumours exactly similar to human cancer could be Induced by the application of pure chemical compounds of known molecular structure to animals. This went further to help elucidate the nature of chemical carcinogens.

The era of cancer chemotherapy began in the 1940s when the Germans introduced a chemical warfare agent called mustard gas during World War I. Nitrogen mustard gas and folic acid antagonist drugs where the first to be used to treat malignancies from exposure to the gas. Cancer drug development has exploded since then into a multi-billion dollar industry. Though these interventions were celebrated, many of the principles and limitations of chemotherapy discovered by the early researchers still apply.

2.5 Experimental Cancer Research

In animal studies, cancer is directly induced in test animals using a large sample of animals, usually of two or more species with varying dose and time parameters. Experiments with animals are based on the premise that chemicals that produce cancer in animals will have similar effects on human cells. Most known human carcinogens have been documented. (www.usmra.com/repository/category/.../Chemical_Carcinogens_1.ppt)

2.6 Carcinogens

A carcinogen is a physical, chemical or biological agent known to be directly involved in causing cancer. Their toxicity occur on cellular DNA causing heritable aberrations on oncogenes or tumour suppressor genes after repeated and long-term exposure.

There are many well-known examples of chemicals that can cause cancer in humans. Various classes of human chemical carcinogens classified based on chemical structure have been documented by the U.S 13th report on carcinogens (2011) by the U.S National Toxicology Center, Department of Health Centre (http://ntp.niehs.nih.gov/go/roc12). Some example of carcinogens are given below:

- **Epoxides:** Ethylene oxide, Propylene oxide
- **Organohalogen compounds:** Hydrazine(and salts), 1,2 Dimethylhydrazine
- **Aromatic Amines:** Benzidine, Aniline, o-Anisidine, o-Anisidine
- **N-Nitroso compounds:** N-Nitrosodimethylamine, N-Methyl-N-Nitrosourea, *N*-Nitrosodi-*n*-butylamine, *N*- Methyl-*N′*-Nitro-Nitrosoguanidine.
- **Miscellaneous organic compounds:** Formaldehyde, Acetaldehyde, 1,4-Dioxane.
- **Miscellaneous inorganic compounds:** Arsenic and compounds, Chromium and compound, Thorium dioxide, Beryllium and compounds, Cadmium and compounds, Lead and compounds, Ethyl carbamate, 2-Nitropropane, Thiourea.

The fumes of the metals cadmium, nickel, and chromium are known to cause lung cancer. Vinyl chloride causes liver sarcomas. Exposure to arsenic increases the risk of skin and lung cancer. Leukemia can result from chemically Induced changes in bone marrow from exposure to benzene and cyclophosphamide, among other toxicants. Other chemicals, including benzo[a]pyrene and ethylene dibromide, are considered to be probably

carcinogenic in humans because they are potent carcinogens in animals. Chemically-Induced cancer generally develops many years after exposure to a toxic agent.

2.7 Nitroso Compounds

These are a class of organic compounds having molecular structures in which the nitroso group (-N=O) is attached to a carbon or nitrogen atom. Substances in which this group is attached to an oxygen atom are called nitrates, that is, esters of nitrous acid; those in which the nitroso group is attached to a metal ion are called nitrosyls.

2.7.1 N-Methyl-N-Nitrosourea

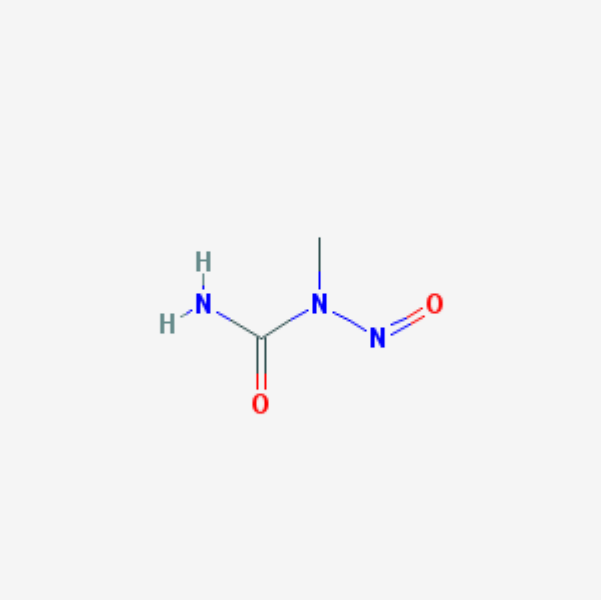


Figure 2.3 : Chemical Structure of N-Methyl-N-Nitrosourea

Also known as N-Nitroso-N-Methylurea, it is a member of Nitroso compounds with molecular weight of about 103.8g/mol and also with alkylating, carinogenic and mutagenic properties.

Administration of N-Methyl-N-Nitrosourea by intratracheal instillation caused cancer of the nasopharyngeal tube, pharynx, larynx, bronchi, esophagus, forestomach, and trachea (epidermoid or large-cell anaplastic carcinoma) in hamsters. Intrarectal administration have been known to cause benign or malignant colon tumours (adenoma, adenocarcinoma, or squamous-cell carcinoma) in male rats and female mice and guinea pigs.(Adamson and Sieber 1979, Zimmerman et al. 1982, Eisenberg et al. 1983, Likhachey et al. 1983, Smulow et al. 1983, Yano et al. 1984, Wang et al. 1985, Verdeal et al. 1986, Beland et al. 1988, Bosland et al. 1992, Furukawa et al. 1992, Tatematsu et al. 1992, 1993, 1994, 1998, Lijinsky and Kovatch 1996, Tamano et al. 1996, Yang et al. 1996, IARC 1972, 1978).

2.7.2 Mechanism of action of Nitroso compounds

There is ample evidence from studies in experimental animals that carcinogenicity of N-nitroso compounds arises from the metabolic conversion of the compound into potent electrophilic alkylating agents. These reactive intermediates are formed by spontaneous decomposition in the case of nitrosoureas and related compounds, or by metabolic activation in the case of N-nitrosamines. The electrophiles subsequently react with DNA of target tissues to form altered bases which leads to the initiation of carcinogenesis. Experimental evidence shows that the biological activity of N-nitroso compounds in experimental animals is similar to that of humans (Archer, 1989)

Previous study has also shown that treating Hep2G (human hepatocellular carcinoma cell line) cells with multiple N-nitrosamines cause cell damage and affects the detoxification mechanisms of the cell. These characteristics may be due to lower detoxification enzymes and an increase in oxidative stress (H_2O_2 production), leading to cell death by apoptosis (at low doses) or necrosis (at high doses) (Patterson et al, 2012)

2.8 Chemoprevention as a Novel Strategy in the Approach to Cancer Containment

At present, the prospects for finding a quick solution to the cancer problem seem rather slight. For most varieties of cancer, the existing method of treatment are not very successful and clear cut improvements seem elusive.

The credibility of chemoprevention as a serious practical approach to the control of cancer has been greatly enhanced by the results of three randomized trials in the field of breast cancer. Three different agents, namely tamoxifen, raloxifene and 4-hydroxyphenylretinamide (Fenretinide) have been shown to be effective agents for prevention of breast cancer in women of varying degree of risk (Fischer et al, 1998; Cummings et al 1999; Veronesi, 1999)

2.9 Nutrition and Cancer Problems

Epidemiological studies on animals strongly supports the role of nutrition in cancer causation. However, there is clearly a need for major research emphasis to further define the role of dietary components as determinants of carcinogenesis in man. For example, in experimental animals, deficiency of dietary retinoids enhanced susceptibility to chemical carcinogenesis (Smith et al, 1975). Lipids have been implicated in cancer as their metabolites have been shown to be promoters of carcinogenesis. (Trosko et al 1995, UNSCEAR 1993).

Further evidences from experiments have suggested a positive association between dietary fat and colon cancer. Furthermore, animal model studies have also shown that high fat and

low fiber diet has a colon tumour–promoting effect. It has been estimated that 30-40 percent of all cancers can be prevented by lifestyle and dietary measures alone. In addition, frequent dietary components such as serine and glycine which provide precursors for proteins, nucleic acids and lipids, have been implicated in tumour homeostasis by modulating cellular antioxidant capacity. This findings provides a translational window for drug development, dietary intervention, and biomarker identification in order to provide targeted anti-metabolic therapy (Ivano et al, 2014).

2.10 Colon Cancer

2.10.1 The Colon

The colon is the third-to-last section of the gastrointestinal tract in humans, followed by the rectum and anus. Food is mostly digested by the time it reaches the colon, so the role of this segment of the large bowel is to absorb water, some short chain fatty acids from plant fibre and undigested starch, sodium, and chloride. Compact waste retained are eliminated during defecation. Moreover, colonic bacteria play a central role in metabolic detoxification by secreting chemicals that encourage excretion of toxins and pathogens. Beneficial bacteria in the colon (probiotics) also ferment dietary fibre and generate compounds, such as butyrate, which nourish cells in the colon wall and protect against carcinogenesis.

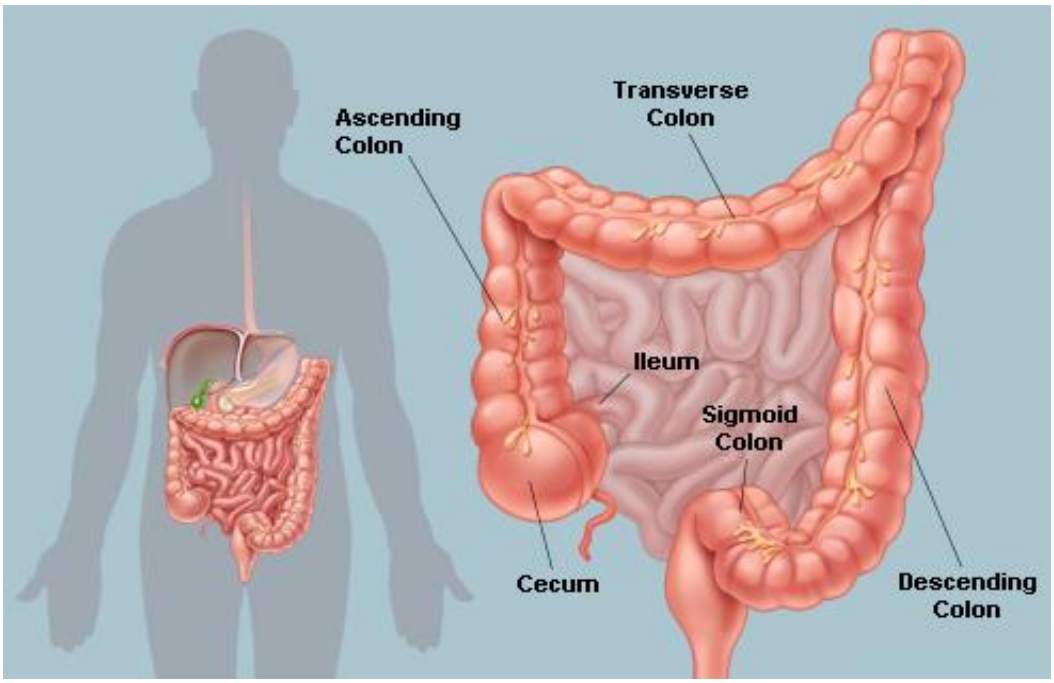


PLATE III: Schematic diagram of the human colon (Source: WebMD image collection: Human Anatomy, http://www.webmd.com/digestive-disorders/picture-of-the-colon)

2.10.2 Carcinogenesis of colon carcinoma

Most colon cancers are adenocarcinomas (malignant epithelial tumour of glandular nature i.e tubular, papillary, etc). First epidemiologic observations indicate that adenoma prevalence within a population as well as the multiplicity of adenomas per person, geographically parallels the prevalence of colon cancer (Sleisenger et al, 1998). Polyps are a risk factor for colon cancer, particularly if they are adenomatous in type. A polyp is a discrete mass of tissue that protrudes into the lumen of the bowel. Like most epithelial cancer pathologies, when the polyp has progressed through the inner lining of the colon to the sub-mucosa. The developmental stages of colon cancer beginning with initiation all require additional mutations to promote and progress to malignant colon cancer (Sleisenger et al, 1998)

2.10.3 Histologic characteristics of colon carcinoma

Cellular differentiation and renewal, resulting in hypercellularity or colonic crypts with cells possessing varying high amounts of mucin and hyperchromatic, elongated nuclei in a picket-fence pattern are some of the histologic characteristics of adenomatous epithelium.

2.11 Colon Cancer and Dietary Implications

Although there is clear evidence of genetic predisposition to colon cancer, much of this variation appears to be related to differences in dietary habits (John, 1996). Works on dietary intervention in cancer using whole plants and or specific plant components have been done. For example an approach for the chemoprevention of various forms of epithelial cancer during the period of pre-neoplasia was reported with the finding that vitamin A and

its synthetic analogues- the retinoids, are potent agents for control of cell differentiation in several epithelial tissues (Sporn, 1976). Conversely, work on Vitamin A on experimental lung cancer showed no protective and adverse effect (Smith et al, 1975).

2.11.1 Dietary factors: cancer promoters

Unlike carcinogens which initiate cancers, some dietary components may accelerate cancers that have already begun to develop. Studies have shown that certain dietary fats eaten in excess may promote cancer, In part by contributing to obesity. More specifically, linoleic acid, the omega-3-fatty acids from fresh oils appear to delay cancer development. (Eleanor, et al 2001)

2.11.2 Dietary factors: anti-Promoters

Epidemiological studies find a link between eating plenty vegetable and low incidence of cancer. The fibre in fruits and vegetables help to protect particularly against colon cancer by speeding up the transit time of all materials through the gastrointestinal tract so that the colon walls are not exposed to cancer causing substances for long. (Eleanor et al, 2001). In addition, Polyphenols present in food have been demonstrated to decrease various types of experimental carcinogenesis (Atawodi et al 2010). In recent years, identification of effective chemo preventive polyphenols in diets or dietary supplements for human use is of much interest. Treatments with such polyphenols result in cell cycle arrest, thereby reducing the growth and proliferation of cancerous cells through apoptosis or programmed cell death.

In addition, frequent dietary components such as serine and glycine which provide precursors for proteins, nucleic acids and lipids, have been implicated in tumour

homeostasis by modulating cellular antioxidant capacity. This findings provides a translational window for drug development, dietary intervention, and biomarker identification in order to provide targeted anti-metabolic therapy (Ivano et al, 2014).

2.11.3 Other etiological factors of colon cancer

Other risk factors known to be associated with colorectal cancer include, smoking, level of physical activities, environmental promoters, hormonal problems, pH of colon, oxidative stress etc.

2.11.4 Diagnosis of colon cancer

The general diagnostic approach has been to identify an alteration that distinguishes colon cancer from normal colonic tissues and then determine how changes in polyps compare with these tissues. This has been achieved through a procedure called colonoscopy. Aneuploidy which may occur in up to half of colorectal cancer and is believed to be a marker of poor prognosis, has been found in approximately 5 to 15% of adenomas, particularly those of larger size with more villous component and with greater degree of dysplasia. Other studies have found that persons with familial polyposis or sporadic adenomatous polyps have circulating mononuclear leukocytes which are deficient in DNA repair synthesis, suggesting that these patients might be more susceptible to environmental mutagens. (Sleisenger et al, 1998).

2.11.5 Biomarkers in colorectal cancer

Major advances in scientific technology have provided an opportunity to investigate at molecular level, the pathobiology of polyp, providing experimental support for the adenoma-carcinoma hypothesis.

At the phenotypic level, several antigens have recently been recognized as cancer-associated antigens in the colorectum. Most of them are oncodevelopmental in nature one of which is the prototype carcinoembryonic antigen (CEA) expressed in variable amounts. Certain qualitative and quantitative alterations of DNA and circulating mononuclear leukocytes deficient in DNA repair synthesis have been noted in patients with adenomas. Radiolabelling of antibodies which may be directed at CEA or other cancer associated antigens, allows for both external scanning of metastatic sites and for intraoperative localization of tumour with the use of hand held Geiger counter (Einhorn, 1989). Monoclonal antibodies especially CA-19-9 and CA 50 may give additional information at diagnostic stages. An integrative approach could give a more precise diagnosis. Additional studies also of these markers are warranted in the future to enhance sensitivity and specificity of diagnosis (Luke et al, 1998) although early detection of limited disease results in reduction in mortality from 60% to 20% or less, this situation is likely to improve as new technologies and ideas emerge for diagnosis and treatment

2.12 Nutritional Manipulation as a Chemopreventive Therapy For Cancer

An exciting area has been opened with observation that nutritional manipulation in chemo preventive strategies per se may be an approach to mitigating certain cancer. For example,

an approach to chemoprevention of forms of caner also has been reported that Vitamin A and its synthetic drugs, the retinoids are potent against control of cell differentiation in several epithelial tissues (Sporn et al, 1976)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 Materials

3.1.1 Chemicals and reagents

All chemicals were of analytical grade. Chemicals used included N-Methyl-N-nitrosourea (MNU), 96 well Rat Carinoembryonic Antigen ELISA Kit (WKEA Med Supplies Corp, China) epinephrine, thiobarbituric acid (TBA), n-butanol, Sodium dodecyl sulphate, pyridine, acetic acid, Monosodium and Disodium phosphates, Potassium dichromate salt, Folin-Ciocalteu reagent, Sodium-Potassium tartarate, Copper II tetraoxosulphate VI pentahydrate, Sodium hydroxide, Sodium trioxocarbonate IV, Egg albumen, distilled water, Sodium chloride and 40% Formaldehyde (10% Formal-saline), tissue paper, cotton wool, graded alcohol, xylene, paraffin, eosin, hematoxylin among others ,all procured from reputable chemical company.

3.1.2 Equipment and apparatus

The required equipment included, dissecting kit, centrifuge(Labofuge 300,Heraeus) mortar and pestle, Jenway 6305 U.V/Visible spectrophotometer, automated Hematological analyser (MINDRAY BC 3200 Series), syringes, centrifuge tubes, EDTA containing blood collection tubes, plain blood collection tubes, Pasteur pipettes, Aluminum paper foil, 0-1000µl capacity Gilson® micropipettes and corresponding tips, cannula, microscope, hand gloves, electrical weighing balance, water troughs, cages, feeders, camera, water bath (Grant JB Series, Serial No:630312002) and thermometer.

3.1.3 Plants collection

Vernonia amygdalina and *Telfairia occidentalis* were obtained from Samaru, Zaira L.G of Kaduna state, identified and authenticated at the herbarium of the Department of Biological Science, A.B.U, Zaria.

3.2 Methods

3.2.1 Sample collection and preparation

Destalked leaves of *Vernonia amygdalina* and *Telfairia occidentalis* leaves were separately rinsed with cold water and shade dried in an airy environment, at room temperature (Bamishaiye et al, 2011). The dried samples were thereafter mechanically ground to fine powder and separately stored in air and water proof containers.

3.2.2 Experimental diet

Basal diet used which was a compounded grower's mash, of Vital® feed brand, bought in Samaru, Zaria was used in the entire study. The basal diet was supplemented with powdered *Vernonia amygdalina* and *Telfairia occidentalis* leaves in the following separate proportions: 2.5%, 5% and 10%.

3.2.3 Experimental animals/grouping

Weanling male Wistar rats of 4 weeks of age weighing 50-80g were obtained from Faculty of Verterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. They were quarantined and acclimatized for 2 weeks at the animal housing facility, Faculty of Pharmaceutical

Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were weighed weekly while they were allowed free access to food (basal diet) and clean water. Distribution and designation of subjects were as follows:

Group 1: Control (0% supplementation of feed + normal saline)

Group 2: Control (0% supplementation of feed + MNU)

Group 3: Test (MNU + 2.5% *Vernonia amygdalina* supplemented feed)

Group 4: Test (MNU + 5% *Vernonia amygdalina* supplemented feed)

Group 5: Test (MNU +10% *Vernonia amygdalina* supplemented feed)

Group 6: Suppemented diet control (10% *Vernonia amygdalina* supplemented feed + normal saline)

Group 7: Test (MNU+2.5% *Telfairia occcidentalis* supplemented feed)

Group 8: Test (MNU +5% *Telfairia occcidentalis* supplemented feed)

Group 9: Test (MNU +10% *Telfairia occcidentalis* supplemented feed).

Group 10: Supplemented diet control (10% *Telfairia occcidentalis* supplemented feed + normal saline)

3.2.4 MNU Exposure and feeding protocols

After six (6) weeks of feeding with respective diets, 0.2ml of 1% (w/v) freshly prepared MNU was administered intra-rectally (IR) for three times weekly for ten week in each

designated group. Concurrent daily feeding with respective diets continued during period of exposure to carcinogen. One week after stoppage of induction, the animals were sacrificed for collection of blood and organs for biochemical analyses and histological examination.

3.2.5 Record of body weights

A record of the weekly body weights of the animals in all the groups were kept during the experiment and finally before sacrifice. The animals were weighed at the commencement of the experiment, and subsequently once a week (Fereshteh et al, 2009; Chinthalapally et al, 2001). The weekly weighing was important for monitoring progressive weight changes in the animals as they respond to the effects of the carcinogen and feed. The results was compared among the animals across the groups.

3.2.6 Record of feed intake

A weekly record of respective feed intake for each group were taken by obtaining the weekly differences in weights of prepared bulk feed for each group.

3.2.7 Tissue collection

Upon completion of the experiment, the rats were sacrificed by decapitation at the 16th week after the first MNU administration. Blood were immediately collected in both EDTA containing tubes and normal blood collection tubes. Sera were obtained and refrigerated at -20^{o}C after centrifuging the normal blood at 3,500 r.p.m for 5minutes. The liver and kidneys were harvested and immediately weighed and portions fixed in 10% Formal-Saline solution for subsequent histopathological analysis. The colons were also harvested and immediately

dissected longitudinally, flushed with normal saline solution, mopped with a paper towel and fixed in a 10% Formal-Saline solution for 24-48 hours at 4°C before being transferred to 70% ethanol. The formalin-fixed tissues were processed, embedded in paraffin, sectioned and mounted onto glass slides. Ten percent (10%) w/v solution of liver and kidney homogenates was also prepared by homogenizing specific organ weights in corresponding buffer volumes, using mortar and pestle and then centrifuged at 3,500 r.p.m for 10 minutes. Supernatants were aspirated into homogenate bottles using clean Pasteur pipettes and kept frozen at -20^{o}C for subsequent analyses.

3.2.8 Histopatological analysis

Following 18-24 hours fixation (1mm/hour) in 10% formal-saline, the colons were dehydrated through ascending grades of alcohol (70%, 90%, 95% and absolute (100%) for 2 hours each. This was followed by clearing in xylene (to remove alcohol) (Balaiya et al, 2010*)* and the tissue was subsequently passed through a molten paraffin wax (impregnation) for 2 hours. The tissues were then immersed in a mold containing molten paraffin wax, which was allowed to solidify (embedding) while the tissue was inside. The tissue trapped in the wax was then trimmed for sectioning in a microtome machine at 3 µm. The sections were finally stained with the staining agents, haematoxylin and eosin, for microscopy (Fereshteh et al, 2009).

3.2.9 Analysis of carcinoembryonic antigen (CEA)

The level of the carcinoembyonic antigen was determined via enzyme-linked immuno sorbent assay (ELISA). The serum sample were added to the pre-coated microtitre plates, followed by the addition of the secondary antibody. The optical density of the

carcinoembryonic antigen were then determined spectrophotometrically using a micro plate reader.

3.2.10 Analysis of endogenous antioxidants and lipid peroxidation

3.2.10.1 Analysis for the Oxidative Stress Marker Malondialdehyde

The formation of TBARS was used as an index of lipid peroxidation according to the method described by Okhawa et al (1979).

Procedure:

The reaction mixtures contained 40µl of Sodium dodecyl sulphate (SDS) 1.5ml/300µl of acetate buffer (19.2% (v/v) stock) 300µl of 0.8% (w/v)TBA and pre-diluted homogenate sample. The mixture was heated in a boiling water bath at 95^0C for 60 mins and then cooled in ice-cold water. Two hundred microliters (200µl) of distilled water, 1.0ml of n-butanol/pyridine (15:1) mixture was added and the reaction mixture vortexed and centrifuged at 3500 rpm for 10 mins. The red (pink) organic layer (upper layer) formed was removed and its absorbance read spectrophotometrically at 532nm. The blank contained all components except the sample (homogenate). Concentration of MDA was calculated using the formular:

$C = (A/\varepsilon l) \times$ dilution factor

A= Absorbance of reaction mixture at 532nm

C= Concentration of TBARS in sample

$\varepsilon = 1.56 \times 10^5 M^{-1} cm^{-1}$ (Molar extinction coefficient of MDA)

l =Path length of cuvette

Values obtained were expressed as µmole per mg protein

3.2.10.2 Analysis for Superoxide Dismutase

The method descried by Misra and Fridovich (1972) and Anyan et al (2009) was adopted.

Procedure:

The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.2 ml of homogenate solution and 0.3 ml of epinephrine in 0.005M acetic acid was used to initiate the reaction. Change in absorbance was recorded at 1 min interval for five minutes (mins).The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.2 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. The slope of the linear part of graph of absorbance against time was used to obtain specific unit of activity which was expressed as U/min/ml per mg protein.

3.2.10.3 Analysis for Catalase

Catalase activity was determined according to the method described by Sinha (1979)

Procedure:

This was done by measuring the decrease in absorbance at 570nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer (Jenway 6305). The reaction mixture (1.32 ml) contained 0.130 ml of tissue homogenate in phosphate buffer (50 mM, pH 7.4) and 0.530 ml of 200 mM H_2O_2 in 0.660ml phosphate buffer pH 7.0. Change in absorbance was used as extent of decomposition of H_2O_2 extrapolated from a standard curve. Rate of

decomposition of catalase was used to obtain activity of catalase. Specific activity of catalase was expressed as milli moles of H_2O_2 decomposed per min/ ml /mg protein.

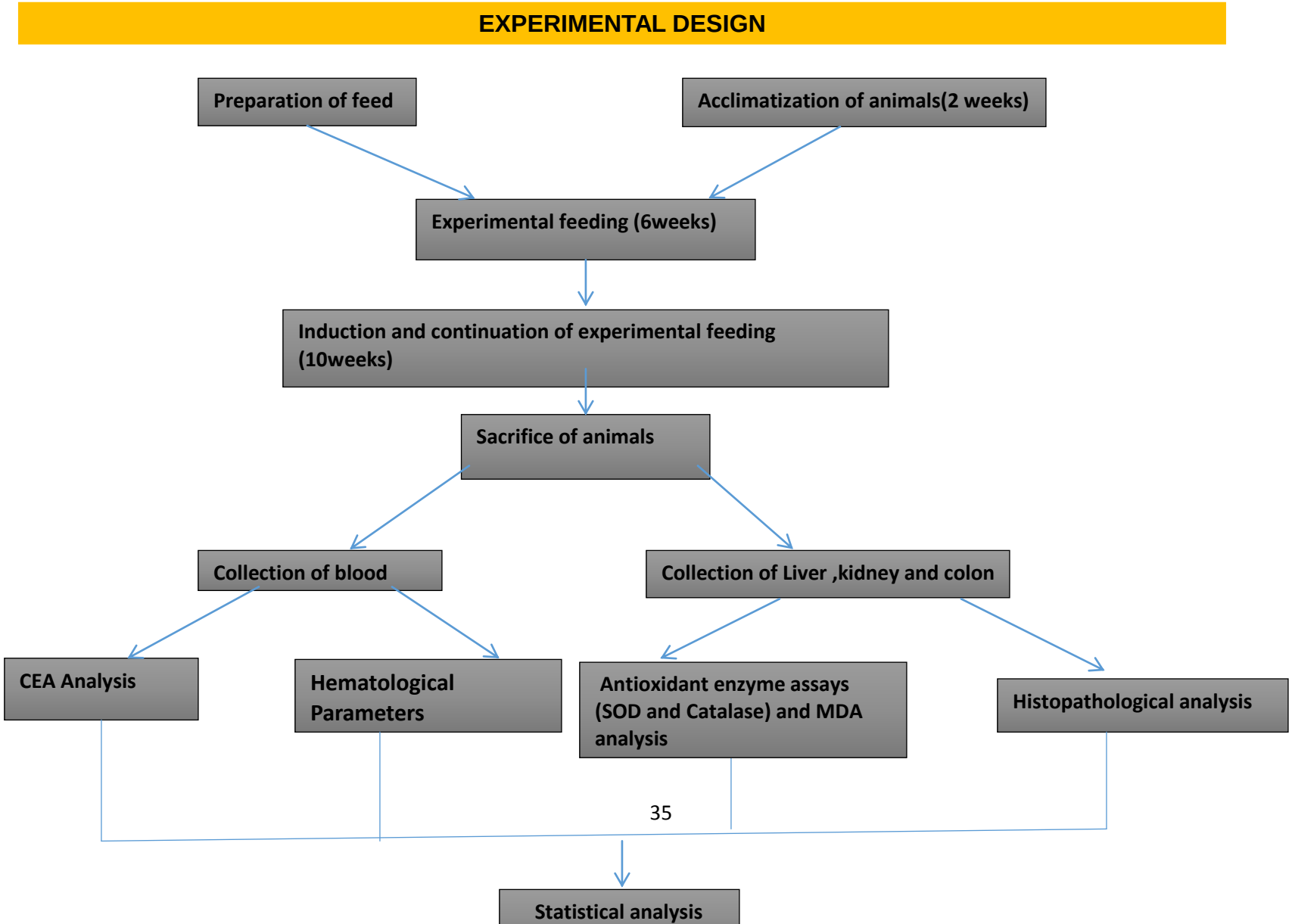
3.2.11 Determination of hematological parameters

The MINDRAY BC 3200 automated Hematological analyzer was used to analyze the hematological parameters like packed cell volume (PCV), white blood count (WBC), red blood count (RBC), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), HGB and PLT.

3.2.12 Statistical analysis

Data was analysed by the method of analysis of variance (ANOVA) using SPSS version 20.0 and results was expressed as mean ± standard deviation (SD) except where otherwise stated. Differences between the various animal groups fed with applicable diets were compared using the Duncan Multiple Range Test and P values less than 0.05 (P□0.05) was taken as significant.

3.3 EXPERIMENTAL DESIGN



CHAPTER FOUR

4.0 RESULTS

4.1 Carcinogenic Indices

4.1.1 CEA levels in sera of animals

Quantitative carcinoembryonic antigen (CEA) was determined. levels of the antigen is expected to be proportional to the stage of disease, and also quantitatively inversely proportional to the amount of anti-carcinogenic treatment administered. Results obtained however, shows a non-proportional relationship to mitigating interventions by *Vernonia amygdalina* (figure 4.1).Levels of CEA increased in sera of animals administered MNU and fed un-supplemented diet by about 41% compared to normal control. No significant ($p>0.05$) reduction in CEA levels was observed in 2.5% and 10% *Vernonia amygdalina* supplementation treated groups, when compared with the MNU control. However, 5% supplementation with *Vernonia amygdalina* showed a reduction of CEA levels by about 45% when compared with MNU control. (figure 4.1)

A significant ($p \square 0.05$) and proportionate decrease in CEA levels, by 15% and 35% respectively was observed in MNU-exposed experimental subjects when treated with, 5% and 10% *Telfairia occidentalis* supplementation respectively when compared with 2.5% supplementation.(figure4.1).



Plate IV:Internal parts of animal (exposed to MNU and Fed *Vernonia amygdalina* supplemented diet) bearing an extra colonic tumour

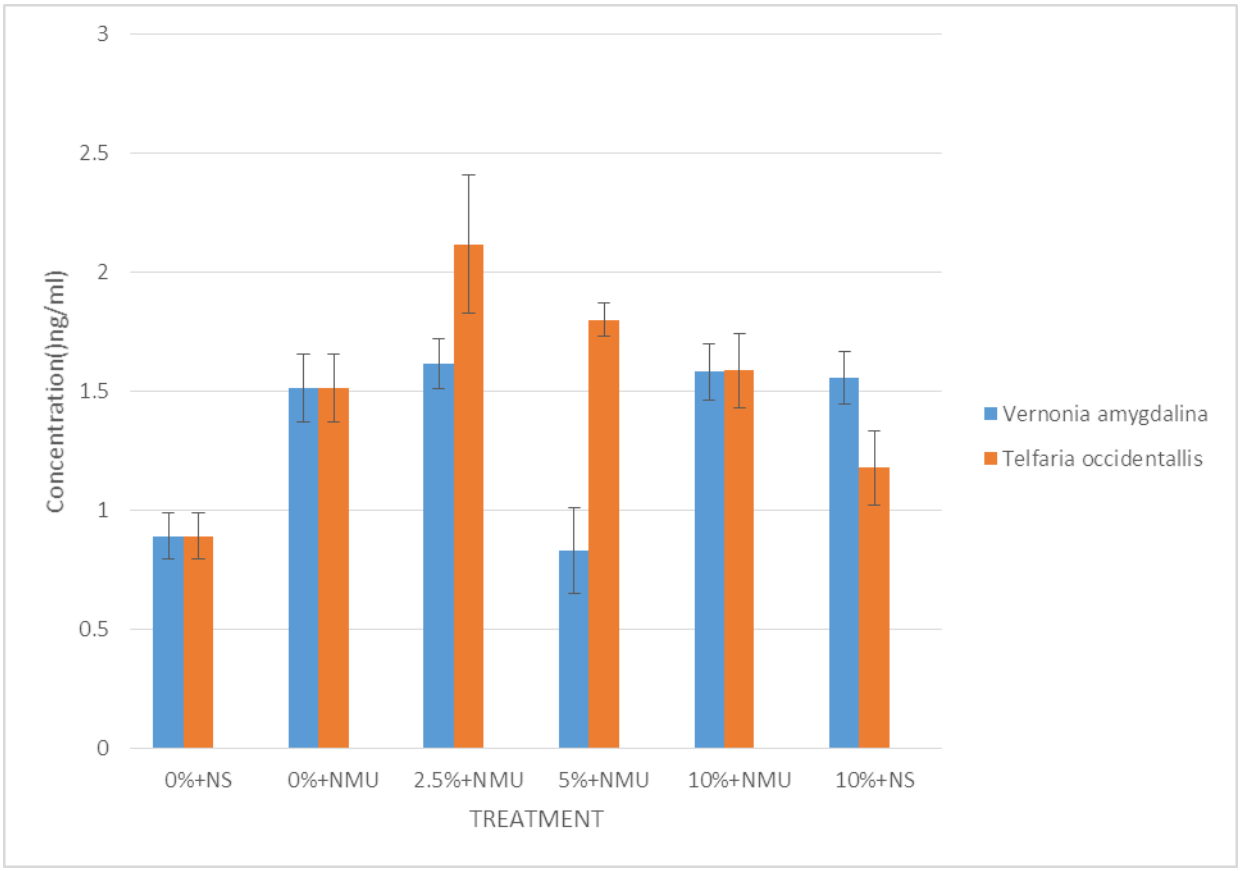


FIG. 4.1: Carcinoembryonic antigen (CEA) concentration in Serum of animals fed with diets supplemented with *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

MNU: N-Methyl-N-Nitrosourea; NS: Normal saline, n%: Level of supplementation

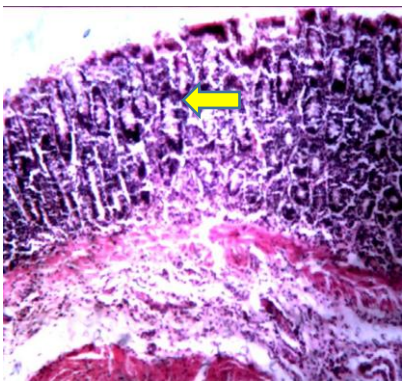
4.1.2 Histopathological results

Colon condition of animals fed with 0% supplement and unexposed group showed no pathology, while slightly epithelial necrosis was observed in group fed with 0% supplemented feed and administered MNU. Animals fed with diet supplemented with 2.5% and 5% *Vernonia amygdalina* and administered MNU showed moderate necrosis of colon epithelium while intense necrosis was observed in those fed with 10% *Vernonia amygdalina* and administered MNU (Plate V).

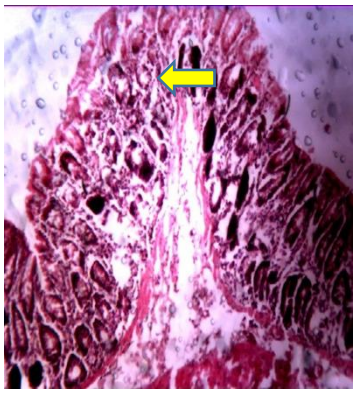
Epithelial necrosis of colon was observed in animal fed with 2.5%, 5% and 10% diet supplemented with *Tefairia occidentallis* and administered MNU, while a moderate necrosis was found in those treated with 10% and administered normal saline. (Plate VI)



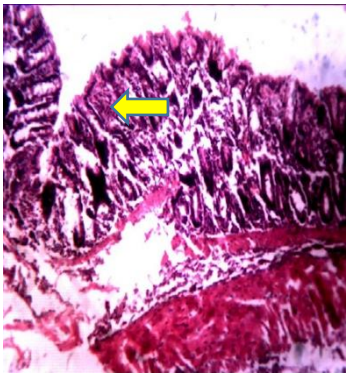
0%+NS (Control): normal mucosa Moderate epithelium



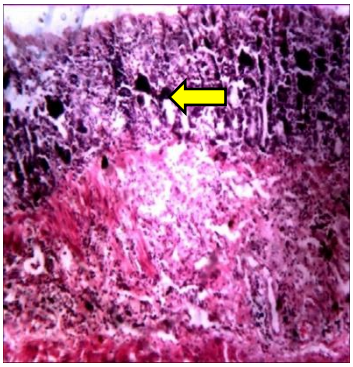
0%+MNU(Control): Colon showing slight necrosis of epithelium



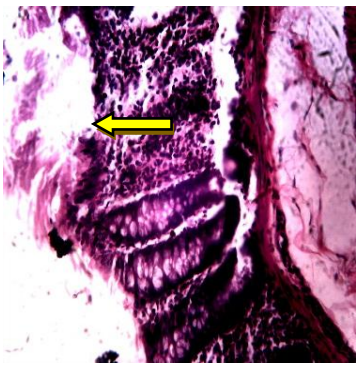
2.5%VA+MNU : necrosis of apical epithelium



10%VA+MNU: Colon showing intense necrosis moderate necrosis of epithilium



10%VA+MNU: Moderate necrosis necrosis with metaplastic changes



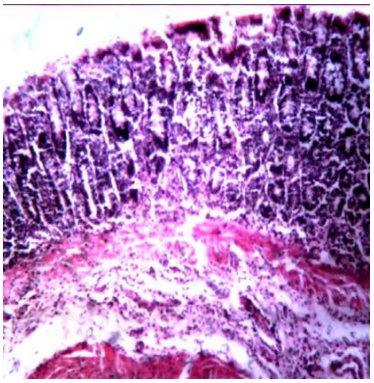
10%VA+NS:Moderate necrosis

Plate V: Photomicrographs of cross section of colon of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and intra-rectally exposed to MNU.

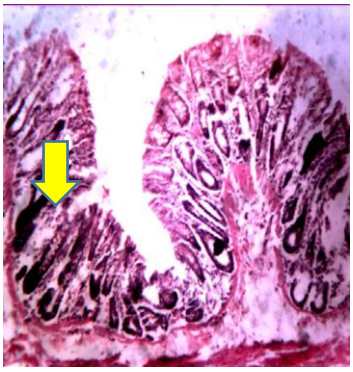
MNU: N-Methyl-N-Nitrosourea; NS: Normal saline; n%: Level of supplementation



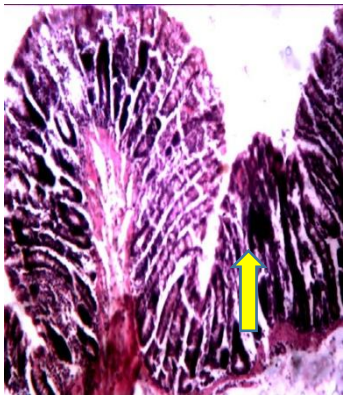
0%+NS (Control): Colon showing normal mucosa of epithelium



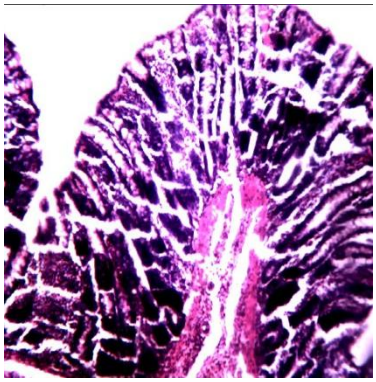
0%+MNU(Control): Colon showing slight necrosis of epithelium



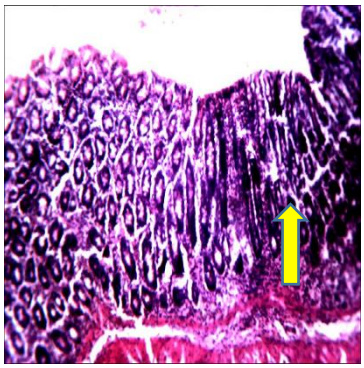
2.5%TO+MNU: Moderate Necrosis of the epithelium



5% TO+MNU: Colon showing Showing necrosis of colonic epithilium



10%TO+MNU: Intense necrosis of the mucosa epithelium



10%TO+NS:Colon slight necrosis

Plate VI: Photomicrographs of cross section of colon of animals fed with diets supplemented with *Telfairia occidentalis* and intra-rectally exposed to MNU

MNU: N-Methyl-N-Nitrosourea; NS: Normal saline; n%:Level of supplementation

4.3 Activities of Antioxidant Enzymes

Antioxidant enzymes were used also as markers of oxidative stress being also an index of carcinogenicity. Catalase and Superoxide dismutase assayed for in liver and kidney organs, showed varied responses to treatments

4.3.1 Catalase activity in liver

4.3.1.1 Effect of Vernonia amygdalina

Significantly (p□0.05) elevated levels (about 33%) in catalase activity was observed in MNU Control group compared to normal saline control group. Test groups showed a steady decrease in activity in groups fed with 2.5%, 5% and 10% *Vernonia amygdalina* and MNU-exposed, except in animals fed 10% and administered normal saline where also there was an elevation in activity similar to that observed in the MNU control group.(figure 4.2)

4.3.1.2 Effect of Telfairia occidentalis

Test groups fed with *Telfairia occidentalis* on the other hand showed a significant (p□0.05) Progressive increase in activity with increasing supplementation, while the group fed 10% *Telfairia occidentallis* and unexposed (supplemented diet control), had the highest overall activity (figure 4.2).

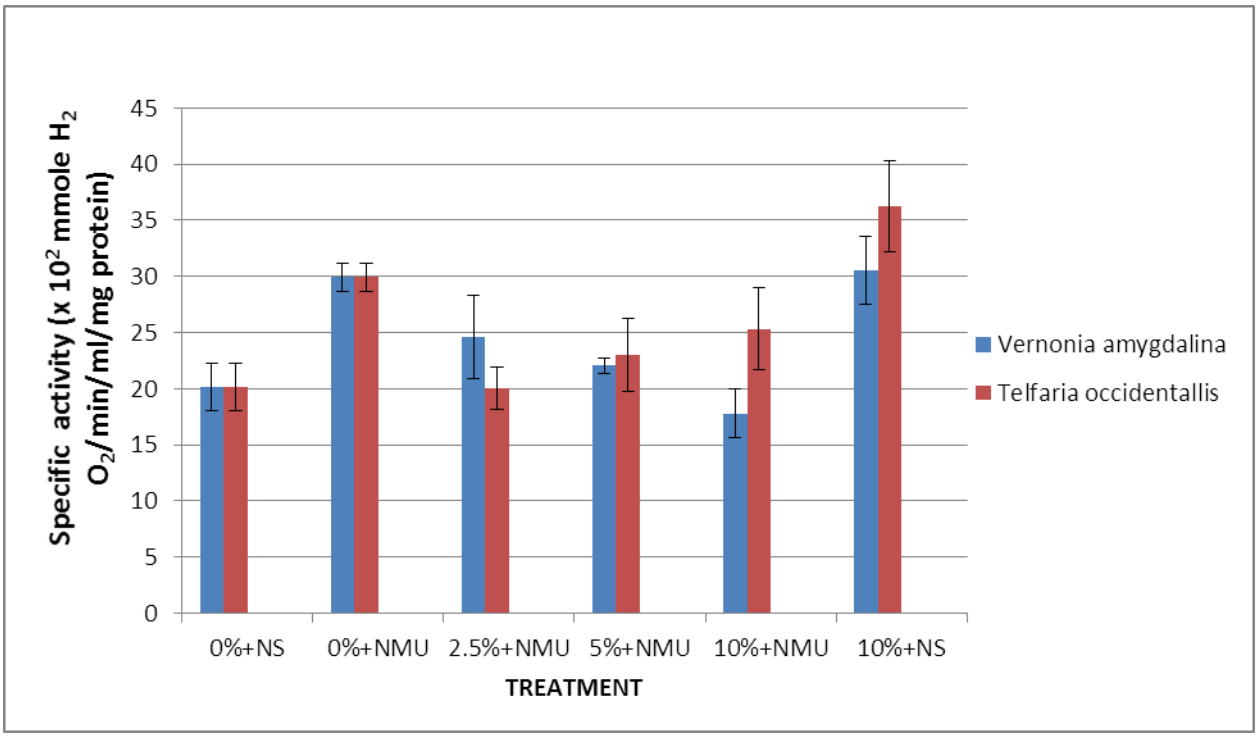


FIG. 4.2: Catalase activity in liver of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU
MNU: N-Methyl-N-Nitrosourea; NS: Normal saline; n%: Level of supplementation

4.3.2 Catalase activity in kidney

4.3.2.1 Effect of Vernonia amygdalina

About 50% increase above the activity of normal saline control was observed in the kidney of MNU control group as seen also in the liver. Test groups fed with 2.5%, 5% and 10% *Vernonia amygdalina* showed a near normal catalase activity compared to the normal control group treated with normal saline, though animals exposed to MNU and fed with 2.5% and 10% showed an increase of about 11% and 39% above the normal control respectively. (figure 4.3).

4.3.2.2 Effect of Telfairia occidentalis

A significant (p□0.05) progressive increase in catalase activity with increasing supplementation above the normal control was observed in test groups, with 5% supplementation having the overall highest activity.(figure 4.3).

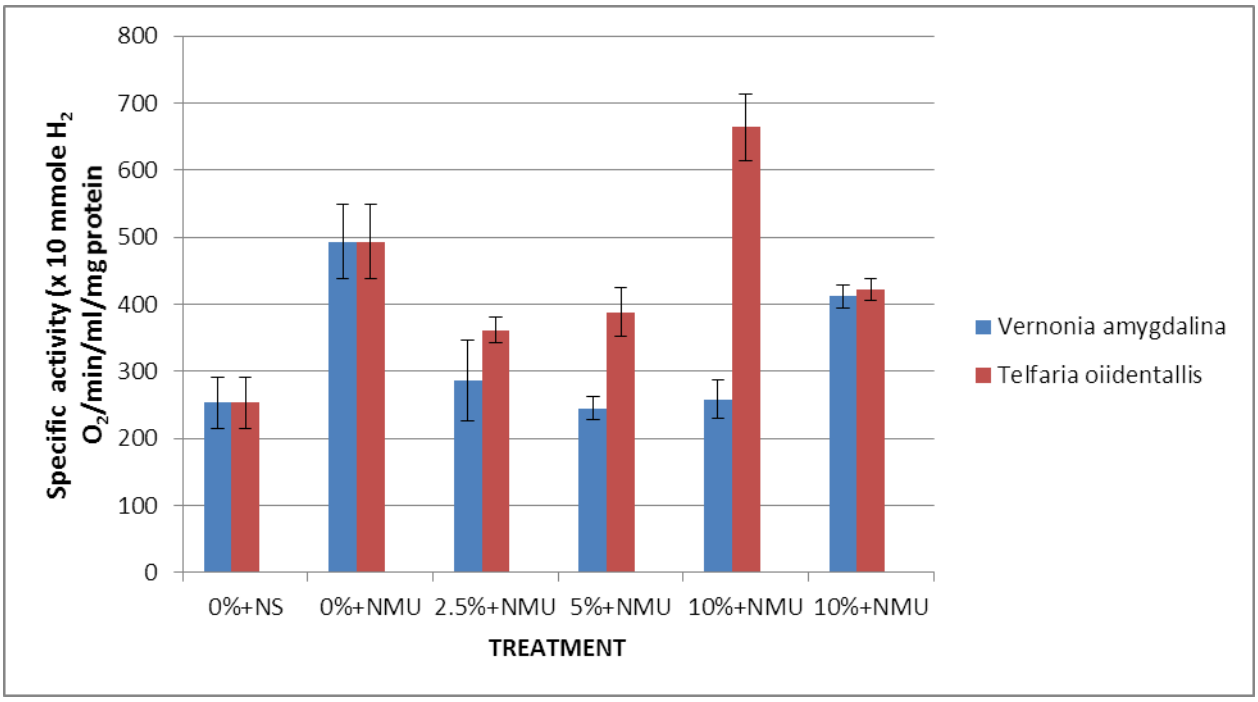


FIG. 4.3: Catalase activity in kidney of animals fed with diets supplemented with varying levels of with *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU
MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline

4.3.3 Superoxide dismutase (SOD) activity in liver

An elevated activity of liver superoxide dismutase MNU control was about 50% more than normal saline control (figure 4.4).

4.3.3.1 Effect of Vernonia amygdalina

There was no significant ($p>0.05$) change in liver SOD activity in animals fed with 2.5%

Supplemented diet and MNU-exposed compared with normal saline (unexposed) control. However a significantly (p□0.05) elevated level was observed in MNU-exposed animals fed with 5% and 10% (MNU-exposed) *Vernonia amygdalina* supplemented diet with the group fed with 10% supplemented diet and MNU-exposed having the least activity.(figure 4.4).

4.3.3.2 Effect of Telfairia occidentalis

It was observed in test (MNU-exposed) animals that there was a significant and progressive decrease in activity with increasing supplementation with 2.5% supplementation having the overall highest activity and10% supplemented diet control (normal saline control) having the overall least activity (figure 4.4).

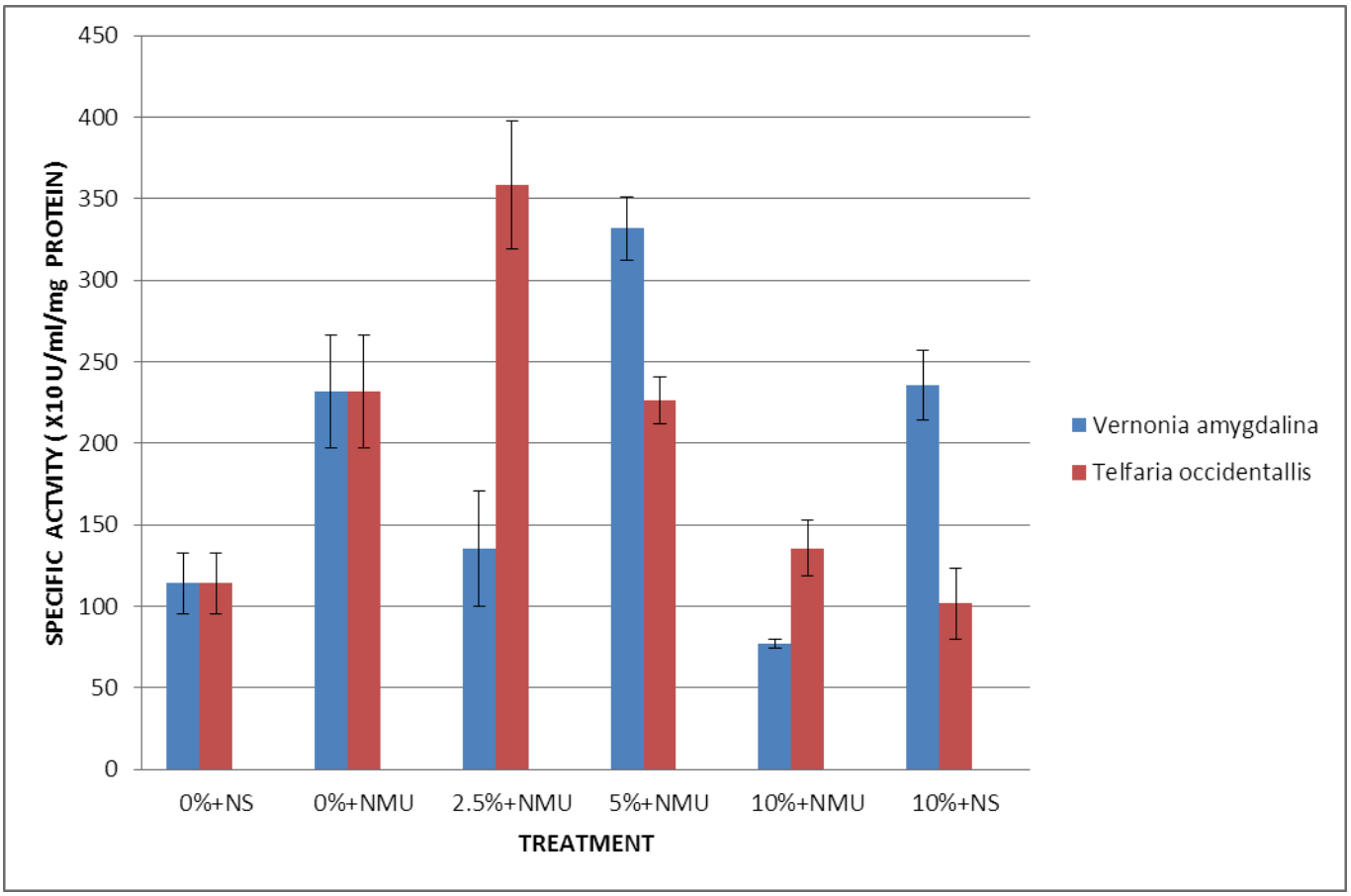


FIG. 4.4: Superoxide dismutase activity in liver of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline; n%:Level of supplementation

4.3.4 Superoxide Dismutase (SOD) activity in kidney

The MNU-exposed and un-supplemented control showed the overall least activity in kidney and also when compared with unexposed supplemented diet control (figure 4.5)

4.3.4.1 Effect of Vernonia amygdalina

A significant (p□0.05) but slight elevation in SOD activity above control groups was observed in MNU-exposed groups fed with 2.5% and 5% supplemented diets. Both MNU-exposed and unexposed animals fed with 10% supplemented diet showed relatively lower levels compared with other test groups but higher than control groups..(figure 4.5)

4.3.4.2 Effect of Telfairia occidentalis

An increase in activity occurred in both MNU-exposed and unexposed animals when they were fed 10% *Telfairia occidentalis* supplemented diets with 10% fed but unexposed to MNU having the highest overall activity while those fed with 5% diet supplemented with *Telfairia occidentalis* having the least activity. (figure 4.5)

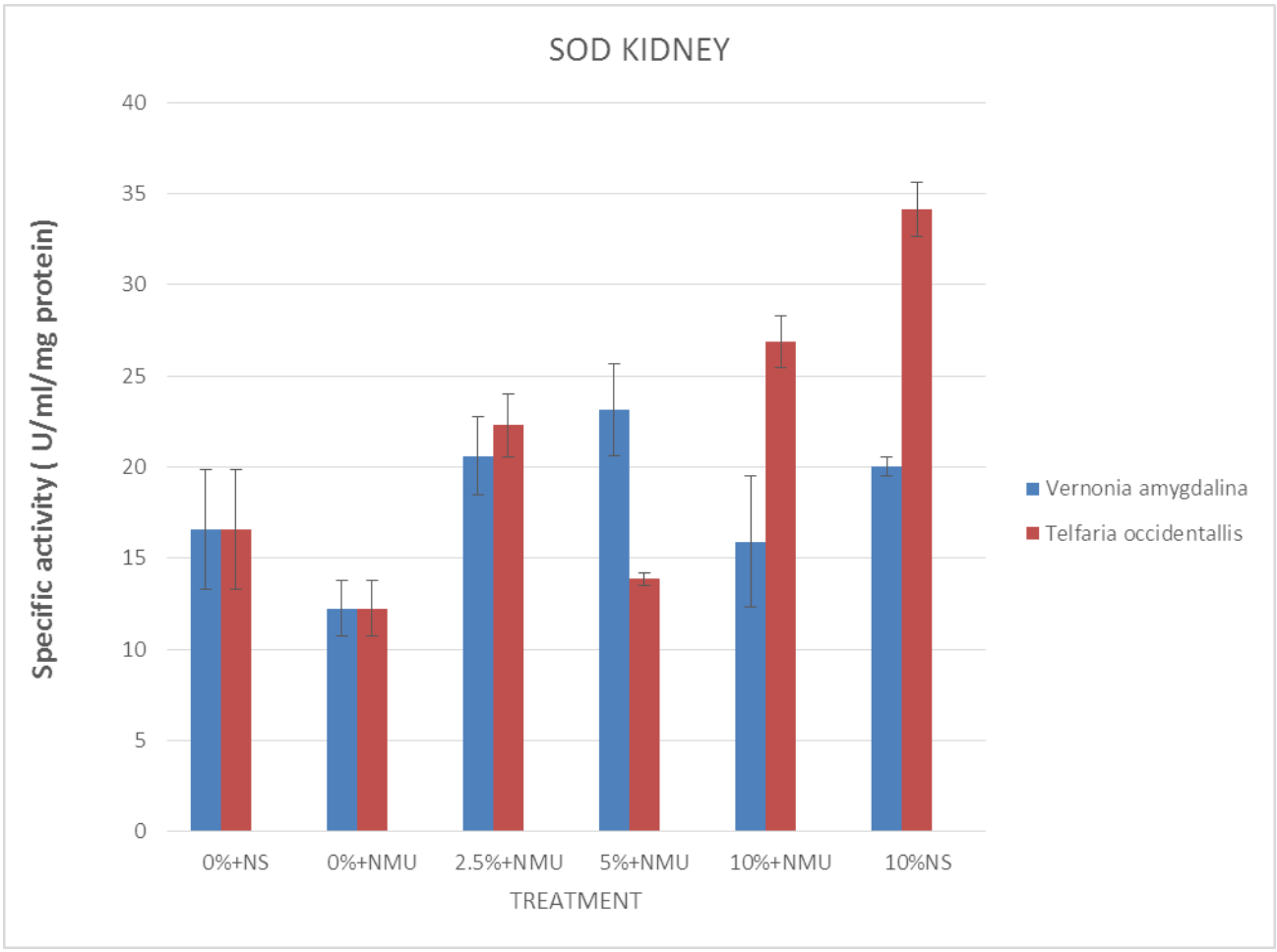


FIG. 4.5: Superoxide dismutase activity in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU
MNU: N-Methyl-N-Nitrosourea; NS: Normal saline; n%:Level of supplementation

4.4 Lipid Peroxidation Index

Formation of thiobarbituric reactive substances is used as an index of lipid peroxidation. It is also used as a marker of oxidative stress. High Levels of lipid peroxidation may predispose cells to genotoxic free radicals. The DNA damaging effect often lead to mutation of susceptibility genes which may eventually lead to cancer. The various levels of supplementation with plants had varying effects on TBARS levels. Lipid peroxidation in liver and kidney may be caused by effect of xenobiotic (diet and MNU) exposure.

4.4.1Thiobarbituric reactive substances (TBARS) levels in liver

Control animals fed with un-supplemented diet had high concentration of TBARS in liver with MNU control having the overall highest concentration all compared to test groups (figure 4.6)

4.4.1.1 Effect of Vernonia amygdalina

Though MNU-exposed animals fed with diets supplemented with 5% and 10% *Vernonia amygdalina* had roughly equal concentrations, theirs were significantly lower than that of control groups followed by animals fed with 10% supplemented diet but unexposed. Animals that were MNU-exposed but fed with 2.5% *Vernonia amygdalina* had the significantly least concentration. (figure 4.6)

4.4.1.2 Effect of Telfairia occidentalis

Animals fed with 5% and 10% *Telfairia occidentalis* supplemented diet and MNU-exposed had similar concentrations but theirs were significantly ($p \square 0.05$) lower than both controls. Though concentrations of TBARS in animals fed with 2.5% and MNU-exposed as well as

those fed 10% but MNU-exposed had roughly equal concentrations, they had the overall lowest concentration (figure 4.6).

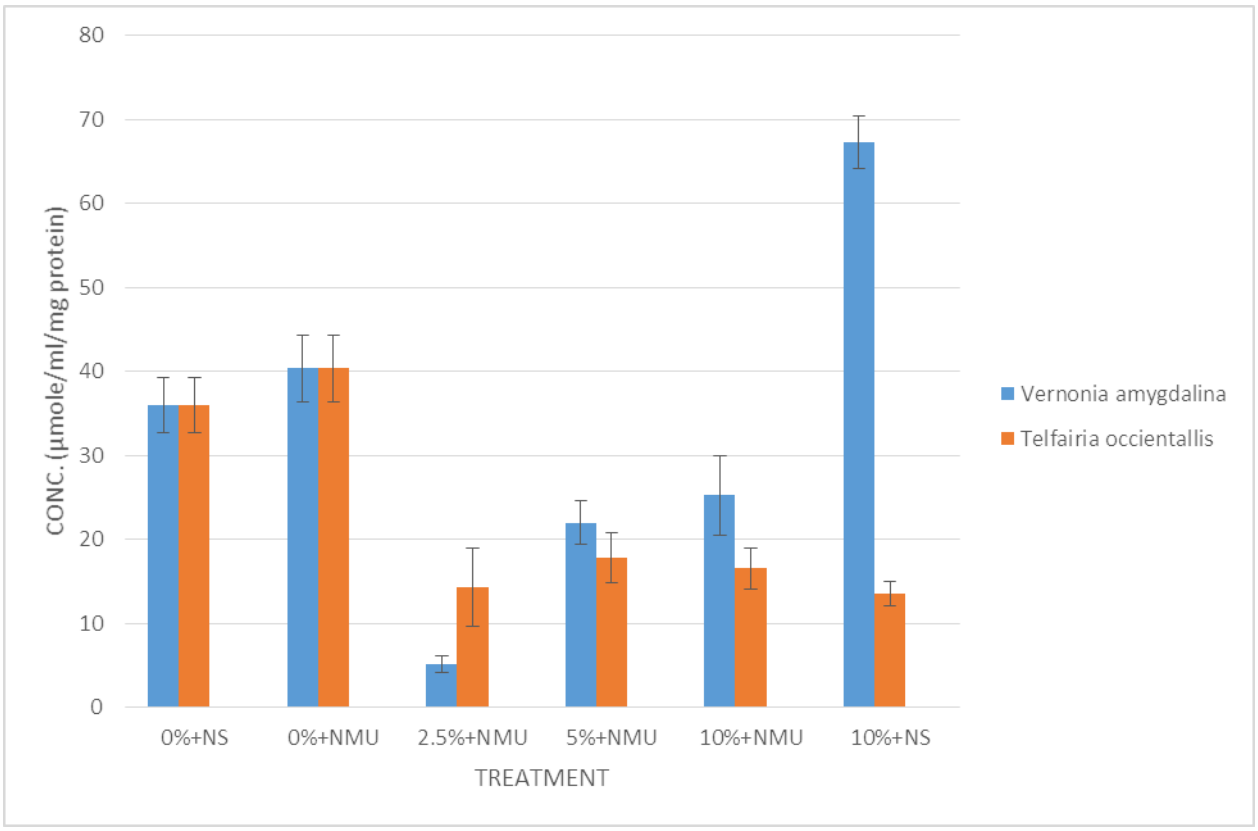


FIG.4.6: TBARS levels in liver of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU
MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline; n%:Level of supplementation

4.4.2 Thiobarbituric reactive substances (TBARS) levels in kidney

Though TBARS levels were generally comparatively lower than that observed in liver, MNU-exposed control group had slightly but significantly (p□0.05) higher TBARS levels than that of unexposed control group. Test groups had a generally lower TBARS compared to both control groups. (figure 4.7)

4.4.2.1 Effect of Vernonia amygdalina

Treatment with *Vernonia amygdalina* on test groups showed that levels of TBARS in group given 2.5% supplementation had the overall highest levels followed by those given 5% and 10% (unexposed). MNU-exposed animals given 10% supplementation had the lowest concentration of kidney TBARS (figure 4.7)

4.4.2.2 Effect of Telfairia occidentalis

Though comparatively lower than control groups, TBARS concentration progressively increased with increase in supplementation at 5%, 10% and 10% (unexposed) respectively. 2.5% had the highest (about 66% higher than the normal control) concentration and 5% having the least concentration (about 79% lower) when compared to the normal control (figure 4.7).

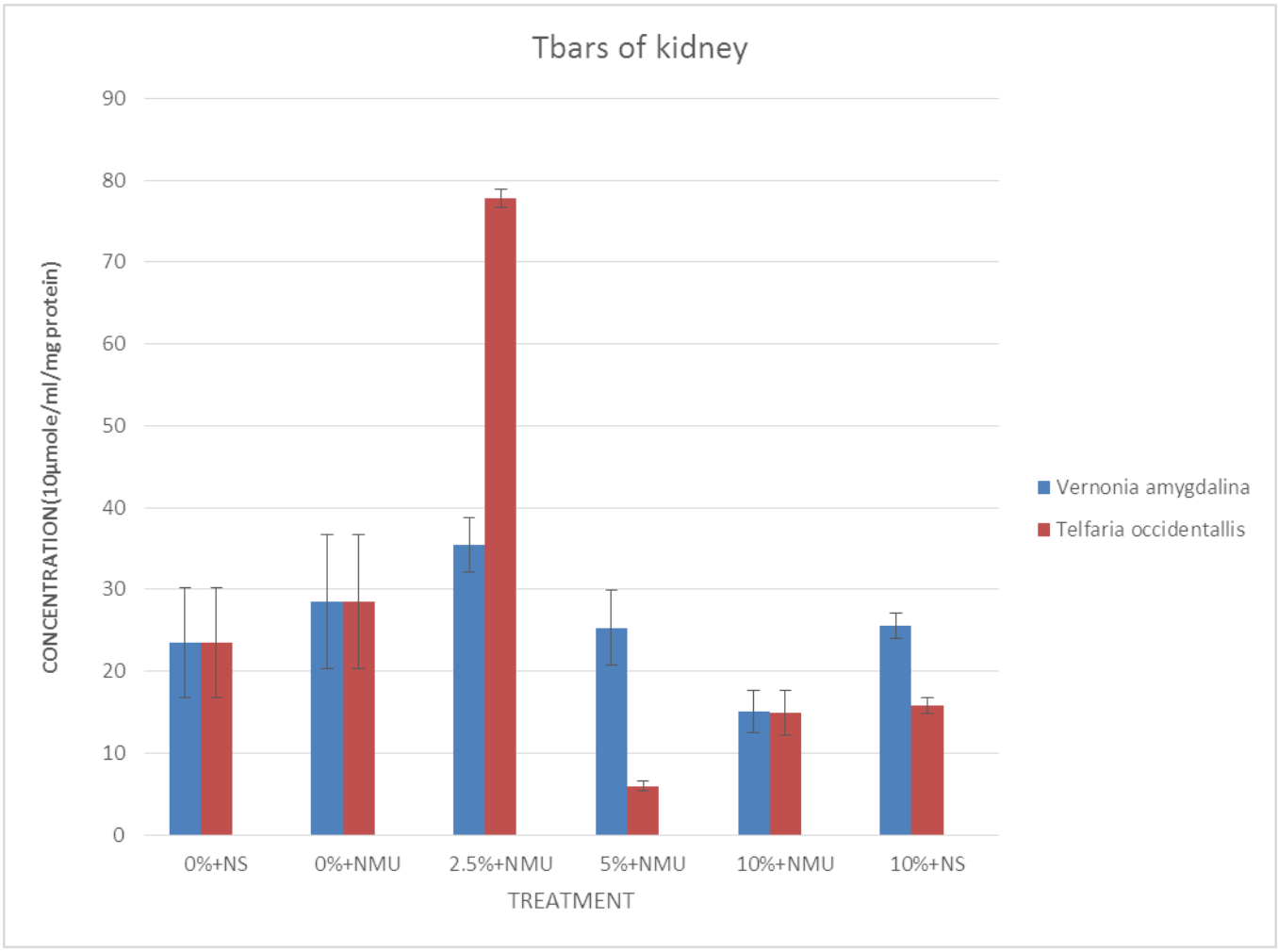


FIG. 4.7: TBARS levels in kidney of animals fed with diets supplementated with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU. MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline; n%:Level of supplementation

4.5 Total Protein

4.5.1 Total protein concentration in liver

Normal control group appeared to have the highest liver protein concentration followed by MNU control, though insignificantly higher than test groups.(figure 4.8)

4.5.1.1 Effect of Vernonia amygdalina

All test groups fed with 5% and 10% *Vernonia amygdalina* had similar concentrations comparable to the MNU control group with 2.5% treated group having a slightly higher concentration compared to the other test groups mentioned above (figure 4.8)

4.5.1.2 Effect of Telfairia occidentalis

Though insignificantly ($p>0.05$) lower than control groups, liver protein concentration progressively decreased in all MNU-exposed test groups given 2.5%, 5% and 10% *Telfairia occidentalis* supplemented diets, with the unexposed group given 10% supplementation having concentration similar to that of the MNU control. (figure 4.8)

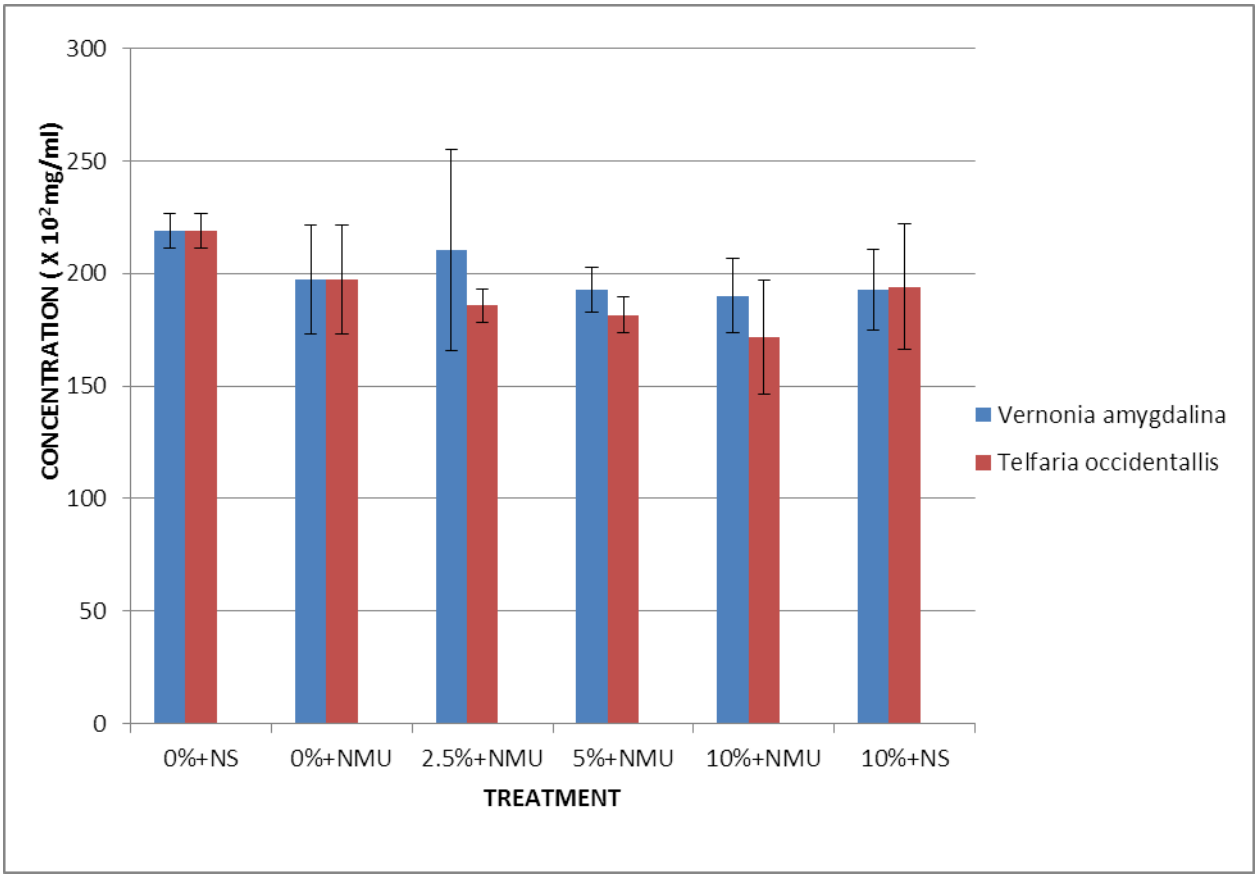


FIG.4.8: Total Protein concentration in liver of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU.
MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline; n%:Level of supplementation

4.5.2 Total protein concentration in kidney

No significant difference ($p>0.05$) in protein concentration was found between the control groups, but slightly higher in test groups. (figure 4.9)

4.5.2.1 Effect of Vernonia amygdalina

Animals fed with 2.5% *Vernonia amygdalina* supplemented diet showed no significant difference ($p>0.05$) in concentration and had the overall lowest protein concentration when compared with the control groups. However an elevated level in total protein concentration found in animals fed with 5%,10% (both MNU-exposed) and 10% (unexposed) were not significantly different ($p>0.05$) within themselves when compared with control group (figure 4.9).

4.5.2.2 Effect of Telfairia occidentalis

Though protein concentrations in those animals treated with *Telfairia occidentalis* were comparatively higher than those treated with *Vernonia amygdalina*, there was a progressive decrease, though insignificantly ($p>0.05$), in those fed with diet supplemented with 5% (MNU-exposed) *Telfairia occidentalis* and those fed with 10% *Telfaria occidentalis* (MNU-exposed) and 10% *Telfairia occidentalis* (un-exposed).

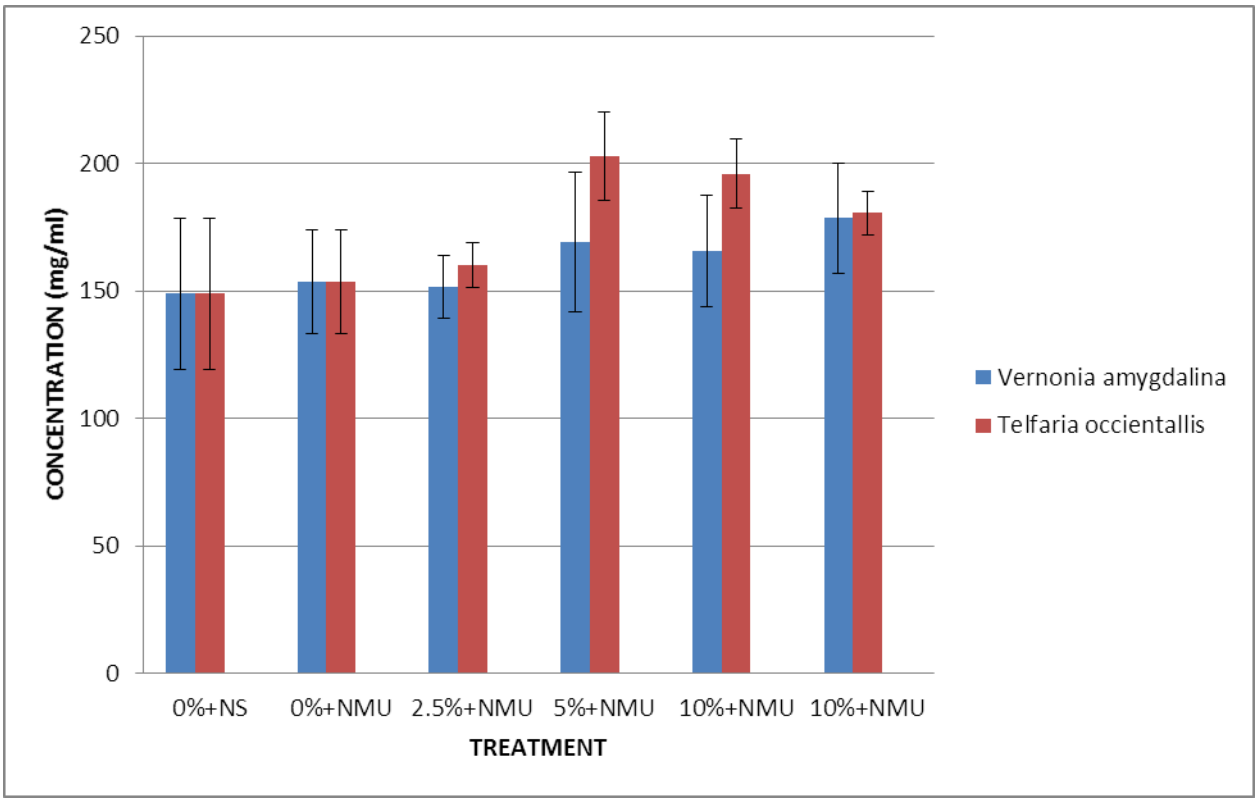


FIG. 4.9: Total Protein levels in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU
MNU: N-Methyl-N-Nitrosourea ;NS: Normal saline; n%:Level of supplementation

4.6 Hematological Parameters

4.6.1 Effect of *Vernonia amygdalina*

White Blood Cells

The MNU-exposed and unexposed controls had no significant difference. ($p>0.05$) in their levels of WBC. Animals fed with 2.5% and 5% diet supplementation and MNU-exposed had similar values but significantly ($p \square 0.05$) higher than the control group. Groups fed with the highest supplementation had the same values but were lowest in value compared to both un-supplemented controls and other test groups (Table 1).

Lymphocytes

Controls having the overall highest levels, had no significant differences ($p>0.05$) between their values. MNU-exposed animals fed with 2.5%, 5% and 10% (unexposed) also showed no significant differences between themselves but however showed a much lower value compared with un-supplemented control groups.10% supplemented diet fed and MNU-exposed group had slightly lower values compared to control groups (Table 1).

Red Blood Cells

There was no significant difference ($p>0.05$) in values between groups except for the MNU-exposed group fed with 2.5% *Vernonia amygdalina* supplemented diet having the highest RBC count. (Table 1).

Mean Corpusclar Volume

Normal saline controls had a significantly higher (p□0.05) Mean Corpuscular Volume (MCV) than MNU control. MNU-exposed group fed with 2.5% *Vernonia amygdalina* and unexposed group fed with 10% *Vernonia amygdalina* had similar MCV values which were the lowest among all the groups while MNU-exposed animals fed with 5% and 10% had similar values but slightly lower than control groups and slightly higher than the 2.5% fed and MNU-exposed and 10% fed and unexposed (Table 2)

Mean Corpusclar Hemoglobin

No significant difference (p>0.05) was observed in Mean Corpuscular Hemoglobin (MCH) for both normal saline and MNU control groups.

MNU-exposed animals fed with 2.5% and 10% *Vernonia amygdalina* as well as those unexposed and fed with 10% *Vernonia amygdalina* had similar MCH concentration though significantly lower than control groups. The MNU-exposed group fed with 5% supplemented diet had the same concentration as that shown by both controls. (Table 2)

Mean Corpusclar Hemoglobin Concentration

Mean corpuscular hemoglobin concentration (MCHC) between control groups and all MNU MNU-exposed groups were significantly different (p□0.05). All groups which were MNU-exposed with MNU and or respectively fed with supplemented diets, had higher values compared to normal saline control. However among all fed and MNU-exposed groups, there was no significant difference (p>0.05) in MCHC values. Other parameters

such as, HCT, HGB, PLT and MPV showed no significant differences in their values between groups. (Table 2)

4.6.2 Effect of *Telfairia occidentalis*

White Blood Cells

5% and 10% *Telfairia occidentalis* supplementation gave similar values as controls. Whereas 2.5% supplementation gave the lowest value, unexposed and 10% supplementation gave the overall highest WBC count (Table 1).

Lymphocytes

MNU and normal saline controls having the overall highest amount, had no significant differences ($p>0.05$) between their values. (Table 1)

Among the MNU-exposed groups, 2.5% supplementation group had the highest lymphocytes in circulation. There was however a significant ($p \square 0.05$) decrease in values observed in groups fed with 5% and10% supplementation and then MNU-exposed. Unexposed animals fed with 10% *Telfairia occidentalis* had similar value as those fed 2.5% *Telfairia occidentalis* and MNU-exposed (Table 1).

Red Blood Cells

MNU and normal saline controls had no significant differences in their RBC counts.

No significant differences ($p>0.05$) was seen between the values of groups fed with 2.5%, 5%, 10% and MNU-exposed compared with control groups. However, there was a

significant decrease (p□0.05) in value observed in group fed 10% *Telfairia occidentalis* and unexposed.

Mean Corpuscular Volume

Normal saline controls had a significantly higher Mean corpuscular volume (MCV) than MNU control. (Table 2)

Values were similar to the control groups but generally on the lower side. No significant (p>0.05) difference in MCV was observed between groups fed with 2.5%, 5%, 10% and MNU-exposed. The group fed with 10% *Telfairia occidentalis* but unexposed had a significantly much lower value compared to the other treated groups. (Table 2)

Mean Corpuscular Hemoglobin

No significant difference (p>0.05) was observed in Mean Corpuscular Hemoglobin (MCH) for both normal saline and MNU control groups. However, significant (p□0.05) but relatively lower MCH values were observed between MNU-exposed groups fed 2.5% and10%. MNU-exposed animals fed with 5% *Telfairia occidentalis* had similar value to the control groups and while unexposed groups fed with diet supplemented with 10% *Telfairia occidentalis* had the lowest significant value. (Table 2)

Mean Corpuscular Hemoglobin Concentration

MNU-exposed control was observed to have a significantly (p□0.05) higher value of Mean Corpuscular Hemoglobin Concentration (MCHC) than unexposed control. Animals fed with 2.5% and 10% *Telfairia occidentalis* and MNU-exposed had MCHC values similar to the MNU-exposed control. MNU-exposed animals given 5% supplementation appeared to

have produced the overall highest concentration while unexposed animals given 10% supplementation had the overall lowest concentration of MCHC. All groups showed no significant ($p>0.05$) differences in HCT, PLT and MPV values (Table 2)

Table 1: Some Hematological parameters of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

	WBC(x10^{9}/ L)	LYM(%)	HGB(g/L)	RBC(x10^{12}/L)	HCT (%)
0%+NS(control)	12.40±1.78^{ab}	75.83±2.12^{c}	143.0±5.00^{a}	8.22±0.03^{ab}	37.27±1.16^{a}
0%+MNU(control	15.25±3.03^{ab}	75.80±2.13^{c}	150.50±10.67^{a}	8.77±0.55^{ab}	38.60±2.59^{a}
2.5%VA+MNU	18.26±7.90^{b}	71.13±4.50^{abc}	147.75±5.50^{a}	8.87±0.205^{c}	38.50±0.73^{a}
5%VA+MNU	19.73±9.21^{b}	67.97±.47^{abc}	142.00±6.08^{a}	8.33±0.25^{ab}	36.77±1.69^{a}
10%VA+MNU	9.13±1.60^{a}	75.50±1.25^{bc}	137.50±5.07^{a}	8.24±0.41^{ab}	36.03±1.24^{a}
10%VA+NS	9.18±1.97^{a}	67.00±4.15^{abc}	141.50±5.26^{a}	8.46±0.33^{ab}	36.48±0.96^{a}
2.5%TO+MNU	9.08±3.52^{a}	75.10±6.99^{abc}	141.00±12.89^{a}	8.29±0.65^{ab}	36.35±3.05^{a}
5%TO+MNU	13.95±4.32^{ab}	66.05±6.29^{a}	149.50±6.37^{a}	8.79±0.078^{ab}	37.95±1.62^{a}
10%TO+MNU	15.90±2.62^{ab}	66.75±8.89^{ab}	142.25±7.93^{a}	8.32±0.13^{ab}	37.18±1.59^{a}
10%TO+NS	19.03±3.00^{b}	70.60±1.44^{abc}	137.33±16.04^{a}	8.11±0.38^{a}	36.03±3.20^{a}

VA: *Vernonia amygdalina;* TO*: Telfairia occidentalis*; MNU: N-Methyl-N-Nitrosourea; NS: Normal Saline; WBC: White blood cell; LYM: Lymphocyte; HGB: Hemoglobin; RBC: Red blood cell; HCT: Hematocrit.

Values are Mean ±Standard deviation. Comparison of values with different superscript letters down a column show significant difference between groups for applicable parameter.

Table :2 Some Hematological parameters of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

GROUP	MCV(fL)	MCH (pg)	MCHC(g/L)	PLT x10^9 (/L)	MPV (fL)
0%+NS(control)	45.40±1.39^{b}	17.33±0.55^{b}	383.33±5.51^{b}	783.00±172.03^{a}	7.43±0.80^{a}
0%+MNU(control)	44.10±0.716^{ab}	17.13±0.22^{b}	389.50±3.42^{ab}	865.25±122.46^{a}	7.60±0.25^{a}
2.5%VA+MNU	43.45±0.252^{a}	16.60±0.37^{ab}	383.25±8.78^{ab}	831.50±77.43^{a}	7.18±0.47^{a}
5%VA+MNU	44.17± 0.72^{ab}	17.00±0.27^{b}	386.00±1.00^{ab}	908.33±324.20^{a}	6.87±0.29^{a}
10%VA+MNU	43.80±1.30^{ab}	16.65±0.45^{ab}	381.25±2.22^{ab}	865.00±86.65^{a}	7.25±0.76^{a}
10%VA+NS	43.15±0.733^{a}	16.65±0.24^{ab}	387.50±4.12^{ab}	899.75±247.08^{a}	7.10±0.50^{a}
2.5%TO+MNU	43.92±1.30^{ab}	16.95±0.55^{ab}	387.25±3.59^{ab}	817.00±166.72^{a}	7.15±0.49^{a}
5%TO+MNU	44.15±1.06^{ab}	17.30±0.42^{b}	393.50±0.71^{b}	922.00±24.042^{a}	7.35±0.35^{a}
10%TO+MNU	43.65±0.94^{ab}	16.63±0.60^{ab}	382.25±9.03^{ab}	945.25±71.89^{a}	7.68±0.26^{a}
10%TO+MNU	42.73±1.16^{a}	16.16±0.569^{a}	380.00±13.00^{a}	920.67±55.78^{a}	7.63±0.74^{a}

VA: *Vernonia amygdalina;* TO*: Telfairia occidentalis*; MNU: N-Methyl-N-Nitrosourea; NS: Normal Saline; MCV: Mean Corpuscular Volume; MCH:Mean Corpuscular Hemoglobin;MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelet; MPV: Mean Platelet Volume.

Values are Mean ±Standard deviation. Comparison of values with different superscript letters down a column show significant difference between groups for applicable parameter.

CHAPTER FIVE

5.0 DISCUSSION

A comparative in-vivo study carried out to investigate how dietary modification influences Induced colon carcinogenesis showed that 2.5%, 5% and 10% levels of supplementation with raw dried leaves of *Vernonia amygdalina* and *Telfairia occidentalis* had varying degrees of effects.

Because it is known that some plant's phytochemicals holds potential for cancer mitigation (Atawodi et al, 2010), it is expected that quantitative treatments with these plants should demonstrate a directly proportional mitigation. However this investigation revealed that a general threshold of 5% supplementation with *Vernonia amygdalina* had the highest ameliorating activity but with a deteriorating effect at the highest supplementation at 10 %, while *Telfairia occidentalis* demonstrated anti- cancer activity at 10% when compared with the control groups. A dose independent explanation for such varied effect is being proposed. This is supported by similar disproportionate behaviour observed in various works done with emphasis on some plant's antioxidants, majorly flavonoids and polyphenols.

The results of this investigation demonstrating anti-proliferative properties of antioxidants of plant, shows that at higher and lower quantities of plants antioxidants, growth inhibition was observed, thereby corroborating with results of previous findings where antioxidant supplementation experiments have failed to show any protective effect against cancer. In fact in several trials antioxidant supplementation has been linked with increased rates of

certain cancers. In one trial, smokers taking extra beta carotene had higher, not lower rates of cancer (Tuveson and Chandel, 2014).

The research done by Tuveson and Chandel proposes that taking antioxidant pills or eating vast quantities of foods rich in antioxidants may be failing to show a beneficial effect against cancer because they do not act at the mitochondrion which is a critical site in cells where tumour-promoting ROS are produced. Rather, supplements and dietary antioxidants tend to accumulate at scattered distant sites in the cell, leaving tumour-promoting ROS relatively unperturbed. Quantities of both Reactive Oxygen Species (ROS) and natural antioxidants are higher in cancer cells -- the paradoxically higher levels of antioxidants being a natural defense by cancer cells to keep their higher levels of oxidants in check, so growth can continue. In addition, Tuveson and Chandel, further proposed that therapies that raise the levels of oxidants in cells may be beneficial, whereas those that act as antioxidants may further stimulate the cancer cells.

A genetic or pharmacologic inhibition of antioxidant proteins, which is a concept tested successfully in rodent models of lung and pancreatic cancers may be a reasonable explanation for observed low levels of some antioxidant enzymes in un-exposed animals and exposed animals fed with highest supplementation or plain diet. This could be a useful therapeutic approach in humans (Tuveson and Chandel, 2014). It is possible that some components of supplemented diet may have elicited a pharmacologic inhibition of these enzyme proteins. The key challenge in this concept, is to identify antioxidant proteins and pathways in cells that are used only by cancer cells. Impeding antioxidant production in healthy cells will upset the delicate redox balance upon which normal cellular function depends.

Apart from its well-known chemopreventive attributes such as free radical scavenging, detoxification, many studies had been conducted to determine the effects of *Vernonia amygdalina* on the activity of cancer cells in particular the human breast cancer and prostatic cancer. It was found that *Vernonia amygdalina* was able to inhibit the growth of MCF-7 (Michigan Cancer Foundation-7) cells in a dose dependent manner upon exposure for 48 hours. The findings also indicated that the plant Induced minimal genotoxic damage in MCF-7 cells. Vernodalinol, a sesquiterpene lactone, isolated from the leaves of *Vernonia amygdalina* effectively inhibited breast cancerous cell growth at a dose of 25 – 50µg/ml. The LC50 being 70 – 75 µg/ml in MCR-7 cells. (Luo et al, 2011; Yedjou , 2008; Gresham et al, 2008; Oyugi, 2009; Luo, 2010).

Similarly, administration of quercetin, a common polyphenolic component of many vascular and edible plants including vegetables, fruits and tea significantly reduced the tumour volume in rats Induced for mammary carcinoma using dimethyl benz (a) anthracene (DMBA). An effective dose of 25mg/kgbw was obtained when a dose response assessment was carried out by treating animals with different doses (15–45mg/kgbw) of quercetin. Serum levels of carcino embryonic antigen (CEA), a potent marker for tumour growth invasion was also significantly decreased on quercetin treatment (Sankarasharma et al, *2006*)

Myricetin is one of the principal phytochemicals in *Vernonia amygdalina* as well as onions, berries and red wine. Previous studies showed that myricetin exhibits potent anticancer and chemopreventive effects. One study examined the effect of myricetin on ultraviolet (UV) B-Induced angiogenesis in an SKH-1 hairless mouse skin. Topical treatment with myricetin indicated that myricetin suppresses UVB-Induced angiogenesis by regulating PI-3 kinase

activity *in vivo* in mouse skin (Sung et al, 2010). Though Myricetin is a potent anticarcinogen and antimutagen, it has also been shown to promote mutagenesis with the use of the Ames Test. However, from the three tested flavonols (myricetin, quercetin and kaempferol) myricetin showed the strongest inhibitory effect on cell transformation. (David et al, 2009)

Other investigations of effect of antioxidants on cancer was done on prostate cancer. Five Ranomised clinical trials (RCTs) reported no significant effects compared with placebo. Two RCTs reported that a combination of antioxidants, isoflavones, lycopenes, minerals, plant oestrogens and vitamins significantly decreased PSA levels compared with placebo.(Posadzki et al, 2013)

Telfairia occidentalis on the other hand showed anti-carinogenic activity at 10% supplementation but no amelioration of intense epithelial necrosis of colon. 2.5% and 5% had a higher levels of CEA with both having necrotic colon epithelia. The observed conditions of these colons despite their exposure to the therapeutic agents may be due to high amounts of cytotoxic phytochemicals that may be destructive to mucosa cells at high amounts. Isorhamnetin being an essential phytochemical in *Telfairia occidentalis*, was found to be a potent anti-proliferative agent in a dose- and time-dependent manner, with an IC_{50} of 72 μM after 48 h of incubation as estimated by MTT assay. Flow cytometry and fluorescence microscopy analysis showed that isorhamnetin exerted a stimulatory effect on apoptosis and necrosis, similar to that observed in photomicrographs of sections of colon indicated above. Isorhamnetin also increased the number of cells in G2/M phase. Serum deprivation appeared to potentiate the effects of isorhamnetin on cell death and facilitated cell cycle progression to G0/G1 phase (Jaramillo et al, *2010*). This kind of dose-time

sensitive therapy gives a premise for explaining the effect observed in this study. Colonic retention time, bowel movement and level of supplementation become critical factors when determining effect of carcinogen and efficacy of dietary supplementation. These factors are however applicable and critical at lower supplementations (2.5% and 5%) at which critical amounts of Isohermentin could have been lost during bowel movements. Higher supplementation (10%) may however still have had sufficient Isohermentin to elicit anti-carcinogenic activity.

A more detailed effect investigated on selected parameters including hematological, TBARS, antioxidant enzymes Catalase and Superoxide dismutase as well as carcinoembryonic antigen (CEA) corroborated with the generally observed effect. Photomicrographs of colons revealed that animals fed with 5% *Vernonia amygdalina* had moderate necrotic changes in their colon epithelia compared with the normal control group. This observation was also supported with their significantly lower CEA levels. Animals fed with 2.5% and 10% *Vernonia amygdalina* showed comparatively higher CEA levels with colonic condition ranging from moderate to intense necrosis of epithelium suggesting a growth inhibiting effect on cells.

Hematological indicies as observed from the results reflects expected responses to respective exposures (MNU and therapeutic diets). Levels in WBC is often used as a prognostic factor in variety of cancers (Smith et al, 2008). Higher levels, above normal ranges, of WBC may indicate Cancer condition. Lymphocyte count has also been used as an independent prognostic factor in lung, colorectal and gastric cancer (Blake et al, 2004,Fumagalli et al, 2003,Brunker et al, 1982). low lymphocyte counts (less than 1500/ ml) indicate cell-mediated immunodeficiency which is common in cancer condition but

also plays a relevant prognostic role for survival (Brunker et al, 1982,). Cancer related lymphocytopenia occurs in advanced stage cancer such as colorectal cancer (Brunker et al, 1982). Hematological result showed significantly higher levels of circulating WBC in animals fed with 2.5% and 5% *Vernonia amygdalina*. This is not far from expectation as this may be an immunomodulatory effect by plant supplements. However, a significantly (about 50%) lower WBC and higher lymphocyte count found in animals fed wth the highest supplementation of 10% *Vernonia amygdalina* compared with those with 5% *Vernonia amygdalina* may indicate an improving immunity due to the amounts of therapeutic agents administered. The WBC counts in 5% and 10% fed group were also about 30- 53% higher than the 2.5% fed group.

Similarly elevated neutrophil-lymphocyte ratio (NLR) have been repeatedly demonstrated as a significantly prognostic factor for some various types of cancer (Noh et al, 2013; Pichler et al, 2013; Shibutani et al, 2013). Patients with elevated NLR have a relative lymphocytopenia and, as a result, may exhibit a poorer lymphocyte-mediated immune response to malignancy; thereby worsening their prognosis. Preoperative elevated NLR was correlated with overall and cancer-specific survival in colorectal cancer (Walsh et al, 2005). It was also observed in previous cancer related study carried out compared to baseline values, NLR values was found to decrease in groups receiving chemotherapeutic agents.

Platelets have additional roles in wound healing, inflammation and angiogenesis. Studies have shown that platelet count can be a prognostic factor; with patients presenting thrombocytosis having a poor survival in a variety of cancers (Monreal et al, 1998; Sun et al, 1979). Platelets store angiogenic factors in granules and that these granules can be differentially released in the presence of the thrombin agonists (Italiano et al, 2008). Mean

platelet volume (MPV) and platecrit (PCT) are crucial indicators, which are associated with platelets functions. MPV level was considerably higher in pre-operative gastric cancer patients compared to healthy subjects (Kılınçalp et al, 2013). In the research undertaken, there appeared no significant difference in the PLT, MPV and PCT values.

Cell proliferation is a characteristic feature of cancer and cancer itself imparts oxidative stress on the host. This is illustrated by the rapid elevation of lipid peroxidation and impaired antioxidant status of mice with various types of tumours as demonstrated in tumour bearing animal which can experience systematic change of antioxidant enzymes in organs distant from the tumour (Devasena et al, 2002; Raju ,et al, 2011).Tumour cells have relatively low amounts of superoxide dismutase (SOD), which quenches superoxide anion (O^{2-}.), and as a result of a higher level of aerobic metabolism, higher concentrations of O^{2-}., compared to normal cells (Das, 2002). Depression of catalase activity in liver is a well-established phenomenon in tumour bearing animals. Elevated levels of lipid peroxidation (measured as quantitative TBARS) at 2.5% and 10% supplementations may be due to corresponding sequestration of catalase in response to excessive generation of hydrogen peroxide. Treatment with effective dose of supplements may have significantly decreased the level of lipid peroxidation showing its protective role. Antioxidant enzymes, Catalase and Superoxide dismutase, also showed a similar response at 5% supplementation with elevated liver and kidney enzyme activities with also a corresponding low level of TBARS in this group. Results of previous experiments have demonstrated that altered oxidant/antioxidant balance favoured free radicals and DNA damage in gastrointestinal cancer patients. Significant decrease in SOD, CAT activities and antioxidant molecules suggested the possible involvement of oxidative stress in gastrointestinal cancer (Aymelek

et al, 2012). Similarly, another investigation to determine antioxidant action of *Terminalia arjuna* monitored by the activities of catalase, superoxide dismutase and glutathione S transferase which constitute major antioxidant defense system by scavenging ROS. These enzyme activities are low in lymphoma bearing mice indicating impaired antioxidant defense system. Oral administration of different doses of aqueous extract of *Terminalia arjuna* causes significant elevation in the activities of catalase, superoxide dismutase and glutathione S transferase (Verma and Vinayak, 2008). This might possibly be the explanation for the observed elevated activities of these enzymes of some group of animals as described in result above.

.

CHAPTER SIX

6.0 SUMMARY, CONLUSION AND RECOMMENDATION

6.1 SUMMARY

Some Dietary components influence development of certain gastrointestinal malignancy such as colon cancer. These components may have cancer promoting or preventing abilities. Effects of diets Supplemented with *Vernonia amygdalina* and *Telfairia occidentalis* raw leaves on animals intra-rectally exposed to N-Methyl-N-Nitrosourea, an established carcinogen, explored in this study showed that carcinoembryonic antigen (a biomarker for colon cancer), colonic histology and oxidative stress status of animals were as follows:

i. Five percent (5%) supplementation with *Vernonia amygdalina* decreased Carcinoembronic antigen levels by about 45% when compared with MNU control.

ii. Animals fed with diet supplemented with 2.5% and 5% *Vernonia amygdalina* and administered MNU showed moderate necrosis of colon epithelium while intense necrosis was observed in those fed with 10% *Vernonia amygdalina* and administered MNU.

iii. Increasing supplementation with *Telfairia occidentalis* showed a stimulatory activity on liver and kidney catalase while increasing supplementation with *Telfairia occidentalis* on the other had an inhibitory effect on liver SOD. Increase in liver SOD at 5% supplementation with *Vernonia amygdalina* was observed.

iv. *Vernonia amygdalina* and *Telfairia occidentalis* generally had a lowering effect at 2.5%, on animal liver TBARS while there was a significant (p□0.05) lowering effect at higher *Vernonia amygdalina* supplementations (5% and 10%) in kidney of animal.

v. 2.5% and 5% supplementation with *Vernonia amygdalina* and 2.5% and 10% supplementation with *Telfairia occidentalis* stimulated white blood cells but decreased levels of lymphocytes.

vi. Corpuscular quantity was observed to increase at 2.5% and 5% *Vernonia amygdalina* supplementation.

6.2 CONCLUSION

The following parametric inferences could be drawn from results obtained:

i. *Vernonia amygdalina* and *Telfairia occidentalis* leaves possesses some anti-ctyoproliferative potential. This is supported by both histologic findings and CEA analysis. However therapeutic effect holds only at specific levels of supplementation of, suggesting a dose independent effect i.e Therapeutic at 5% for *Vernonia amygdalina* and 10% for *Telfairia occidentalis* which showed ameliorative activity on CEA, while 10% *Telfairia occidentalis* only had a similar effect on colon but not CEA.

ii. A number of the findings of this study supports a number of related work done by others. Notably is the corroborative finding that effective therapy of most antioxidants of plant origin were mostly dose dependent.

iii. Hematological finding showed adaptive and evasive attempts by immune system of groups treated with 2.5% and 5% *Vernonia amygdalina* and 2.5% and 10% *Telfairia occidentalis*. Dietary contribution to hematological status was evaluated as quantitative state of corpuscules where 2.5% and 5% *Vernonia amygdalina* supplementation significantly improved quantity.

iv. Elevated level of antioxidant enzymes in MNU controls apparently supports the adaptive response theory of animals to oxidative stress due to a Xenobiotic exposure such as MNU. Treatment with plants showed varied effects. However, a more definitive effect was observed in results from liver where an antoxidant effect by *Telfairia occidentalis* was generally inferred

as supplementation increased, whereas *Vernonia amygdalina* had antioxidant effect at 5% supplementation.

Integrative inferences from the above parametric results suggests a anti-cytoproliferative potential possessed by both *Vernonia amygdalina* and *Telfairia occidentalis* .The foregoing therefore proposes *Vernonia amygdalina* and *Telfairia occidentalis* as potentially useful prophylactic dietary anti-cytoproliferative agents against malignancy such as cancer.

6.3 RECOMMENDATION

The similitude of a dose independent anti-cancer activity inferred from this research seem corroborative. These findings thus provides a window for further exploration using other isolated active flavonoids and polyphenols abundant in these plants, in this case vernonioside, myricetin and isoharmentin, in the hope of standardizing active principles in them and other potential plants.

REFERENCES

Adamson R, Sieber S. (1979). The use of nonhuman primates for chemical carcinogenesis studies in Regulatory Aspects of Carcinogenesis and Food Additives: the Delaney Clause.San Francisco: Academic Press. pp. 275-302.

Adaramoye O, Achem J, Akintayo O, Fafunsho N(2007) Hypolipidemic Effect of *Telfairia occidentalis* (Fluted pumpkin) in Rats fed a cholesterol-Rich Diet. *Journal of Medicinal Food* 10(2):330-336

American cancer Society (2014), *Coloretal cancer and figures* 2014-2015

Anyan, G. N., Ogunowo, A. and Omotosho O. O. (2009).Oxidative Stress status in vegetarians and non-vegetarians. *Journal Life & Physical Sciences. Acta SATECH* 3 (1): 110-113.

Archer MC. (1989) Mechanism of action of N-Nitroso compounds; *Cancer Survey*; Review; 8 (2):241-50.\

Asuku O, Atawodi, S.E and E. Onyike (2012) Antioxidant, Hepatoprotective, and Ameliorative Effects of Methanolic Extract of Leaves of *Grewia mollis* Juss. On CarbonTetrachloride Treated Albino Rats *Journal of Medicinal Food1*5(1):83-88

Atawodi S E (2011) Nigerian foodstuffs with prostate cancer chemopreventive polyphenols *Epub*, 6 (Suppl 2):S9

Atawodi S., Atawodi J., Idakwo G., Beate P, Roswitha H, Gerd W, Helmut B and Robert W (2010) Evaluation of the polyphenol content and antoxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam *journal of Medicinal food.* 13(3):710-16

Aymelek Gönenç, Aysun Hacışevki, Sabahattin Aslan, Meral Torun, Bolkan Şimşek (2012) Increased oxidative DNA damage and impaired antioxidant defense system in patients with gastrointestinal cancer. *European Journal of Internal Medicine.*23(4):350-4.

Balaiya V., Rana P. S, Alpna T. and Rajesh A (2010): Inhibition of Azoxymethane-Induced Colonic Aberrant Crypt Foci Formation by Silibinin in Male Fisher 344 Rats, p1 10

Bamishaiye E. I., Olayemi F.F., Awagu E. F. And Bamishaiye O. M., (2011) Proximate and phytochemical composition of Moringa oleifera Leaves at Three Stages of

Maturation. *Advanced Journal of Food Society and Technology* 3 (4): 233-237, ISSN: 2042-4876

Beland FA, Dooley K.L, Sheldon W.G, Delongchamp R.R. (1988). Circadian variation in the induction of intestinal tumours by *N*-methyl-*N*-nitrosourea in male C57BL/6N mice. *Journal of National Cancer Institute* 80(5): 325-330.

Blake M, Stephon S, Carlson R, Stites D, Speigel D.(2004) Cytotoxic T lymphocyte count and survival time in women with metastatic breast cancer. *The Breast Journal:*10(3):195-199

Bosland M, Prinsen M., Rivenson A, Silverman J, Fiala E, Williams GM, Kroes R, Weisburger JH. (1992). Induction of proliferative lesions of ventral prostate, seminal vesicle, and other accessory sex glands in rats by *N*-methyl-*N* nitrosourea: effect of castration, pretreatment with cyproterone acetate and testosterone propionate and rat strain. *Prostate* 20(4): 339-353.

Brunker H, Lavin P, Plaxe S, Storch J, Livingstone E(1982)Absolute granulocyte, lymphocyte and monocyte counts. Useful determinants of prognosis for patients with metastatic Cancer of the stomach. *Journal of the American Medical Association* 247:1004-1006.

Cairns J (1978) *Cancer and Society* W.H Freeman and Company. Pgs 89,157,61. ISBN 0 7167 0098-2

Chang C, Yang M, Wen H and Chen (2002) Estmation of total flavonoid content in propdis by 2 complimentary colorimetric methods. *Journal of Food Drug Analysis* 10:178-182

Chen J, Stampfer M J, Hough H L, Garcia-Closas M, Willett WC, Hennekens C H, Kelsey K T, Hunter D J (1998): A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. *Cancer Resource*, 58:3307-3311.

Chintalapally V. R.,Wang C.Q, Barbara S, Jose G.R, Indranie C, Karam E, Bandaru S.R, (2001) Chemo-prevention of Colon Cancer by a Glutathione Conjugate 1,4-Phenylenebis(methylene) selenocyanate,; A Novel Organo-selenium Compound with Low Toxitcity pp. 1-12 Decker Inc.

Clement G., Ernest B, and Paul B. (2013) *Vernonia amygdalina*-Induced Growth Arrest and Apoptosis of Breast Cancer (MCF-7) cells *Pharmacology & Pharmacy* 4(1): 93-99

Committee on the Effects of Atomic Radiation (UNCEAR), (1993). Report to the General Assembly. United Nations, New York. ISBN 978-92-1-142274-0

Cummings S., Eckert S, Krueger K, (1999). The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the more randomized trial.

Multiple Outcomes of Raloxifene Evaluation; *Journal of American Medical Association,* 281: pp. 2189–2197

Cuthbert E. Dukes, (1965). The origin and early history of the imperial cancer research fund: A lecture given to the Staff of the fund. *Annals of Royal College of Surgeons of England*. 36(6): 325–338.

Das U.N. (2002) A radical approach to cance**r** *Medical Science Monitor*, 8(4), RA79–RA92

David L, Mathieu P, Sylvie L, Dominique B, Denis G and Richard B (2009) The Flavonols Quercetin, Kaempferol, and Myricetin Inhibit Hepatocyte Growth Factor-Induced Medulloblastoma Cell Migration. *Journal of Nutrition.* 139(4) :646-652

Devasena T, Rajasekaran K., Menon V (2002). Bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene 3,5-dione (A curcumin analogue) ameliorates DMH-Induced hepatic oxidative stress during colon carcinogenesis. *Pharmacological Resource*; 46(1): 39-45.

Doyle V (2007)Nutrition and Colorectal Cancer Risk: A Literature Review *Gastroenterology Nursing*:30(3):178-182

Einhorn L H, Improvements in Fluorouracil chemotherapy? (1989) *Journal of Clinical Oncology*; 7:1377-1379

Eisenberg E, Murthy AS, Vawter GF, Krutchkoff DJ. (1983). Odontogenic neoplasms in Wistar rats treated with *N*-methyl-N-nitrosourea. *Oral Surgery, Oral Medicine, Oral Pathology* 55(5): 481-486.

Eleanor N, Corinne B, Linda K, Sharon R (2001) *Nutrition for health care.* Peher Marshal (Second edition) pp 565-566.

Eseyin O A, Ebong P, Ekpo A, Igbasoiyi A, Oforah E (2007) *Pakistani Journal of Biological Sciences* 10(3):498-501

Evropi T, Geraldine M,Roseanne C, Susan F, Albert T, Rebecca B, Mary P, Malcom D,Harry C (2007) Dietary Fatty Acids and Colorectal Cancer: A Case-Control study. *American Journal Epidemiology* 166 (2):181-195. doi: 10.1093/aje/kwm063

Fereshteh E. G, Abdollah R. G, Shalmoli B, Devinder K.D, (2009): Selenium as a chemopreventive agent in experimentally Induced colon carcinogenesis; *World Journal of Gastrointestinal Oncology*,1 (1):74-81

Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L,

Wolmark N (1998) Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *Journal of the National Cancer Institute;* 90**:**1371-1388.

Fumagalli L, Vinke J, Hoff W, Ypma E, Brivio F, Nespoli A (2003) Lymphocyte counts independently predicts overall cancer patients: a biomarker for IL-2 immunotherapy. *Journal of immunotherapy* 26:394-401

Furukawa F, Sato H, Imaida K, Toyoda K, Imazawa T, Takahashi M, Hayashi Y. (1992), Induction of pancreatic tumours in male Syrian golden hamsters by intraperitoneal N-methyl-*N*-nitrosourea injection. *Pancreas* 7(2): 153-158.

Gordian C. Obute and Godswill O. Adubor (2007), Chemicals detected in plants used for folk medicine in south eastern Nigeria. *Ethnobotanical Leaflets 11: 173-194*

Gresham L J, Ross J, Izevbigie E B (2008) *Vernonia amygdalina*: anticancer activity, authentication, and adulteration detection. *International Journal Environmental Resources and Public Health* (5):342-8.

Grubben (2004) Vegetables, *PROTA Wageningen*, 3: 543-544

Howlader N, Noone A.M, Krapcho M, Garshell J, Miller D, Altekruse S.F, Kosary C.L, Yu M, Ruhl J, Tatalovich Z,Mariotto A, Lewis D.R, Chen H.S, Feuer E.J, Cronin K.A (eds) (2014). SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov.csr/1975_2011/, based on November 2013.

Ibrahim K O, Anjorin A S, Afolayan A E, Badmo K B (2011), Morphology of colorectal carcinoma among Nigerians: A 30-year review. *Nigerian Journal of clinical practise* 14: 432-435

International Agency for Reseach on Cancer. 1972. Nitrosomethylurea. In *Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines,* N-*Nitroso Compounds and Natural Products*: IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 1. pp. 125-134.

International Agency for Research on Cancer (1978) N-Nitroso-N-Methylurea in some N nitroso compounds: IARC monographs on Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 17. Lyon, France, *International Agency for Research on Cancer*: pp 227 255

Italiano J E Jr, Richardson J L, Patel-Hett S (2008) Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood,* 111: 1227-33

Ivano A, Francesca C, Alexy A, Massimiliano A, Gerry M (2014) Serine and glycine metabolism *Trends in Biochemical sciences in Cancer*, 39(4):191-198

Jaramillo Sara, Lopez Sergio, Valera M. Lourdes, Rodriguez-Arcos Rocio Abia, Jimenez , Rafael Guilenand, Francisco JG (2010)The Flavonol Isorhamnetin Exhibits Cytotoxic Effects on Human Colon Cancer Cells , *Journal of Agricultural Food Chemistry 58* (20), pp 10869–10875

Jemal A, Bray F, Centre M, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *A Cancer Journal for Clinicians, 6*1:69-90

Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun M (2003) Cancer statistics .*A Cancer Journal for Clinician* 53:5–26.

John D (1996),Nutrition and colorectal cancer. *Cancer,causes and control* (7) 1:127-146

June C (2010) From incurable to curable. Source: Mind your body, The Straits Times. Retrieved from: http://www.healthexchange.com.sg/News/Pages/Cancer-From incurable-to curable.aspx

Kılınçalp S, Ekiz F, Başar O, Omer B, Mehmet A, Sahin C, Bans Y, Akif A, Nurcan B, Bora T, Hail E, Engin U, Elife E and Osman Y(2013). Mean platelet volume could be possible biomarker in early diagnosis and monitoring of gastric cancer. *Platelets* 25(8):592-594

Koshimizu K, Ohigashi H, Huffman M.A (1994). Use of *Vernonia amygdalina* by wild chimpanzee: possible roles of its bitter and related constituents. *Physiology and Behaviour*. 56(6):1209-16.

Kupchan SM, Hemingway RJ, Karim A, Werner D (1969) Tumour inhibitors. XLVII. and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del *Journal of Organic Chemistry*; 34 (12):3908-11.

Laekeman G M, De Clerck F, Vlietinck A J, Herman A G (1985). Vernolepin: an antiplatelet compound of natural origin. Naunyn Schmiedebergs *Archive of Pharmacology*; 331(1):108-13.

Laekeman G M, Mertens J, Totté J, Bult H, Vlietinck A J, Herman A G (1983). Isolation and pharmacological characterization of vernolepin. *Journal of Natural Products*.; 46(2):161-169.

Lijinsky W, Kovatch RM. (1996). Similar carcinogenic actions on nitrosoalkylureas of varying structure given to rats by gavage. *Journal of Clean Technology Environmental Toxicology and Occupational Medicine* 5(2): 155 165

Likhachey AJ, Ohshima H, Anisimov VN, Ovsyannikov AI, Revskoy SY, Keefer LK, Reist E J. (1983) Carcinogenesis and aging. 2. Modifying effect of aging on metabolism of methyl(acetoxymethyl)nitrosamine and its interaction with DNA of various tissues in rats. *Carcinogenesis* 4(8): 967-973

Lucio T,Angelo Z,Sergio S, Colonoscopy(2014), Pain and fears: Is it an indissoluble trinormal? *World Journal of Gastrointestinal Endoscopy*; 6(6):227-233

Lui K, Li G, Fan C, Zhou X, Wu B and Li J,(2011)Increased expression of Micro Mrna 21 and its Association with chemotherapeutic response in Human colorectal cancer. *The Journal of International Medical Research, 39:2288-2295.*

Luke G D, Desai T K, Conteas C N, Moshier J A,Silverman A L(1998); *Gastroenterology Clinics of North America*; 17(4): 931-940

Luo X, Jiang Y, Fronczek F. R, Lin C, Izevbigie E .B, Lee K. S (2011). Isolation and structure determination of a sesquiterpene lactone (vernodalinol) from *Vernonia amygdalina* extracts. *Pharmaceutical Biology*.; 49(5):464-70. doi: 10.3109/13880209.2010.523429.

Luo X, Oyugi DA, Lin C, Izevbigie EB, Lee KS (2010). Isolation and characterization of the anti-breast carcinoma cell growth components of *Vernonia amygdalina*extracts. *Experimantal Biology and Medicine (Maywood);235(12):1472-1478.* doi: 10.1258/ebm.2010.010124. *Epub*

Michael D (2004) Nutrition and cancer: A review of the evidence for an anti-cancer diet *Nutrition Journal* 3:19

Misra H. P. and Fridovich I. (1972) Chemistry and Metabolism of Substances of low molecular weight. *Journal of Biological Chemistry* 277: 3170-3175.

Monreal M, Fernandez-Llamazares J, Piñol M, (1998). Platelet count and survival in patients with colorectal cancer-a preliminary study. *Thrombosis and Haemostasis*, 79: 916-918.

Mueller M.S., Mechler E. (2005) Medicinal Plants in Tropical Countries, Traditional Use Experience--Facts, Georg Thieme Verlag, Stuttgart, Germany, ISBN 1-58890-253-6

Mutoh M, Takahasi M, Fukuda K, (2000). Suppression of nitrosourea Induced Colon nitrosourea Induced Colon Carcinogenesis in Rats by Oxygenated Carotenoid Capsanthin and Capsanthin-Rich Paprika Juice. *Experimental Biology and Medicine* 224 (2);116 122

Noh H, Eomm M, Han A (2013). Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients. *Journal of Breast Cancer*, 16: 55-59

Ogunbinu A.O, Flamini G, Cioni P.L, Ogunwande IA, Okeniyi SO (2009). Essential oil constituents of Eclipta prostrata (L.) L. and *Vernonia amygdalina* Delile. *Natural Product Communication*. ; 4(3):421-4

Ohkawa W, Ohishi N, Yagi K. (1979) Assay For Lipid Peroxides In Animal Tissues By Thiobarbituric Acid Reaction. *Analytical Biochemistry*. ;95:351–358.

Okoli E., and Mgbeogu M (1983). Fluted Pumpkin, Telfairia occidentalis: West African Vegetable Crop. *Economic Botany* 37(2):145-149

Oyewole O. A., Abalaka M. E. (2012,) Antimicrobial Activities of *Telfairia occidentalis* (fluted pumpkins) Leaf Extract against Selected Intestinal Pathogens. *Journal of Health Science* 2(2): 1-4 DOI: 10.5923/j.health.20120202.01

Oyugi DA, Luo X, Lee KS, Hill B, Izevbigie EB (2009). Activity markers of the an-breast carcinoma cell growth fractions of *Vernonia amygdalina* extracts. *Experimental Biology and Medicine* (Maywood).;234(4):410-7. doi: 10.3181/0811-RM 325.

Patterson J, Boateng J, Walker L.T. and Verghese M., (2012). Cytotoxic Effects of Multiple N-nitrosamines in Human Liver Cell Line Hep2G: Possible Mechanisms of Action. *Journal of Pharmacology and Toxicology, 7: 114-127*

Pichler M, Hutterer G.C, Stoeckigt C, (2013). Validation of the pre-treatment neutrophil Lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. *British Journal of Cancer*, 108, 901-907. DOI: 10.1038/bjc.2013.28

Posadzki P, Lee M S, Onakpoya I, Lee H W, Ko B S, Ernst E (2013) Dietary supplements and prostate cancer: a systematic review of double blind, placebo-controlled randomised clinical trials. *Maturitas*.75(2): 125-130.

Raju K, Balasubramanian R, Perumal P (2011) In vitro and in vivo anticancer activity of *Indigofera cassioides* Rottl. Ex. DC. *Asian Pacific Journal of Tropical Medicine pp.* 379- 385

Ruldera E, Irazu C. E, Rajagopalam P, Orak K, Fitts C, Singh I (1988) Fatty acid Metabolism in Renal Ischemia. *LIADS*, (23)9, pp882-884

Sankarasharma D, Vani G, Chennam S,(2006) Suppression of tumor growth and invasion in 9,10 dimethyl benz(*a*) anthracene Induced mammary carcinoma by the plant bioflavonoid quercetin. *Chemio-Biological interaction CHEM-BIOL INTER* 162(2):106 113

Shibutani M, Maeda K, Nagahara H, et al (2013). A High preoperative neutrophil-to lymphocyte ratio is associated with poor survival in patients with colorectal cancer. *Anticancer Research*, 33, 3291-4.

Singha, S.C (1965). Medicinal Plants of Nigeria; Nigerian National Press: Lagos, Nigeria .

Sinha.A K. Calorimetric Assay of Catalase (1972). *Analytical*. *Biochemistry*. 47, 389-394

Sleisenger H Marvin, John S. Fiordtran (1998) Gastro intestinal Disease-Pathophysiology diagnosis managemet Fourth edition vol 2. Published by W,B Saunders company;1483,1484,1487-1488

Smith D.M, Rogers A.E, Newberne P.M. (1975) Vitamin A and benzo(a)pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. *Cancer Research*:35(6):1485 1488

Smulow JB, Konstantinidis A, Sonnenschein C. (1983) Age-dependent odontogenic lesions in rats after a single i.p. injection of *N*-nitroso-*N*-methylurea. *Carcinogenesis* 4(9): 1085 1088

Sporn M.D, Dunlop N.M, Newton D.L, Smith J.M (1976); Nutritional manipulation as a therapy for caner, *Federation proceeding.* 35, 1332

Steward W, Brown K (2013) Cancer chemoprevention: a rapidly evolving field, *British Journal of cancer*, 109(1):1-7

Sun N.C, McAfee W.M, Hum G.J, Weiner J.M (1979). Hemostatic abnormalities in malignancy, a prospective study of one hundred eight patients. Part I. Coagulation studies. *American Journal of Clinical Pathology*, 71: 10-6.

Sung Keun Jung, Ki Won Lee,sanguine Byun, Eun Jung Lee, Jong-Eun Lee,Jong-Eun Kim,Ann Bode, Ziang Dong, and Hyong Joo Lee(2010). Myricetin inhibits UVB Induced angiogenesis by regulating PI-3 kinase in vivo. *Carcinogrnesis* 10.1093/carcin/bgp221

Tamano S, Rehm S, Waalkes MP, Ward JM. (1996). High incidence and histogenesis of seminal vesicle adenocarcinoma and lower incidence of prostate carcinomas

in the Lobund Wistar prostate cancer rat model using *N*-nitrosomethylurea and testosterone. *Veterinary Pathology* 33(5): 557-567

Tatematsu M, Ogawa K, Hoshiya T, Shichino Y, Kato T, Imaida K, Ito N.(1992) Induction of adenocarcinomas in the glandular stomach of BALB/c mice treated with *N* methyl-*N*-nitrosourea. *Japan Journal of Cancer Resourc* 83(9): 915-918

Tavani A, Pelucchi C, Parpinel M, Negri E, Franceschi S, Levi F, La Vecchia (2003) C. n-3 polyunsaturated fatty acid intake and cancer risk in Italy and Switzerland. *International Journal of Caner* 105:113–116,

Trosko J.E, Chang C.L, Madhukar B.V, Emmanuel D (1995). Intercellular communication: a paradigm for the interpretation of the initiation, promotion, progression model of carcinogenesis. *Chemical induction of cancer, SpringerLink* 205-225

Tuveson D, Chandel N (2014) The promise and preils of Antioxidants for cancer patients. *New England Journal of Medicine*, 371(2):177-178

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (1993). Sources and Effects of Ionising Radiation, 1993 Report to the General Assembly, United Nations, New York.

Verma N, Vinayak M (2008).Effect of *Terminalia arjuna* on antioxidant defense system in cancer. *Molecular Biology Reports.* 36 (1):159-64.

Veronesi U, Maisonneuve P, Costa A. (1998) Italian Tamoxifen Prevention Study. Prevention of breast cancer with tamoxifen. preliminary findings from the Italian randomised trial among hysterectomised women. *Lancet*. ;352:93-97

Walsh SR, Cook EJ, Goulder F, (2005). Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *Journal of Surgical Oncology*, 91: 181-184.

Wang CX, Watanabe K, Weisburger JH, Williams GM. (1985) Induction of colon cancer in inbred Syrian hamsters by intrarectal administration of benzo[*alpha*]pyrene, 3 methylcholanthrene and *N*-methyl-*N*-nitrosourea. *Cancer Letters* 27(3): 309-314.

Woisky, R. and Salatino, A. (1998). Analysis of propolis:some parameters and procedures for chemical quality control. *Journal of. Apicultural. Research.* 37: 99-105.

Yang J, Shikata N, Mizuoka H, Tsubura A.(1996). Colon carcinogenesis in shrews by intrarectal infusion of *N*-methyl-*N*-nitrosourea. *Cancer Letters* 110(1-2): 105 112.

Yedjou C, Izevbigie E, Tchounwou P. Preclinical assessment of *Vernonia amygdalina* leaf extracts as DNA damaging anti-cancer agent in the management of breast cancer. *International Journal for Environmental Research and Public Health*. 2008;5 (5):33741.

Zimmerman JA, Trombetta LD, Carter TH, Weisbroth SH. (1982) Pancreatic carcinoma induced by *N*-methyl-*N'*-nitrosourea in aged mice. *Gerontology* 28(2): 114-120.

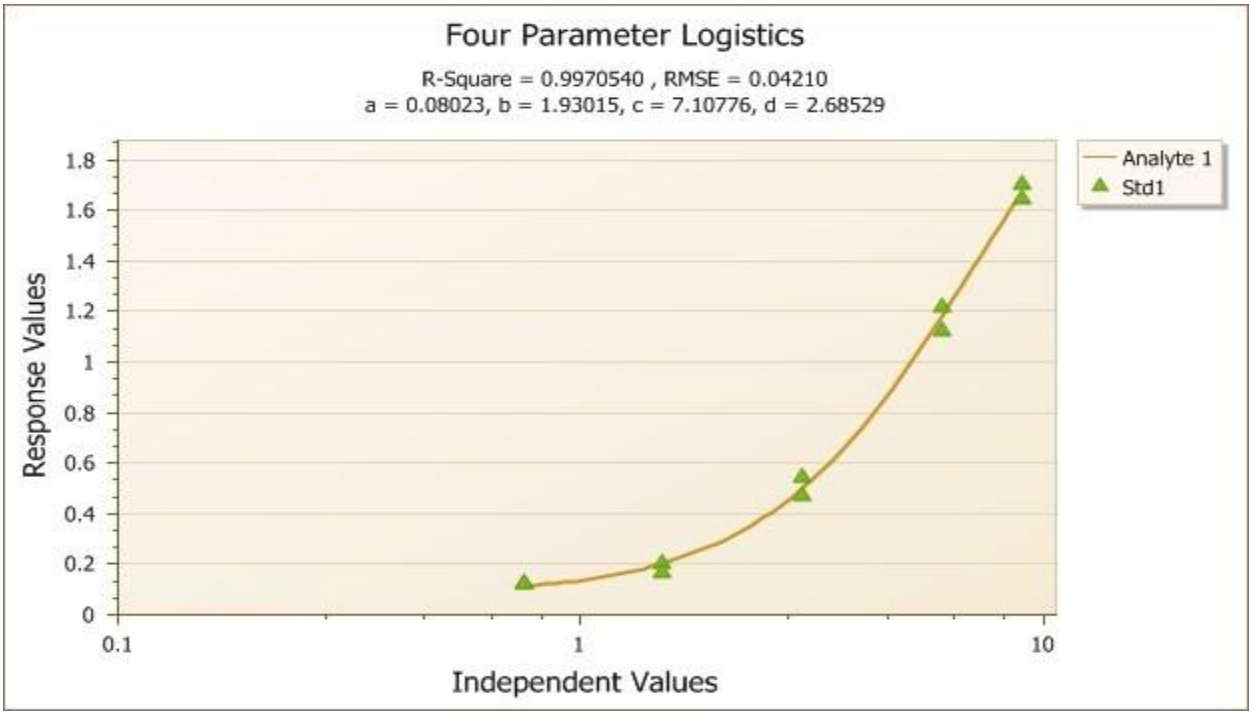
APPENDICES

APPENDIX 1.0

Carcinoembryonic Antigen Concentration in Serum of animals fed with diets supplemented with *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

CEA CONCENTRATION (ng/ml)		
TREATMENT	***Vernonia amygdalina***	***Telfairia occidentallis***
0%+NS	0.89±0.10	0.89±0.10
0%+MNU	1.51±0.14	1.52±0.14
2.5%+MNU	1.62±0.11	2.12±0.29
5%+MNU	0.83±0.18	1.80±0.07
10%+MNU	1.58±0.11	1.18±0.16
10%+NS	1.56±0.11	1.59±.16

APPENDIX 2.0



Well	Sample Name	Ignored	Absorbance	Calculated:	Standard concent	Residuals	% Recovery
Analyte: Analyte 1							
Group Name: Stadard							
E1	ss	☐	0.12	0.85	0.75	0.10	113.33
E2		☐	0.13	0.87	0.75	0.12	116.00
D1		☐	0.17	1.25	1.50	-0.25	83.33
D2		☐	0.20	1.50	1.50	0.00	100.00
C1		☐	0.47	2.88	3.00	-0.12	96.00
C2		☐	0.54	3.21	3.00	0.21	107.00
B1		☐	1.12	5.75	6.00	-0.25	95.83
B2		☐	1.21	6.21	6.00	0.21	103.50
A2		☐	1.65	8.79	9.00	-0.21	97.67
A1		☐	1.71	9.24	9.00	0.24	102.67

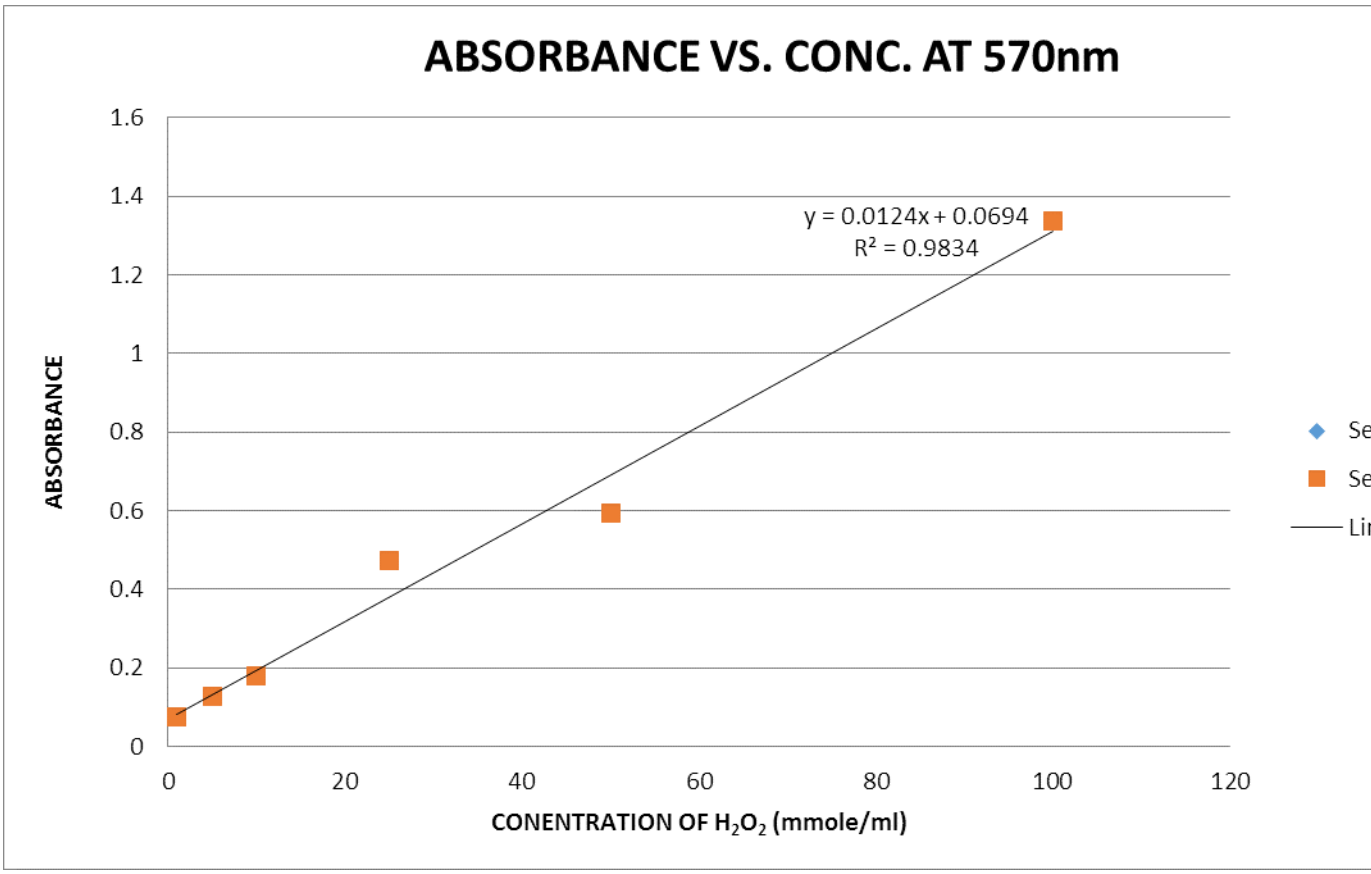
ELISA Standard curve for CEA determination Using Mastrplex 2010® software

APPENDIX 3.0

Catalase activity in liver and kidney of animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

	CATALASE ACTIVITY (mmole H_2O_2/min/ml/mg Protein)			
	LIVER		**KIDNEY**	
TREATMENT	***Vernonia amygdalina***	***Telfairia occidentalis***	***Vernonia amygdalina***	***Telfairia occidentalis***
0%+NS	2012.30±210.25	2012.309±2.10	253.55±56.31	253.55±56.31
0%+MNU	2996.23±125.25	2996.234±1.25	493.19±55.03	493.19±55.02
2.5%+MNU	2461.69±368.47	2004.507±1.84	286.80±80.13	361.16±27.80
5%+MNU	2206.74±70.03	2300.9±3.28	244.63±42.04	388.39±94.87
10%+MNU	1779.83±214.03	2533.92±3.68	258.39±55.77	663.70±103.09
10%+MNU	3053.03±298.23	3622.092±4.04	412.04±17.43	422.26±15.66

APPENDIX 4.0



Standard curve used for catalase activity determination

APPENDIX 5.0

Superoxide dismutase activity in Liver and kidneyof animals fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

	SOD ACTIVITY (U/ml/mg protein)			
	LIVER		**KIDNEY**	
TREATMENT	***Vernonia amygdalina***	***Telfairia occidentalis***	***Vernonia amygdalina***	***Telfaria occidentallis***
0%+NS	114.10±18.68	114.10±18.68	147.09±19.42	147.09±19.42
0%+MNU	231.69±34.38	231.69±34.38	164.96±71.33	164.96±71.32
2.5%+MNU	135.53±35.30	358.52±39.50	206.20±21.64	222.93±17.14
5%+MNU	331.62±19.25	226.54±14.39	173.36±73.56	138.71±3.47
10%+MNU	77.10±46.70	135.66±17.39	273.35±65.79	268.71±14.15
10%+NS	235.32±21.43	101.52±21.41	200.46±5.16	341.31±14.87

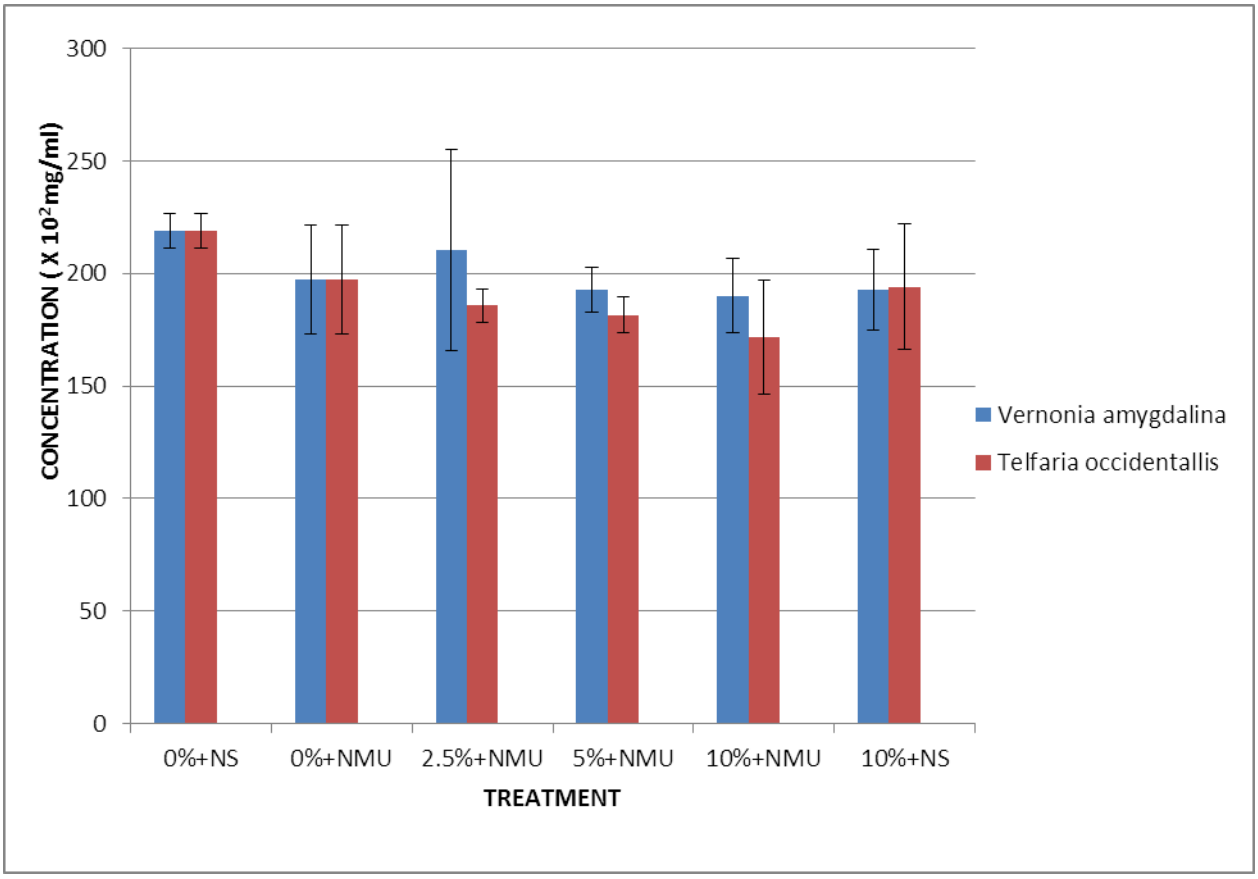
APPENDIX 6.0

TBARS Levels in Liver and kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

TBARS CONCENTRATION (µmole/ml/mg protein)

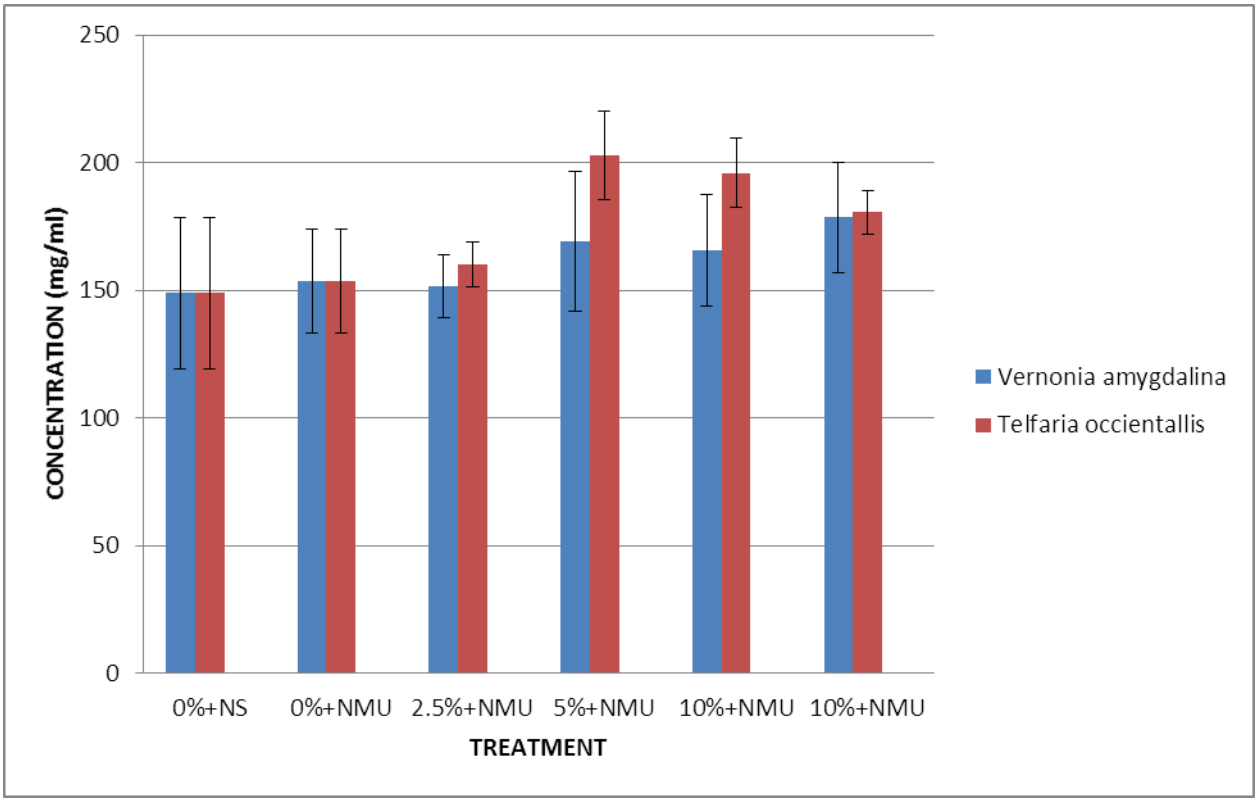
	LIVER		**KIDNEY**	
TREATMENT	***Vernonia amygdalina***	***Telfairia occientallis***	***Vernonia amygdalina***	***Telfairia occientallis***
0%+NS	35.95±3.27	35.95±3.27	23.45±6.69	23.45±6.69
0%+MNU	40.37±3.97	40.37±3.97	28.44±8.19	28.45±8.19
2.5%+MNU	5.14±1.00	14.29±4.62	35.47±3.28	77.84±1.12
5%+MNU	21.96±2.62	17.83±2.93	25.30±4.56	5.99±0.62
10%+MNU	25.28±4.74	16.50±2.45	15.04±2.60	14.93±2.79
10%+MNU	67.27±3.11	13.536±1.51	25.53±1.55	15.76±0.98

APPENDIX 7.0



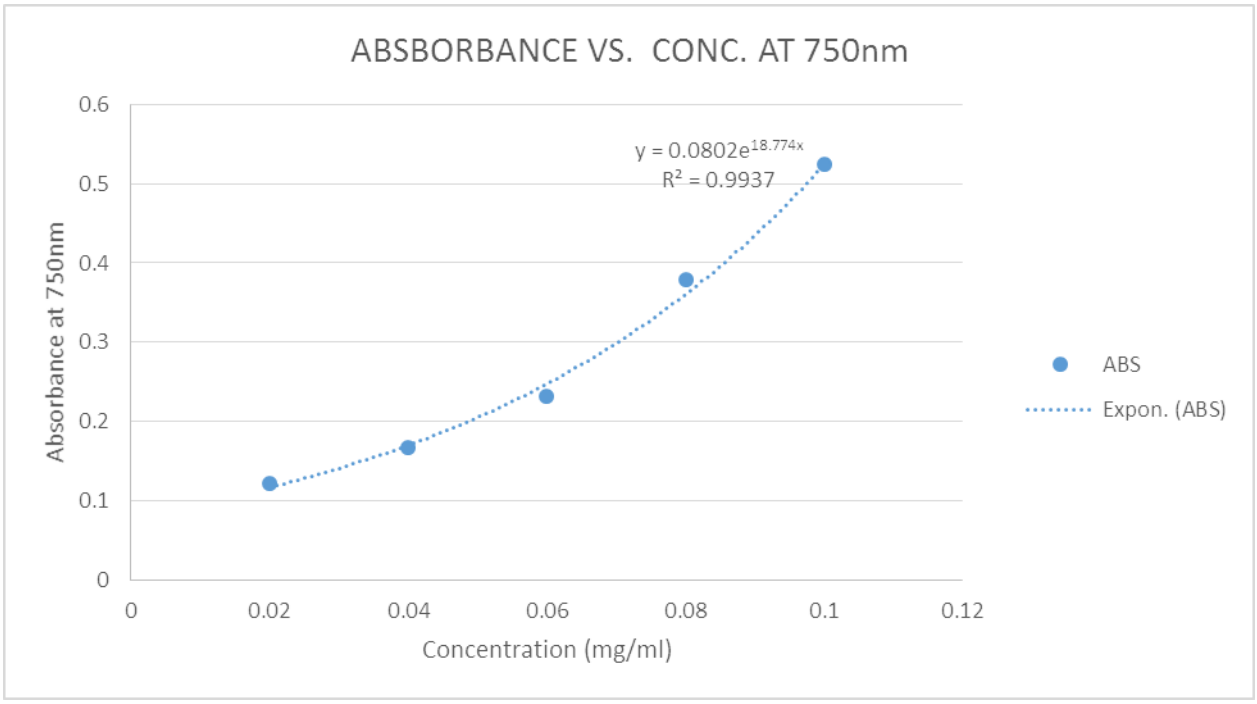
Total Protein concentration in Liver of subjects fed with diet supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU.

APPENDIX 8.0



Total Protein levels in kidney of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

APPENDIX 9.0



Standard curve for Total protein determination using Egg albumen .

APPENDIX 10.0

Hematological Parameters of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

	WBC(x10^9/L)	LYMP(%)	HGB(g/L)	RBC(x10^{12}/L)	HCT (%)
0%+NS(control)	12.40±1.78^{ab}	75.83±2.12^{c}	143.0±5.00^{a}	8.22±0.03^{ab}	37.27±1.16^{a}
0%+MNU(control)	15.25±3.03^{ab}	75.80±2.13^{c}	150.50±10.67^{a}	8.77±0.55^{ab}	38.60±2.59^{a}
2.5%VA+MNU	18.26±7.90^{b}	71.13±4.50^{abc}	147.75±5.50^{a}	8.87±0.205^{c}	38.50±0.73^{a}
5%VA+MNU	19.73±9.21^{b}	67.97±.47^{abc}	142.00±6.08^{a}	8.33±0.25^{ab}	36.77±1.69^{a}
10%VA+MNU	9.13±1.60^{a}	75.50±1.25^{bc}	137.50±5.07^{a}	8.24±0.41^{ab}	36.03±1.24^{a}
10%VA+NS	9.18±1.97^{a}	67.00±4.15^{abc}	141.50±5.26^{a}	8.46±0.33^{ab}	36.48±0.96^{a}
2.5%TO+MNU	9.08±3.52^{a}	75.10±6.99^{abc}	141.00±12.89^{a}	8.29±0.65^{ab}	36.35±3.05^{a}
5%TO+MNU	13.95±4.32^{ab}	66.05±6.29^{a}	149.50±6.37^{a}	8.79±0.078^{ab}	37.95±1.62^{a}
10%TO+MNU	15.90±2.62^{ab}	66.75±8.89^{ab}	142.25±7.93^{a}	8.32±0.13^{ab}	37.18±1.59^{a}
10%TO+NS	19.03±3.00^{b}	70.60±1.44^{abc}	137.33±16.04^{a}	8.11±0.38^{a}	36.03±3.20^{a}

APPENDIX 11

Table 2: Hematological parameters Cont'd

Hematological parameters of animals fed with diets supplemented with varying levels of *Vernonia amygdalina* and *Telfairia occidentalis* and exposed to MNU

GROUP	MCV(fL)	MCH (pg)	MCHC(g/L)	PLT x10^9 (/L)	MPV (fL)
0%+NS(control)	45.40±1.39^{b}	17.33±0.55^{b}	383.33±5.51^{b}	783.00±172.03^{a}	7.43±0.80^{a}
0%+MNU(control)	44.10±0.716^{ab}	17.13±0.22^{b}	389.50±3.42^{ab}	865.25±122.46^{a}	7.60±0.25^{a}
2.5%VA+MNU	43.45±0.252^{a}	16.60±0.37^{ab}	383.25±8.78^{ab}	831.50±77.43^{a}	7.18±0.47^{a}
5%VA+MNU	44.17± 0.72^{ab}	17.00±0.27^{b}	386.00±1.00^{ab}	908.33±324.20^{a}	6.87±0.29^{a}
10%VA+MNU	43.80±1.30^{ab}	16.65±0.45^{ab}	381.25±2.22^{ab}	865.00±86.65^{a}	7.25±0.76^{a}
10%VA+NS	43.15±0.733^{a}	16.65±0.24^{ab}	387.50±4.12^{ab}	899.75±247.08^{a}	7.10±0.50^{a}
2.5%TO+MNU	43.92±1.30^{ab}	16.95±0.55^{ab}	387.25±3.59^{ab}	817.00±166.72^{a}	7.15±0.49^{a}
5%TO+MNU	44.15±1.06^{ab}	17.30±0.42^{b}	393.50±0.71^{b}	922.00±24.042^{a}	7.35±0.35^{a}
10%TO+MNU	43.65±0.94^{ab}	16.63±0.60^{ab}	382.25±9.03^{ab}	945.25±71.89^{a}	7.68±0.26^{a}
10%TO+MNU	42.73±1.16^{a}	16.16±0.569^{a}	380.00±13.00^{a}	920.67±55.78^{a}	7.63±0.74^{a}