EVALUATION OF THE PREVENTIVE EFFECT OF DIETARY INCLUSION OF *HYPHAENE THEBAICA* FRUIT (LINN) ON N-METHYL-N-NITROSOUREA-INDUCED COLON CARCINOGENESIS IN MALE WISTAR RATS

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ZARIA, NIGERIA

OCTOBER 2017

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A DISSERTATION 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 NUTRITION

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

OCTOBER, 2017

DECLARATION

I declare that the work in this Dissertation entitled **"Evaluation of the Preventive Effect of Dietary Inclusion of *Hyphaenethebaica* fruit (L.) on N-methyl-N-nitrosourea-induced Colon Carcinogenesis in Rats"** has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution

YUSUF ABDULRAZAQ	Signature	Date
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CERTIFICATION

This dissertation entitled **"Evaluation of the Preventive Effect of Dietary Inclusion of *Hyphaene thebaica* fruit (L.) on N-Methyl-N-nitrosourea-induced Colon Carcinogenesis in Rats"** by **ABDULRAZAQ YUSUF** meets the regulations governing the award of the degree of Master of Science in Nutrition of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. S.E. Atawodi, FAS

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Dean, School of Postgraduate Studies _____ Signature _____ Date

DEDICATION

This research work is dedicated to Almighty Allah for the gift of life, Strength and enablement to undertake this programme successful, and my caring and beloved mother HajiaFatimatAbdulsalam and father Alhaji Yusuf Abdulsalam.

ACKNOWLEDGEMENTS

With sincere gratitude to Almighty Allah for giving me the profound strength wisdom and grace throughout my period of study at Ahmadu Bello University, Zaria. I whole heartedly give thanks to Almighty Allah for guiding and protecting me throughout my study period I will forever live to glorify your name, oh! Allah.

My profound gratitude goes to my supervisor, father and mentor Professor S.E. Atawodi, FAS, for all the support and encouragement both financially and strength throughout the course of this work, He really took his time, energy and resources to see that this project work is successfully completely. May Almighty Allah reward him abundantly and upgrade his honour. Also, I'm equally grateful to my co-supervisor Dr. I. A. Aimola for his immense contribution. May Almighty Allah reward him abundantly.

I sincerely appreciate the Head of Biochemistry Department for permission to use available facilities. My unreserved gratitude goes to Mr. Apeh for his tireless and physical support rendered to me during the course of this research work. May God increase him in health and wealth. Lastly, my appreciations to all the entire lecturers in Biochemistry Department, May Allah upgrade their honour.

My special thanks go to my caring father Alh. Yusuf A. Abdulsalam and my dear mother HajiaFatimatAdelani and HajiaAishatAdelani, whom their support both financially and morally can never be estimated. May Allah reward them abundantly. Also, my appreciation goes to my entire family members, particularly my brother MutiuAdelani, HaneefAyinla, Ayub, Lukman, AbdulqodeerAdelani, HajiaBaliqeesSheriffdeen, MuneerAdelani, HajiaRofiatMutiu, MutiatAdelani, MujidatAdelani, HajiaMonshedatAbdulwahab, HajiaNafisatAbdulwahab and my lovely sister Princess BaliqeesAbdulwahab for their tireless support. May Allah increase them in health and wealth, AMEEN.

My unreserved gratitude goes to my friends Mr. Abdulfatah Ismail, Mr. AbdulazizYunus, Mr. UsmanSani Suleiman, Mr. Mumini S. Adeiza, Mallam Abdulrahman Al-hadib, Mr. AbdulwasiuAjibola, Mr. Abdulrazaq T. Abdullahi,Mr. HamisuYerima,Mr. QozeemAdeyemo, HajiaSherifat A. Misbah and to my colleagues in this renowned research work (the cancer research group, a.k.a My MNU family), Dr. Mubarak, Dr. Uju, Mr. Ochaie, Dr. Ojo, Ishaq Ibrahim, Ofodileh John Ejimofor, Solomon Alhassan, Onuche Job, JamilaLawal, and Mr. Labaran for their moral support. May Allah increase them in health and wealth, AMEEN.

ABSTRACT

The preventive effect of dietary inclusion of *Hyphaene thebaica* fruit at 0%, 2.5%, 5% and 10% on colon carcinogenesis initiated with N-methyl-N-nitrosourea (MNU) induction for twelve (12) weeks was evaluated. After twelve (12) weeks of treatment, the colon and liver of rats administered *H. thebaica* fruit included diet showed a significant ($p \leq 0.05$) increase in the activity of endogenous antioxidant enzymes (catalase and superoxide dismutase) when compared with MNU control group. A dose-dependent decrease in the extent of lipid peroxidation was observed in the colon and liver of dietary inclusion of *H. thebaica* fruit treated groups. A significant ($p \leq 0.05$) increase in packed cell volume, haemoglobin count, red blood cell, platelet level were observed in dietary inclusion of *H. thebaica* fruit treated groups when compared with MNU control group, while a significant ($p \leq 0.05$) decrease in white blood cell, neutrophil and lymphocyte concentration were observed in dietary inclusion of *H. thebaica* fruit treated groups when compared with MNU control group. Histological observation of colon and liver tissue sections showed focal area of inflammatory cells in the MNU control group, while dietary inclusion of*H. thebaica* fruit fed groups, showed milder to no tissue damage, while a statistically significant ($p \leq 0.05$) decrease in Carcino-Embryonic Antigen (CEA) levels was observed in the group fed with dietary inclusion of *H. thebaica* fruit compared with MNU control group. Immunohistochemical observation of the colon tissue sections showed no expression of *Mut L-Homolog* gene (MLH1) in MNU control group, while mild to moderate expression ofMLH1 in the groups treated with dietary inclusion of *H. thebaica* fruit. These results indicate the positive potential of dietary inclusion of *H. thebaica* fruit to prevent oxidative stress caused by free radical generation and hence, preventing the process of chemically induced colon carcinogenesis by boosting the endogenous antioxidant system. A better preventive effect was observed in the group treated with highest percentage of dietary inclusion of*H. thebaica* fruit (10% of *H. thebaica* fruit).

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LIST OF ABBREVIATIONS

ABC	Avidin Biotin Complex
ACF	Aberrant crypt foci
ANOVA	Analysis of Variance
AORTIC	African Organization for Research and Training in Cancer
APC	Adenomatous polyposis coli
AST	Aspartate aminotransferase
BHA	Butylated Hydroxyl Anisole
BHT	ButylatedHydroxyl Toluene
CAT	Catalase
CEA	Carcinoembrayonic antigen
CIN	Chromosomal instability
CIMP	CpG island methylator phenotype
CRC	Colorectal cancer
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EVF	Erythrocyte volume fraction
FAP	Familial adenomatous polyposis
GLOBOCAN	Global burden on cancer
GPx	Glutathione Peroxidase
GSHPx	Glutathione peroxidase

Hb	Haemoglobin
HDL	High Density Lipoprotein
HNPCC	Hereditary non-polyposis colon cancer
HNE	Hydroxynonenal
Ht or Hct	Haematocrit
IBD	Inflammatory bowel diseases
LDL	Low Density Lipoprotein
LYMPHO	Lymphocyte
MDA	Malondialdehyde
MSI	Microsatellite instability
MLH1	*Mult L homologue* 1
MMR	Mismatch repair gene
MNU	N-methyl nitrosourea
NADPH	Nicotinamide Adenine dinucleotide Phosphate Reduced
NCI	National Cancer Institute
NICE	National Institute for Health and Care Excellence
NUETRO	Nuetrophil
PCV	Packed cell volume
PG	Propyle gallate
PUFAs	Polyunsaturated Fatty Acids
RBC	Red blood cell count
RNS	Reactive nitrogen specie
ROS	Reactive Oxygen Species

SOD	Superoxide Dismutase
TAG	Triacylglyerol
TBA	Thiobarbituric Acid
WBC	White blood cell count
WHO	World health organization

CHAPTER ONE

1.0 INTRODUCTION

Cancer is a leading cause of death worldwide. An estimated 14.1million new cancer cases occurred in 2012 (GLOBOCAN, 2012). Lung, female breast, colorectal and stomach cancers accounted for more than 40% of all cases diagnosed worldwide (GLOBOCAN, 2012). In men, lung cancer was the most common cancer (16.7% of all new cases in men). Breast cancer was by far the most common cancer diagnosed in women (25.2% of all new cases in women), (Srijita-Dutta, 2015). Approximately, 32.5 million People diagnosed with cancer in 2008 were alive at the end of 2012. Most were women after their breast cancer diagnosis (6.3 million), men after their prostate cancer diagnosis (3.9 million), and men and women after their colorectal cancer diagnosis (3.5 million) (Popoola *et al*., 2013).

The incidence of colorectal carcinoma has been on the increase in the developing countries, including Nigeria, as a result of change in diet and adoption of western lifestyle (Echendu *al.*, 2015). A total of 241 cases of colorectal carcinoma were reported, 144 cases (60%) in males and 96 cases (40%) in females with a male: female ratio of 1.5:1. The peak age of occurrence for males was between 51 and 60 years, while that of the females was between 41 and 50 years. The malignancy was found in the rectum in 60.2% of the cases, while the least affected site is the descending colon (1.2%) (NJCP, 2011).

However, colon cancer is a disease of the large intestine which begins at a structure called the caecum, located in the right lower quadrant of the abdomen and continues through all portions of the abdomen to its junction with the rectum, located in the deep pelvis (Yeatman, 2001). Colon cancer begins from colon polyps that turn cancerous and individuals who develop polyps are at the highest risk of colon cancer (College of American pathologists, 2011). Most colorectal cancers develop slowly over several years. Before a cancer develops,

a growth of tissue or tumor usually begins as a non-cancerous polyp on the inner lining of the colon or rectum (American Cancer Society, 2014).

Colorectal cancer is a disease in which abnormal cells in the colon or rectum divide uncontrollably, ultimately forming a malignant tumor (American Cancer Society, 2014), the colon and rectum are parts of the body's digestive system, which takes up nutrients from food and water and stores solid waste until it passes out of the body.

Colorectal cancer is the third most common type of non-skin cancer in both men (after prostate cancer and lung cancer) and women (after breast cancer and lung cancer). It is the second leading cause of cancer death in the United States after lung cancer (American Cancer Society, 2014). The rates of new colorectal cancer cases and deaths among adults aged 50 years or older are decreasing in United States. However, the incidence is increasing among younger adults (American Cancer Society, 2014). It is estimated that a total of 136,830 people in the United States will be diagnosed with colorectal cancer and 50,310 people will die from it in 2014 (American Cancer Society, 2014). It's also estimated that a total of 50,260 deaths from colorectal cancer will occur in 2017 (American Cancer Society, 2017).

Although the major risk factors for colorectal cancer are a family history and older age, several other factors have been associated with increased risk, including excessive alcohol use, obesity, being physically inactive, cigarette smoking, and possibly diet. In addition, people with a history of inflammatory bowel disease (such as Ulcerative colitis or Crohn disease) have a higher risk of colorectal cancer than people without such conditions, and people who have a family history of colorectal cancer or certain inherited conditions (such as Lynch syndrome and familial adenomatous polyposis) also have an increased risk of colorectal cancer (Sankaranarayanan *et al.*, 2010).

Several screening tests have been developed to help doctors find colorectal cancer early, when it may be more treatable. Some tests that detect adenomas and polyps can actually prevent the development of cancer because these tests allow growths that might otherwise become cancer to be detected and removed. That is, colorectal cancer screening may be a form of cancer prevention, not just early detection (Sankaranarayanan *et al.*, 2010).

Natural plants, vegetables, herbs and spices used in folk and traditional medicine remain the most potent and promising vehicles of chemopreventive effects with these now the main sources of leading compounds for discovery and development of chemo-preventive therapies (Dwivedi *et al.*, 2011). Several plants and vegetables highly consumed in Africa have been reported by several studies to possess potent chemopreventive properties. Plants such as *Moringa oleifera*, Palm oil, *Canarium schweinfurthii* oil, *Syzygium aromaticum, Hibiscus esculentum,* and *Dacryodes edulis* contain potent phytochemicals that have been used to suppress gastric and skin carcinomas, also have been tested in various cell culture and animal based models in the prevention of cancers of the colorectum, renal, skin and lungs. More have been observed to possess potent antioxidant potential able to prevent or limit the effects of oxidative stress which is an important precursor of cancers (Atawodi *et al*., 2011).

1.1 Statement of Research Problem

Cancer is still one of the most deadly diseases that cause serious health problems, physical disabilities, mortalities, and morbidities around the world (GLOBOCAN, 2012). Although great advancement has been made in the treatment of cancer progression, there is still significant deficiencies and room for improvement (Sankaranarayanan *et al.*, 2010). Colorectal carcinoma is the commonest malignancy of the gastrointestinal tract worldwide, with about 140,000 cases each year in the United States, according to the National Cancer Institute. Incidence and death rates for colorectal cancer increase with age. In Africa, the

incidence is said to be relatively low, because of the young age of the population, low incidence of precancerous lesions and high intake of fiber diet, which results in passage of bulky stool with short intestinal transit time. This decrease might be attributable to differences in dietary intake and environmental exposures. For instance, more people tend to eat more of refined, preserved food rather than freshly prepared high fibre food which they are originally used to (Center *et al*., 2009). Other reason might be due to urbanizations or movement of people from rural to urban leading to high intake of food high in carcinogens.

The treatment of colorectal cancer constitutes a great diagnostic and therapeutic challenge in resource-limited countries such as Nigeria (Abdulkareem *et al*., 2008). Late presentation of the disease, lack of an adequate screening program, lack of endoscopic facilities and lack of adjuvant therapy are among the hallmarks of the disease in most developing countries. Some of the major problems of colon cancer treatment are the unpredictable post operative survival rate and the reoccurrence (Atkin *et al.*, 2010). Others are the complications that accompany resection and the damages caused by radiotherapy; recently resistance to anticancer drugs has been observed (Abdulkareem *et al*., 2008). Therefore, more research is required to explore the anticancer or preventive potential of dietary supplementation with medicinal plants.

1.2 Justification

Most current drugs such as cisplatin, adriamycin and bleomycin designed for the treatment of colon cancer are very expensive, inaccessible and unaffordable to most poor patients (Boni *et al.*, 2009). Thus, natural preventive agents that are safe, available, accessible, affordable and sustainable are urgently needed for the prevention of chronic diseases such as colon cancer. Epidemiological and experimental studies demonstrated the importance of some vegetables, fruits and herbs in diets and medicinal plants in inhibiting the development and multiplicity of tumours and hence reducing the risk of colon cancer (Abdulkareem *et al.*, 2008). Plants,

vegetables, fruits, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemo preventive drugs (*Canarium schweinfurthii* oil, *Syzygium aromaticum, Dacryodes edulis* and *Hibiscus esculentum*) and can protect against a wide range of cancers, heart diseases and other chronic diseases (Dwivedi *et al.,* 2011).

Thus, dietary inclusion of *Hyphaene thebaica* fruit in meals for the prevention of colon cancer and also cancers of other parts of the body like the breast, prostate and rectum would be culturally acceptable in many countries like Western World and Africa, including Nigeria where these fruit are already part of their cultural practice.

1.2 General Aim

The aim of this work is to evaluate the preventive effect of dietary inclusion of *Hyphaene thebaica* fruit on MNU induced colon carcinogenesis in albino wistar rats.

1.4 Specific Objectives

(i) To determine the effect of *Hyphaene thebaica* fruit inclusion diet on haematological parameters in MNU-induced colon carcinogenesis in rat model.

(ii) To establish the possible role of Hyphaene *thebaica* fruit inclusion diet on oxidative stress and antioxidant status in MNU-induced colon carcinogenesis in rat model.

(iii) To assess the effect of *Hyphaene thebaica* fruit on MNU-induced colon tissue damage in rat model.

1.5 Null Hypothesis

Dietary inclusion of *Hyphaene thebaica* fruit has no preventive effect on colon carcinogenesis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Colon Cancer

Colorectal cancer (CRC) is the third most common cancer (Jemal *et al.,* 2011) and the second leading cause of cancer deaths in the United States (Jemal *et al.,* 2010). Overall risk of colon cancer is approximately 5% in the general population without family history, with 92% of colorectal cancers diagnosed after age 50 (Parkin, 2008). Unfortunately, 20% of those diagnosed with colon cancer are diagnosed in late, less treatment-responsive stages (Parkin, 2008). It is estimated that routine screening of everyone over the age of 50 could save 18,800 lives annually (Popoola *et al*., 2013). Primary care practitioners are uniquely positioned to influence adherence to screening procedures, recognize those at highest risk of disease, and address modifiable risk factors in their patients. Those at greatest risk for colon cancer are carriers of 1 of 2 familial genetic disorders, familial adenomatous polyposis (FAP) or hereditary non-polyposis colon cancer (HNPCC), also known as Lynch syndrome (Foley *et al.,* 2006). These genetic disorders account for 1–2% and 3–5% of all colorectal cancers, respectively. While the incidence of colorectal cancer cases directly attributable to these genetic syndromes is small, they are highly penetrant, with a lifetime risk of 100% and 80% of developing colorectal cancers in those with familial adenomatous polyposis and Lynch syndrome respectively (Foley *et al*., 2006).

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, also raise the risk of colorectal cancers considerably, while inflammatory bowel diseases related colorectal cancers account for only 1–2% of all Colorectal cancer cases, colorectal cancer accounts for 10–15% of deaths in patients with inflammatory bowel diseases (Jemal *et al*.,

2011). Risk reduction for these patients should focus on controlling the underlying disease, thus reducing the chronic inflammation that is an integral part of the carcinogenic process.

In addition to genetic syndromes and inflammatory bowel diseases, established risk factors include age, personal history of adenomas, family history of colorectal cancer, diets high in fat and low in fruit/vegetables, obesity, sedentary lifestyle, smoking, and excessive alcohol intake (Parkin, 2008). A recent meta-analysis has suggested that diabetes mellitus is also an independent risk factor (Atkin *et al.,* 2010). Up to 70% of colorectal cancers may be due to preventable habits involving diet and lifestyle (Anand *et al*., 2008). The National Cancer Institute (NCI) of America has created an online risk assessment tool to calculate the risk of colon cancer in patients over 50 years old. The National Cancer Institute (2011) of America, identifying the risk factors relevant for individual patients provides an opportunity for clinicians to educate patients on how to modify their risk.

2.2 Incidence of Colon Cancer

There were an estimated 14.1 million cancer cases around the world in 2012 (GLOBOCAN, 2012). Of those cancers, 7.4 million were in men, while 6.7 million were in women (GLOBOCAN, 2012). Nearly, 1.4 million of those new cancer cases were from colorectal cancer (GLOBOCAN, 2012).

According to American Cancer Society (2015), colorectal cancer is the third most common cancer in both women and men after breast and prostate cancer, respectively, and lung cancer, the breakdown between genders is similar in the United States (American Cancer Society, 2015). Among both gender groups, it is the second leading cause of cancer deaths (Behind lung cancer), with peak incidence being in the seventh decade of life (Ferri, 2015). In 2015, it was estimated that there will be 848,200 new cases of cancer among men and

810,000 among women in 2015 (American Cancer Society, 2015). Of those new cancer cases, 8% will comprise of colon and rectal cancer, with an estimated 69,090 in men and 63,610 in females (American Cancer Society, 2015).

The incidence of colorectal cancer is increasing in certain countries where risk was historically low (Japan) (Center *et al*., 2009). In high-risk/high-income countries, trends are either gradually increasing (Finland, Norway), stabilizing (France, Australia), or declining (United States) with time (Center *et al*., 2009). The greatest increases in the incidence of colorectal cancer are in Asia (Japan, Kuwait and Israel) and Eastern Europe (Czech Republic, Slovakia and Slovenia). In fact, rates among males in the Czech Republic, Slovakia, and Japan have not only exceeded the peak rates observed in long-standing developed countries such as the United States, Canada, and Australia but they continue to increase (Center *et al*., 2009). In contrast to the stabilizing rates observed in most Western and Northern European countries, relatively large increases have been observed in Spain, which may be related to the increasing prevalence of obesity in recent years in that country (Martin *et al*., 2008). The decrease in colorectal cancer incidence in the United States partially reflects the increase in detection and removal of precancerous lesions (Center *et al*., 2009). The increase in several Asian and Eastern European countries may reflect changes in risk factors for colorectal cancer that are associated with westernization such as elevated obesity and smoking prevalence (Center *et al*., 2009). In contrast to incidence trends, decreasing colorectal cancer mortality rates have been observed in a large number of countries worldwide and are most likely attributed to colorectal cancer screening and/or improved treatments. However, increases in mortality rates are still occurring in countries that have more limited resources, including Mexico and Brazil in South America and Romania and Russia in Eastern Europe (Center *et al*., 2009).

Colorectal cancer is the third most common cancer in men and the second in women. Worldwide, an estimated 1.2 million cases of colorectal cancer occurred in 2008. The highest incidence rates were in North America, Australia, New Zealand, Europe, and Japan. Rates were low in Africa and South Central Asia. Rates were substantially higher in men than in women.

Colorectal cancer (CRC) is a malignancy with a relatively long preclinical phase which provides a unique opportunity for screening and early detection. Many developed countries have either established programmatic colorectal cancer screening endeavors or use healthcare systems that can facilitate early detection and treatment of colorectal cancer (Center *et al*., 2011). The story is quite different in continental Africa, although the burden of colorectal cancer is relatively lower in most countries in Africa when compared to developed countries; there is very little investment in colorectal cancer control. There is a general lack of colorectal cancer screening programs and poor access to standard treatment for this disease. Under the auspices of the African Organization for Research and Training in Cancer (AORTIC), the challenges and possible solutions to enhance colorectal cancer control were discussed at the 9th scientific meeting of the organization in Durban, South Africa in November 2013. Although inadequate research opportunities and data collection systems raise concerns of possible under-reporting, available data suggest a lower burden of colorectal cancer in Africa (Center *et al*., 2011). While the overwhelming majority of the population in Africa is of indigenous African origin (black), countries with substantial other racial groups, such as in South Africa, report significant population (racial) differences in colorectal cancer burden. The lowest burden of disease has been noted among blacks, and especially those who have maintained their traditional lifestyle including diet, an intermediate burden among Indians and those of mixed ancestry (coloureds) and the highest burden among persons of European origin (whites). This contrasts the findings in the United States where

African Americans suffer a disproportionally higher burden of colorectal cancer when compared to other population groups (American Cancer Society, 2015).

Colorectal carcinoma (CRC) is relatively uncommon in Nigeria, compared to the developed countries. In 2008, colorectal cancer was the third commonest cancer in males and the second in females worldwide. However almost 60% of these cases occurred in developed countries and only 2% of new cases and 3% of deaths due to colorectal cancer occurred in Africa (GLOBOCAN, 2008). Recent studies of colorectal carcinoma indicate an increasing trend, which has been attributed to the adoption of sedentary western lifestyle and diet, migration patterns and the improved availability of requisite specialized medical services (Ogunbajo *et al*., 2009). Patients with colorectal carcinoma in Nigeria and other developing countries often present at an earlier age than in developed countries, which has important implications for the molecular pathogenesis of colorectal carcinoma in our patients.

2.3 Causes and Mechanism of Colon Carcinogenesis

Carcinogenesis or oncogenesis or tumorigenesis is the formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by changes at the cellular, genetic, and epigenetic levels and abnormal cell division, in some cancers forming a malignant mass (Lim *et al*., 2006).

Cell division is a physiological process that occurs in almost all tissues and under many circumstances. Under normal circumstances, the balance between proliferation and programmed cell death, usually in the form of apoptosis is maintained by regulation of both processes to ensure the integrity of tissues and organs (Adams *et al.,* 2007). According to the prevailing accepted theory of carcinogenesis, the somatic mutation theory, mutations and epimutations in DNA that lead to cancer (only certain mutations and epimutations can lead to

cancer and the majority of potential mutations and epimutations will have no such effect) disrupt these orderly processes by disrupting the programming regulating the processes, upsetting the normal balance between proliferation and cell death (Adams *et al.*, 2007). This results in uncontrolled cell division and the evolution of those cells by natural selection in the body.

The uncontrolled and often rapid proliferation of cells can lead to benign tumors; some types of these may turn into malignant tumors (cancer). Benign tumors do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they compress vital structures or are physiologically active, for instance, producing a hormone. Malignant tumors can invade other organs, spread to distant locations (metastasis) and become life-threatening (Fearon and Vogelstein, 1990). More than one mutation is necessary for carcinogenesis. In fact, a series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell (Fearon and Vogelstein, 1990).

Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes that regulate cell growth and differentiation must be altered (Croce, 2008). Genetic and epigenetic changes can occur at many levels, from gain or loss of entire chromosomes, to a mutation affecting a single DNA nucleotide, or to silencing or activating microRNA that controls expression of 100 to 500 genes (Lim *et al.*, 2006; Balaguer *et al.*, 2010). There are two broad categories of genes that are affected by these changes. Oncogenes may be normal genes that are expressed at inappropriately high levels, or altered genes that have novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. Tumor suppressor genes are genes that inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are

required to transform a normal cell into a cancer cell (Wood *et al.*, 2007 and Daniel *et al.*, 2011).

2.3.1 Mechanisms of Carcinogenesis

Colorectal carcinoma can arise from one or a combination of three different mechanisms, namely chromosomal instability (CIN), CpG island methylator phenotype (CIMP), and microsatellite instability (MSI). According to Ferlay *et al*., 2015, the classical CIN pathway begins with the acquisition of mutations in the adenomatous polyposis coli (*APC*), followed by the mutational activation of oncogene *KRAS* and the inactivation of the tumor suppressor gene, *TP53*. Aneuploidy and loss of heterozygosity (LOH) are the major players in chromosomal instability tumors, which not only constitute most of the sporadic tumors (85%) but also involve familial adenomatous polyposis cases associated with germline mutations in the *APC* gene3 (Ferlay *et al*., 2015). The CIMP pathway is characterized by promoter hypermethylation of various tumor suppressor genes, most importantly *MGMT* and *MLH1*. This hypermethylation is often associated with *BRAF* mutation and microsatellite instability (Lopez-Lazaro, 2015). The MSI pathway involves the inactivation of genetic alterations in short repeated sequences. This activation occurs in CRCs in DNA mismatch repair (MMR) genes, and is a hallmark condition in familial Lynch syndrome (LS), which also appears in 15% of the sporadic CRC cases. In addition, the hypermethylation of the MMR genes may lead to microsatellite instability. This mechanism is often associated with the CIMP pathway (East *et al*., 2008). MSI tumors are often associated with proximal colon and poor differentiation but better prognosis. Moreover, the three mechanisms often overlap in CRC.

2.4 Diagnosis (Biomarkers) Carcinoembryonic antigen and Immunohistochemistry

Carcinoembryonic antigen (CEA) is a glycoprotein, which is present in normal mucosal cells but increased amounts are associated with adenocarcinoma, especially colorectal cancer.

Carcinoembryonic antigen therefore has a role as a tumour marker. Carcinoembryonic antigen (CEA) is one of the most widely used tumor markers worldwide (Richie *et al*., 2007). It main application is mostly in gastrointestinal cancers, especially in colorectal malignancy. Although in use for almost 30 years, the clinical value of Carcinoembryonic antigen in colorectal cancer is still not clear.

According to the 2001 American Association for Clinical Chemistry, Carcinoembryonic antigen (CEA) was first described in 1965 by Gold and Freedman, when they identified an antigen that was present in both fetal colon and colon adenocarcinoma but that appeared to be absent from healthy adult colon. Because the protein was detected in only cancer and embryonic tissue, it was given the name carcinoembryonic antigen, or CEA. Subsequent work showed that carcinoembryonic antigen, or at least a CEA-like molecule, was also present in certain healthy tissues, although concentrations in tumors were on average 60-fold higher than in the nonmalignant tissues (Baucher *et al.,* 2006). In one of the first reports on carcinoembryonic antigen in serum, (Thomson *et al*., 1991), found increased concentrations in 35 of 36 patients with colorectal cancer. In contrast, high values were not found in "normal" subjects, pregnant women, patients with nongastrointestinal cancers, or in patients with miscellaneous benign gastrointestinal diseases. Although these findings were not confirmed, they nevertheless prompted widespread use of carcinoembryonic antigen as a marker for colorectal cancer. Thirty years after its initial detection in serum, carcinoembryonic antigen is one of the most widely used tumor markers worldwide and certainly the most frequently used marker in colorectal cancer (Richie *et al*., 2007).

2.4.1 Conditions which may have elevated Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) may be elevated in colorectal cancer, which is where it is most clinically useful. However, it may also be elevated in a wide variety of other malignant, benign conditions, smoking and other chronic disease conditions.

2.4.2 Normal Range

Individual laboratory normal ranges vary but CEA level is usually deemed to be normal at 2.5-5 μg/L. increasing levels of CEA suggestive of active disease may be more clinically helpful than absolute level, (Sturgeon *et al*. 2008). Levels exceeding 10 μg/L are rarely due to benign disease or the moderate elevation that may occur due to smoking (Fuchs *et al.*, 1999).

2.4.3 Sensitivity

Sensitivity in early-stage colorectal cancer is very low and increases with the stage of the disease. Studies reporting sensitivity vary in the cut off for what constitutes a normal CEA level so results are varied. Using a cutoff point of 5μg/L, the proportions of patients with increased values are 3%, 25%, 45% and 65% for patients with Dukes' A, B, C and D disease respectively. Around 72% of cases with unrespectable or metastatic disease have elevated CEA levels (Richie *et al*., 2007).

2.4.4 Indications for Carcinoembryonic antigen Measurement Colorectal Cancer

Due to the low sensitivity and specificity of the Carcinoembryonic antigen test, it is not recommended to be used for screening of healthy individuals or for the diagnosis of early colorectal cancer. In natural disease, Carcinoembryonic antigen level should be ordered only after malignancy has been confirmed.

2.4.5 Prognosis

The Carcinoembryonic antigen test is of much more use in determining prognosis than it is as an early diagnostic test for colon cancer. Carcinoembryonic antigen levels are more likely to be elevated in advanced disease (Anand *et al*., 2008).

2.4.6 Monitoring Treatment

The major role for Carcinoembryonic antigen levels is in following patients for relapse after intended curative treatment of colorectal cancer. Carcinoembryonic antigen levels typically return to normal within four to six weeks after successful surgical resection. The Carcinoembryonic antigen level can also be used to assess the response to chemotherapy. It is recommended that people with stage II or III colorectal cancer have Carcinoembryonic antigen surveillance every three months for at least three years after treatment (Graham *et al.*, 1998). National Institute for Health and Care Excellence (NICE) guidelines recommend at least every six months, although most other authorities recommend every three months, (Sturgeon *et al*., 2008). Clinical trials have shown improvement in survival after five years in patients who underwent carcinoembryonic antigen monitoring as part of post-treatment management, (Sturgeon *et al*., 2008). However, normal levels do not necessarily indicate that recurrence has not occurred, (Hanna and Kangolle, 2010).

Currently, the most useful application of carcinoembryonic antigen is in the detection of liver metastasis from colorectal cancers. Because of the relative success of surgery in resecting hepatic metastases, serial determinations of the marker are recommended for detecting cancer spread to the liver (Hanna and Kangolle, 2010). In the future, preoperative concentrations of carcinoembryonic antigen may be included with the standard staging procedures for assessing prognosis.

2.4.7 Carcinoembryonic antigen as a Marker for Colorectal Cancer Screening

In screening for colorectal cancer, the aim should be to detect disease at either Dukes' A or B stage. Malignancy detected at more advanced stages is unlikely to be more treatable than that detected through the usual course of events. Using an upper limit of normal of 2.5 mg/L, calculated that carcinoembryonic antigen has a sensitivity of 36% and a specificity of 87% in screening for Dukes' A and B colorectal cancer (Fletcher,1986). These findings, combined with the low heterogeneity, which appears to be attributable to variations in its carbohydrate side chains (Thomas *et al.,* 1990). Which are mostly composed of mannose, galactose, N-acetylglucosamine, fucose, and sialic acid (Thomas *et al.*, 1990).

Carcinoembryonic antigen is a member of the immunoglobulin super family. Two types of immunoglobulin domains are found: an N-terminal domain of 108 amino acids homologous to the immunoglobulin variable domain (IgV-like) and six domains homologous to the immunoglobulin constant domain of the C-2 set (IgC2-like) (Thompson *et al.*, 1991 and Hammerstron *et al.,* 1999).

Carcinoembryonic antigen is attached to the cell membrane by a glycosyl phosphatidylinositol anchor and probably is released as a soluble form by a phospholipase C or phospholipase D, (Thompson *et al.,* 1991). Structural similarity of carcinoembryonic antigen to certain immunoglobulin-related proteins, such as ICAM-1 and ICAM-2, initially suggested that carcinoembryonic antigen might act as an adhesion molecule. *In vitro* experiments showed that carcinoembryonic antigen was capable of both homophilic (CEA binding to CEA) and heterophilic (CEA binding to non-CEA molecules) interactions. Because alterations in cell adhesion are causally involved in cancer invasion and metastasis, it was further suggested that carcinoembryonic antigen may play a role in these processes (Jessup *et al.,* 1989). Further evidence for a role in cancer dissemination was obtained by

(Hostetter *et al.,* 1990), who showed that after transplantation of colorectal tumors into nude mice, the number of liver metastases increased from 2% to 48% following injection of mice with carcinoembryonic antigen. There is, however, no direct evidence that carcinoembryonic antigen is causally involved in cancer dissemination.

Although *in vitro* data implicate carcinoembryonic antigen in cell adhesions, its localization to the apical surface of mature enterocytes in healthy human colon is difficult to reconcile with this role. In the healthy colon, carcinoembryonic antigen has been found to bind certain strains of *Escherichia coli*. According to Thompson *et al.,* 1991, this binding may facilitate bacterial colonization of the intestine. On the other hand, suggested that carcinoembryonic antigen may play a role in protecting the colon from microbial infection, possibly by binding and trapping infectious microorganisms.

2.4.8 Immunohistochemistry

Immunohistochemistry (IHC), or immunocytochemistry, is a method for localizing specific antigens in tissues or cells based on antigen-antibody recognition; it seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light microscopic level. Immunohistochemistry is a method used to determine the expression of biomarkers in tissue. It is used to characterize various surface and intracellular proteins from cells of all tissues as it looks at the pattern of proteins that are in the tumour. Among other things, IHC can be used to determine if you may have a hereditary form of cancer known as Lynch syndrome (Wolff *et al*., 2007). Immunohistochemistry has a long history, extending more than half a century from 1940, when Coons developed an immunofluorescence technique to detect corresponding antigens in frozen tissue sections. However, only since the early 1990s has the method found general application in surgical pathology (Taylor *et al*., 1994). A series of technical developments led eventually to the wide range of immunohistochemistry

applications in use today. The enzymatic label (horseradish peroxidase), developed by Avrameas and by Nakane and colleagues (1976), allowed visualization of the labeled antibody by light microscopy in the presence of a suitable colorogenic substrate system. In Oxford, Taylor and Burns developed the first successful demonstration of antigens in routinely processed formalin-fixed paraffin-embedded (FFPE) tissues (Taylor *et al*., 1994). A critical issue in the early development of immunoperoxidase techniques was related to the need to achieve greater sensitivity. Greater sensitivity would facilitate staining of FFPE tissues from a simple one-step direct conjugate method to multiple-step detection techniques such as the peroxidase antiperoxidase (PAP), avidin-biotin conjugate (ABC), and biotin-streptavidin (B-SA) methods and would eventually lead to amplification methods (such as tyramide) and highly sensitive " polymer-based" labeling systems (Adams *et al*., 2007).

It is the localization of antigens or proteins in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, or colloidal gold. The central reagent common to all immunohistochemical techniques is the antibody. The availability of new monoclonal antibodies, antisera and their immunoglobulin fractions to an ever-increasing number of clinically useful tissue biomarkers (antigens) has enormously expanded the quantity and quality of the immunohistologic repertoire (Hammond *et al*., 2010).

2.4.9 Basic Principles of Immunohistochemistry

Surgical pathologists have long recognized their fallibility, although they have not always publicized it (Taylor *et al*., 1994). They have, however, sought more certain means of validating morphologic judgments. A variety of "special stains" were developed to facilitate cell recognition and diagnosis; most of these early stains were based on chemical reactions of cell and tissue components in frozen sections (histochemistry) (Halliwell, 1997). In certain

circumstances, these histochemical stains proved to be of critical value in specific cell identification. More often, they served merely to highlight or emphasize cellular or histologic features that supported a particular interpretation without providing truly specific confirmation. When the new field of immunohistochemistry was created by combining immunology with histochemistry, a wide variety of truly specific special stains were generated.

The aims of immunohistochemistry are akin to those of histochemistry. Indeed, immunohistochemistry builds on the foundations of histochemistry; it does not replace histochemistry but rather serves as a valuable adjunct that greatly extends the variety of tissue components that can be demonstrated specifically within tissue sections or other cell preparations (Kaur *et al.*, 2011). As emphasized by pioneers in this field of functional morphology, "the objective of all staining is to recognize microchemically the existence and distribution of substances which we have been made aware of macrochemically (Mann, 1902).

The basic critical principle of immunohistochemistry, as with any other special staining method, is a sharp visual localization of target components in the cell and tissue, based on a satisfactory signal-to-noise ratio (Boland *et al.*, 2008). Amplifying the signal while reducing non-specific background staining (noise) has been a major strategy to achieve a satisfactory result that is useful in daily practice (Boland *et al.*, 2008). After more than two decades, advances in immunohistochemistry have provided a feasible approach to performing immunostaining on routinely processed tissues, such that this method is now "routine" for the performance of immunohistochemistry "special stains" in surgical pathology laboratories using formalin fixed paraffin embedded tissues. However, demands for improved reproducibility and for quantification has led to a growing recognition that

immunohistochemistry has the potential to be more than just a special stain. If properly controlled in all aspects of its performance, immunohistochemistry can provide a tissue-based immunoassay with the reproducibility and quantitative characteristics of an ELISA (enzyme-linked immunosorbent assay) test, which not only detects the presence of an "analyte" (protein or antigen) but also provides an accurate and reliable measure of its relative or real amount (Lynch *et al.*, 2008).

2.5 Animal Models in Colon Carcinogenesis

2.5.1 Rodent Colon Tumour Models (History and Overview)

The study of experimental colon carcinogenesis in rodents has had a remarkably long history, dating back almost 80 years (Crebs, 1928). Perhaps the earliest published study of Lorenz *et al.,* (1941), demonstrated tumorigenesis in the fore stomach and intestine of mice following feeding with the polycyclic aromatic hydrocarbon, methylcholanthrene. Lisco *et al*., (1947) reported that feeding radioactive yttrium to rats induced a high proportion of colon tumors. Walpole *et al.,* (1952) reported that white rats given injections of 4-aminodiphenyl and 3,2-dimethyl-4-aminodiphenyl developed colon tumors.

In summary, there are a number of advantages in studying the pathogenesis of carcinogen-induced colon cancer in rodent models. The models are highly reproducible, they can be readily tested on animals with different genetic backgrounds and the pathogenesis recapitulates human colorectal cancer, at least at the early stages. Historically, the majority of colon carcinogenesis studies have been carried out in rats (Madara *et al.,* 1983). However, the high frequency of tumors generated within the distal colon of mice, as well as the histogenesis of multiple adenomas with subsequent development of adenocarcinomas, also validate the importance of this species for studying the pathogenesis of colon cancer (Nambiar *et al.,* 2003). An advantage of murine models is the availability of extensive

genetic information on individual mouse lines, the existence of recombinant inbred mouse panels and the ever-increasing number of transgenic, knockout and knocks in genetic models that are available for study.

2.6 Role of MNU in Colon Carcinogenesis

N-methyl-nitrosourea (MNU), are among the primary causes of cancer (Verma *et al.* 2013). N-methyl nitrosourea an alkylating agent was reported to induce retinal degeneration in mice, rats, and other animals following systemic administration, providing a simple model of retinal degenerative diseases as well as tumours of other sites. It exhibits its toxicity by transferring its methyl group to nucleo bases in nucleic acids which can lead to AT: GC transition mutation (Swanson *et al.*, 1996).

2.7 Oxidative Stress and Cancer/ Colon Carcinogenesis.

The oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the oxidants potentially leading to damage has been suggested to be that which cause aging and various diseases in humans. In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept of maintaining a healthy biological system (Dreosti, 1991; Ahmad *et al*., 2012; Davies, 2000; Tiwari, 2001; Katalinic *et al.,* 2006). Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, parkinson's disease, immune dysfunction and ageing (Souri *et al*., 2008).

2.8 Free Radicals and Antioxidants

Free radicals, primarily the reactive oxygen species (ROS), superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism. As free radicals are

potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids.

The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT). These block the initiation of free radical chain reactions (Dusica and Vesna, 2002). The nonenzymatic antioxidant components consist of molecules such as glutathione, alpha tocopherol, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions.

However, when free radicals are generated in excess or when the cellular antioxidant defense system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death. Physiologically, antioxidants play a major role in preventing the formation of free radicals (Atawodi *et al.,* 2010), which are responsible for many oxidative processes. Antioxidants may be synthetic, such as butylated hydroxyanisole (BHA), Propyle gallate (PC) and butylated hydroxytoluene (BHT) or of natural origin such as alpha-tocopherol, phenolic compounds as well as polyphenolics (Abas *et al*., 2006).

Natural products of plant origin are major source of vitamin C, vitamin E, and beta –carotene and have been proposed as potential source of natural antioxidants with strong activity (Duh *et al*., 1999). This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, flavonols and proanthocyanidins (Rice-Evans *et al.,* 1996). Polyphenols are the major plant compounds with high level of antioxidant activity. This activity could be due to their ability to absorb, neutralize and to quench free radicals (Duh *et al*., 1999). Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid

peroxidation (Rice-Evans *et al*., 1996). Antioxidant is one of the most essential ingredients of today's therapies since they reduce *in vivo* oxidative damages. Plants are the good resources for natural antioxidants. 1,1- diphenyl-2-picrilhydrazyl (DPPH) radicals scavenging method is widely used to investigate the total antioxidant activities in plants. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease and in the ageing process. Antioxidants can be effective in the prevention of free radical formation by scavenging or promotion of their decomposition and thus, suppress such disorders. Hence, there is growing interest in natural antioxidants from herbal and food plant sources (Atawodi, 2010; Atawodi and Onaolapo, 2010; Ghanbari *et al*., 2012 and Zia-Ul-Haq *et al*., 2012a) as epidemiological and laboratory studies have strongly supported their therapeutic and protective efficacies in biological systems (Souri *et al*., 2008 and Atawodi, 2011). Besides, vegetables, spices and herbs are rich in natural antioxidants that protects against oxidative stress and thus play important roles in the therapy and chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species (Atawodi *et al.,* 2010a; b; Atawodi, 2011a and Manzoor *et al*., 2012).

In traditional Chinese medicine for more than 2000 years, a general recommendation to their consumption is to increase the intake of foods rich in antioxidant compounds (e,g polyphenols, carotenoids) due to their well-known healthy effects (Kitts, *et al*., 2000). As a consequence, these evidences accelerated the search for antioxidants principles, which led to the identification of natural resources and isolation of active antioxidant molecules. Many plants have been identified as having potential antioxidant activities and their consumption recommended (Kitts, *et al*., 2000; Lee *et al.*, 2003; Piao *et al*., 2008; Kilani *et al.*, 2008 and Wang *et al.,* 2009).

Bioactive phenols are very interesting as antioxidants because of their ability to act as efficient free radical Abbreviations: Butylated hydroxytoluene (BHT), 1,1- diphenyl-2-picrilhydrazyl (DPPH), acute myeloid leukemia (AML), scavenging (Langley-Evans, 2000). In the last two decades the number of publications on the potential health benefits of polyphenols, have increased enormously (Tiwari, 2001; Lee *et al*., 2003; Hinneburg *et al*., 2006 and Katalinic *et al*., 2006).

2.9 Haematological Parameters in Colon Carcinogenesis and their Applications as Possible Cancer Biomarker.

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood, the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Onyeyili *et al*., 1992 and Togun *et al.*, 2007). Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004), and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Mmereole, 2008 and Isaac *et al*., 2013). Haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). Haematological parameters are those parameters that are related to the blood and blood forming organs (Waugh *et al*. 2001 and Bamishaiye *et al*., 2009). Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions (Olafedehan *et al.*, 2010). As reported by Isaac *et al.*, (2013) animals with good blood composition are likely to show good performance.

Thus, laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body (Ogunbajo *et al*., 2009). The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004 and Doyle, 2006). According to Olafedehan *et al.,* (2010), examining blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals and humans. Blood constituents change in relation to the physiological conditions of health (Togun *et al.,* 2007). These changes are of value in assessing response of animals to various physiological situations (Khan and Zafar, 2005). According to Afolabi *et al.,* (2010), changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors.

2.9.1 Haematological Components and their Functions

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.,* 2013). Haematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Oyawoye and Ogunkunle, 2004). Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris, 1996 and Chineke *et al*., 2006). According to Isaac *et al*., (2013), red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be

carried to the tissues as well as the level of carbon dioxide returned to the lungs (Soetan *et al*., 2013 and Isaac *et al.,* 2013).

The major functions of the white blood cells and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al*., 2013) and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al*., 2011; Okunlola *et al*., 2012; Iwuji and Herbert, 2012 and Isaac *et al.,* 2013). Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury.

Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood (Purves *et al*., 2003). According to Isaac *et al.,* (2013), Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume shows a better transportation and thus results in an increased primary and secondary polycythemia. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Maton *et al.,* 1993), with the exception of the fish family, channichthyldae (Sidell and O 'Brien, 2006), as well as tissues of invertebrates. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al*., 2012; Soetan *et al.,* 2013 and Isaac *et al.,* 2013).

According to Peters *et al*., (2011), previous reports stated that Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al*., 2005 and Chineke *et al.,* 2006). Furthermore, Chineke *et al.,* (2006) posited that high packed cell volume (PCV) reading indicated either an increase in number of red blood cells (RBCs) or reduction in circulating plasma volume. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia (Aster, *et al.,* 2004).

2.10 Plants with Chemo-preventive Effect on Colon Carcinogenesis

2.10.1 *Hyphaene thebaica*

Doum palm (*Hyphaene thebaica*) is a desert palm native to Egypt, sub-Saharan Africa and West India. It is known in Egypt as the doum or gingerbread palm which grows to a height of 6 or 9 m and usually has forked stems with fan shaped leaves, 65–75 cm long. It is listed as one of the useful plants of the world (Fletcher, 1997). The trunk of the palm is used for construction, as well as for manufacture of various domestic utensils and the leaves used to make mats, bind parcels and writing paper. The oblong, yellow-orange apple sized fruit has a red outer skin, a thick, spongy and rather sweet, fibrous fruit pulp that tastes like gingerbread and a large kernel.

2.11 Distribution and Habitat

The doum palm is native to the northern half of Africa. It is widespread in the Sahel and grows from Mauritania and Senegal in the west, through Central Africa, and east to Egypt, Kenya and Tanzania. It tends to grow in areas where ground water is present and is found

along the Nile River in Egypt and Sudan, in riverine areas of northwestern Kenya, and along the Niger River in West Africa. It is also native to the Levant and the Arabian Peninsula (Israel, Sinai, Yemen and Saudi Arabia. and is reportedly naturalized in the Netherlands Antilles in the Caribbean (Facciola, 2014). It grows in wadis and at oases, but sometimes occurs away from water and on rocky hillsides. It dislikes waterlogged soils and is very resistant to destruction by bushfires (Brunken *et al.*, 2014).



Plate 2.1: Picture Showing *Hyphaene thebaica* tree and fruit

2.12 General Uses of *Hyphaene thebaica* fruit

The doum palm flourishes in hot dry regions where little else grows and the tree is appreciated for the shade it provides. All parts of the tree are useful, but probably the most important product is the leaves (El-Gendy *et al.,* 2008). The fibre and leaflets are used by people along the Niger and Nile Rivers to weave baskets, such as in the material culture of the Manasir. Other things made from the leaves are mats, coarse textiles, brooms, ropes, string and thatch (Brunken *et al.,* 2014). The timber is used for posts and poles, furniture manufacture and beehives, and the tree provide wood for fuel. The leaf stalks are used for fencing and the fibre is used for textiles. Other products include fishing rafts, brooms, hammocks, carpets, buttons and beads (Kamis, 2003).

2.13 Nutritional Composition of Different Parts

The doum palm fruit-dates are also known in Eritrea as *Akat,* or *Akaat* in the Tigre language. The thin dried brown rind is made into molasses, cakes, and sweetmeats. The unripe kernels are edible. The shoots of the germinated seeds are also eaten as a vegetable (Facciola, 2014). In Egypt, the fruit is sold in herbalist shops, and is popular among children, gnawing its sweet yet sour hard fibrous flesh beneath the shiny hard crust. In Diu, Una and Saurashtra region of Gujarat (India), the tree is known as *Hoka* Tree and the red ripe edible fruit is known as *Hoka*. In northern part of Nigeria, among the Hausa people, it's known as "*Goruba*". Apart from the use of the fruit as food, juice is extracted from the young fruit and palm wine is prepared from the sap (Kamis, 2003).



Plate 2.2: Picture Showing *Hyphaene thebaica* fruit

2.14 Uses as Recipe in Traditional Medicine

The fruit of the doum palm has been used in folk medicine to treat hypertension. In a trial, a group of patients with raised blood pressure were all given an antihypertensive drug but in half the individuals, this was supplemented with doum fruit extract. It was found that those receiving the supplement had lower systolic and diastolic pressures and lower total cholesterol, and the blood lipids and lipoproteins were changed in such a way as to decrease the risk of cardiovascular disease (El-Gendy *et al.,* 2008).



Plate 2.3: Picture Showing *Hyphaene thebaica* fruit

2.15 Phytochemical Constituents of Different Parts

Phytochemicals are non nutritive bioactive chemical compounds that occur naturally in plants such as polyphenol and flavonoids, which are considered to have beneficial effects on human health and have disease preventive properties. The presence of these compounds in *H. thebaica* fruit proved that the plant can be used as a potential food and drug (Hsu *et al.,* 2006, Faten, 2009). The doum palm is among the more important plant families that supplies human with dietary fibers, carbohydrates and anti-hypertension substances.

2.16 Culinary Uses of *Hyphaene thebaica* fruit

The Doum palm is among the more important plant families that supplies human with dietary fibers, carbohydrates and anti-hypertension substances. The covering of the fruit is edible and can either be pounded to form a powder or cut off in slices; the powder is often dried, then added to food as a flavouring agent (Orwa *et al*., 2011). The fruit pulp is used in cooking, in various ways, and the different varieties differ in their edibility. While the unripe kernel is edible, the ripe kernel is hard and used only as a vegetable ivory (Doren, 1999).

Previous studies on Doum had focused on the fruit because, besides its nutritional value, the fruit drink brewed from a hot water infusion of the dried fruit pulp is widely consumed as a health tonic and has been valued in the region for its many anecdotal medicinal properties. Research on the fruit pulp of *H. thebaica* showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Kamis *et al.,* 2003).

Also, the aqueous extract of doum fruits showed an antioxidant and anticancer activities; this is due to the substantial amount of their water-soluble phenolic contents (Hsu *et al.,* 2006; Faten, 2009).

The phytochemical, called lignans, found in *H. thebaica* fruit have apparent anti-carcinogenic action and antifungal activity against a wide range of fungal isolates as mentioned by Carter in (1993). Also, prominent antibacterial activities of doum fruits were reported against gram positive and gram negative bacteria (Dosumu *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Chemicals

The carcinogen (MNU) was purchased from BDH Chemical Ltd, Poole, England. Carcinoembryonic antigen assay kit was purchased from Diagnostic Automation Inc CA. Others are formal-saline, graded alcohol, xylene, paraffin, eosin and hematoxylin. All other reagents and chemicals of analytical grade obtained from Sigma Alderish.

3.2 Materials and Equipment

Based on the experimental designed, the material and equipment used are listed below:
Dissecting set, temperature regulated centrifuge (TGL-16G. B. Bran Scientific & Instrument England), homogenizer, UV spectrophotometer (Jenway 6305 model, 50/60Hz, U.K.), microscope, hand glove, electrical weighing balance (GF-2000, A&D Instrument Ltd, Japan), water bath (Grant NL-420S series, Grant Instruments Ltd, England), microplate reader (GM-3000, model 200711012; B. Bran Scientific Company England), micropipette, pH meter (Jenway 3150 model, UK.), EDTA bottles, foils paper, razor blades and refrigerator.

3.3 Sample Collection and Preparation

Ripe dried fruit of *Hyphaene thebaica* was purchased from Samaru Market, Zaria and taken to the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification, authentication and a voucher number of the specimen is 3252. The fruit of *Hyphaene thebaica* was sun-dried, pounded with pestle and mortar to obtain their powder form and the powder was stored in air-tight glass containers at room temperature until used in the experimental diet for dietary inclusion with standard feed based on the experimental design.

3.4 Experimental Diet

Grower mash feeds produced by UAC livestock's company (vital feed with registration number RC54291) was purchased and used in the entire experiment. The feeds served as base line for supply of nutrient needed for the growth of the rats, also used to mix the powdered fruit of *Hyphaene thebaica* fruit according to the percentage of dietary inclusion (0%, 2.5%, 5% and 10%) respectively.

3.5 Experimental Animals

A total of fourty-two (42) healthy male Albino rats of the wistar strains of 5-6week of age, weighing 50-80g were purchased from the animal unit of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University Zaria. The animals were housed in standard cages at room temperature at the Department of Biochemistry Animal House, Ahmadu Bello University Zaria and allowed free access to water and grower mash feeds for two weeks to acclimatize.

At the end of the acclimatization period (one week), all animals were weighed and randomly divided into six (6) groups of seven animals each (n=7). The experimental animals were fed according to the group's percentage of dietary inclusion or standard diet for four (4) weeks and subsequently induced with MNU for period of 12 weeks or normal saline. The groups are as follows:

(i) Normal saline + standard feeds.

(ii) MNU control: MNU+ standard feed.

(iii) MNU + dietary inclusion of 2.5% *Hyphaene thebaica* fruit powder.

(iv) MNU + dietary inclusion of 5% *Hyphaene thebaica* fruit powder.

(v) MNU + dietary inclusion of 10% *Hyphaene thebaica* fruit powder.

(vi) Dietary inclusion control normal saline + 10%*Hyphaene thebaica* fruit powder.

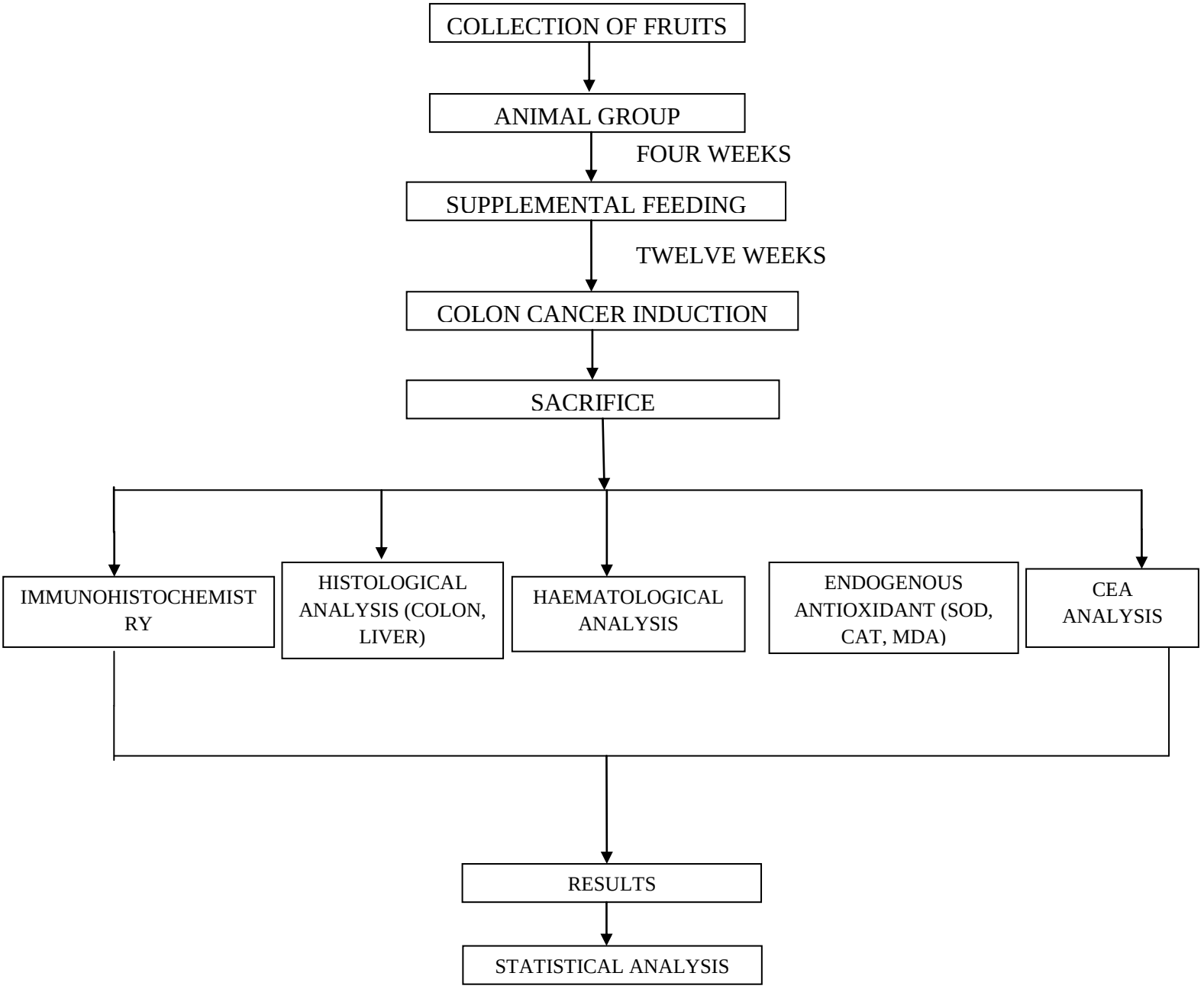


Figure 3.1: Experimental Design for the Dietary Inclusion of *Hyphaene thebaica* fruit for Prevention of Colon Carcinogenesis in Male Albino Wistar Rats.

3.6 Induction of Colon Cancer

After four (4) weeks of supplemental feeding, the rats in the experimental groups received an intra-rectal instillation of freshly prepared MNU solution using a syringe with a cannula, while the rats in the control group also received an intra-rectal instillation of freshly prepared normal saline.

3.6.1 MNU Preparation and Dosage

All experimental rats received 200ul intrarectal instillation of aqueous solution containing 0.2mg of MNU. The MNU administration was performed every 72 hours until the end of the experiment (12 weeks).

3.7 Collection of Organs or Tissues

The animals were sacrificed by decapitation one week after the end of the eighteen week of the last MNU induction and the blood was collected with heparin test tube. The rats were dissected and the colon, liver, and kidney were removed and fixed quickly in 10% Normal-saline for subsequent analyses.

The rat's colon, liver, and kidney were dissected out quickly, weighed and washed immediately with ice-cold saline to remove blood (absolute weight) using a digital weighing scale and refrigerated.

The colon, liver and kidney (100 mg tissue/ml buffer) were crushed using mortar and pestle and homogenized in 50 mM phosphate buffer (pH 7.4); the homogenate was centrifuged at 4000Xg for 10 minutes and the supernatant collected with Pasteur pipette and used for subsequent analysis.

3.8 Estimation of Endogenous Antioxidants

3.8.1 Lipid peroxidation by measuring the malondialdehyde (MDA) level

The concentration of thiobarbituric acid reactive substances (TBARS) in the tissue homogenate was estimated by the method of (Fraga *et al*., 1988). The formation of malondialdehyde is the basis for the well known TBA method used for evaluating the extent of lipid peroxidation. 1ml of the tissue homogenate was treated with 2.0 ml of thiobarbituric acid–trichloro acetic acid–hydrochloric acid reagent and mixed thoroughly. The mixture was incubated in a boiling water bath for 15 min, cooled and centrifuged at 1200g for 10 min and the supernatant was taken and the absorbance was read at 535 nm against the reagent blank.

3.8.2 Superoxide dismutase (SOD) activity assay

The enzyme superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at a high reaction rate. SOD activity assay method is based on monitoring the auto-oxidation rate of haematoxylin as originally described by Martin *et al.,* 1987. In the presence of SOD enzyme, the rate of auto oxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity was determined by measuring ratios of auto-oxidation rates in the presence and absence of the sample.

$$\text{Heamatoxylin} \xrightarrow{\text{Auto-oxidation}} H_20 + \text{Heamatin (active form of heamatoxylin)}$$

The procedure involved exactly 920 µL of phosphate buffer (pH 7.8), which was added into clean test tube containing 40 µL of sample, mixed and incubated for 2 min at 25^0C, before 40 µL of hematoxylin was added, mixed quickly and the absorbance was measured at 560nm.

3.8.3 Catalase activity assay

Catalase (CAT) activity was measured using the procedure reported by Abei (1984). Briefly, the method is as follows: 10 µL of serum was added to test tube containing 2.8 ml of 50 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 ml of fresh 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 min. on a spectrophotometer (Jenway 640 UV/Vis). A molar extinction coefficient of 0.0411 $mM^{-1}cm^{-1}$ was used to calculate catalase activity.

The reaction was measured at 240 nm for 3 min and H_2O_2 consumption was calculated using extinction coefficient, 39.4 $mM^{-1}cm^{-1}$.

3.9 Histological Analysis of the Colon

The colon tissue was rapidly fixed for 18-24 hours in 10 % formal saline and dehydrated in ascending grades of alcohol (70%, 80%, 90%, 95%, and 100 % respectively) for 2 hours each. The tissue was cleared in xylene and subsequently transferred through a pure paraffin in an oven for 1 hour and then into a second pot of melted paraffin for additional 2 hours (infiltration). The tissue were immersed in a mould containing molten paraffin wax, which was allowed to solidify (embedding) while the tissue was inside and ready for sectioning. Sections of 3 µm in thickness was prepared according to standard micro techniques onto glass slide and stained with hematoxylin and eosin. Photomicrographs under light microscope at high power magnifications (× 250) were examined for histopathological changes.

3.10 Haematological Analysis

The whole blood of the sacrificed animals was collected into EDTA bottles for analysis of haematological parameters such as haemoglobin concentration (Hb.), white blood cell count

(WBC), red blood cell count (RBC), packed cell volume (PCV), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). The determination of haematological parameters was done within two hours of sample collection using Automated Haematological analyser.

3.11 Carcinoembryonic Antigen Assay

Carcinoembryonic Antigen is one of the most widely used tumor markers worldwide and certainly the most frequently used marker in colorectal cancer (Sturgeon *et al.,* 2008). The concentration of CEA was determined by enzyme-linked immunosorbent assay (ELISA) test based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CEA molecule was used for solid phase immobilization (on the microtiter wells). A goat anti-CEA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample was allowed to react simultaneously with the two antibodies, resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 1 hour incubation at room temperature, the wells were washed with water to remove unbound labelled antibodies. The colour development was stopped with the addition of Stop Solution changing the colour to yellow. The concentration of CEA is directly proportional to the colour intensity of the test sample. Absorbance was measured spectrophotometrically at 450 nm.

3.12 Immunohistochemical Assay Method

The method used is the Avidin Biotin Complex (ABC) method also referred to as the avidin biotin Immunoperoxidase method. Exactly 2 microns thick of formalin fixed and paraffin embedded tissues were cut for the IHC. Antigenic sites were retrieved using citric acid (pH

6.0) solution in pressure cooker at 100^{o}C for 25 minutes. Peroxidases, protein and biotin blocks were done using hydrogen peroxide, avidine and biotin respectively. *MLH1* antibody was diluted (1.100) and incubated on sections at 6^{o}C for 60 minutes. These were followed by the biotylinated secondary antibody, streptavidine and DAB/substrate reaction as described by Goldstein *et al*., (2007); Wolff *et al*., (2007).

3.12.1 Pre-Analytical Procedure

The processed tissue were sectioned at 2 microns on the rotary microtome and placed on the hot plate at 70^{o}C for 1hour. Sections were brought down to water by passing them on 2 changes of xylene, then 3 changes of descending grades of alcohol (molecular grade) and to water. Antigen retrieval was performed on the sections by heating them in a citric acid solution of pH 6.0 using the Microwave at power 100^{o}C for 15 minutes. The sections were equilibrated gradually with cold water to displace the hot citric acid for at least 5 minutes for the section to cool.

Peroxidase blocking was done on the sections by simply covering section with 3% hydrogen peroxide (H_2O_2) for 15min. Sections were washed with phosphate buffered solution (PBS) and protein blocking were performed using avidin (100 µl to cover the slide) for 15 minutes. Sections were washed with PBS (about 100 µl of pH 7.2) and endogenous biotin in tissue was blocked using biotin for 15 minutes, and then incubated with the respective diluted primary antibody (*MLH1*) for 60 minutes.

Excess antibodies were washed off with PBS and a secondary antibody (LINK) was applied on the sections for 15 minutes. Slide sections were washed and the LABEL which is the horseradish peroxidase (HRP) was applied on the sections for 15 minutes. A working diaminobenzidine (DAB) solution was made up by mixing 1 drop (20 microns) of the DAB

chromogen to 1ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5 minutes. Excess DAB solution and precipitate were washed off with water and sections were counterstained with haematoxylin solution for at least 2 minutes and blued briefly. Sections were dehydrated in alcohol, cleared in xylene, coverslip and mounted.

3.12.2 Post-Analytical Procedure

In the post-analytical process, the stains were interpreted by a pathologist in context with positive and negative tissue controls, using bright field microscopy (maginification at x 40) and the results were reported.

3.13 Statistical Analysis

The results were reported as mean ± SD. The statistical significance between the control and other groups of experimental animals was determined by one-way ANOVA followed by Duncan test for multiple comparisons. Statistical test was performed at $p \leq 0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Assessment of the packed cell volume (PCV), neutrophil, lymphocyte in the liver and colon homogenate revealed that the MNU control group showed a lower percentage level of packed cell volume (PCV) concentration which was significantly ($p \leq 0.05$) different from the normal control group and the groups that received 2.5%, 5% and 10% of the dietary inclusion of *H. thebaica* fruit with MNU or normal saline respectively. The MNU control group showed higher percentage level of neutrophil and lymphocyte concentration which was significantly ($p \leq 0.05$) different from the normal control group and the group that received 2.5%, 5%, 10% of the dietary inclusion of *Hyphaene thebaica* fruit (Figure 4.1).

Effect of *H. thebaica* fruit on haemoglobin and platelet concentration in the blood of different experimental group revealed that the MNU control group showed lower level of haemoglobin and platelet concentration which was significantly ($p \leq 0.05$) different from the normal control group. However, dietary inclusion of *H. thebaica* fruit raised the level of these parameters to the levels that there were no significant ($p \geq 0.05$) difference with respect to haemoglobin and platelet concentrations among the groups treated with dietary inclusion of *H. thebaica* fruit (Figure 4.2).

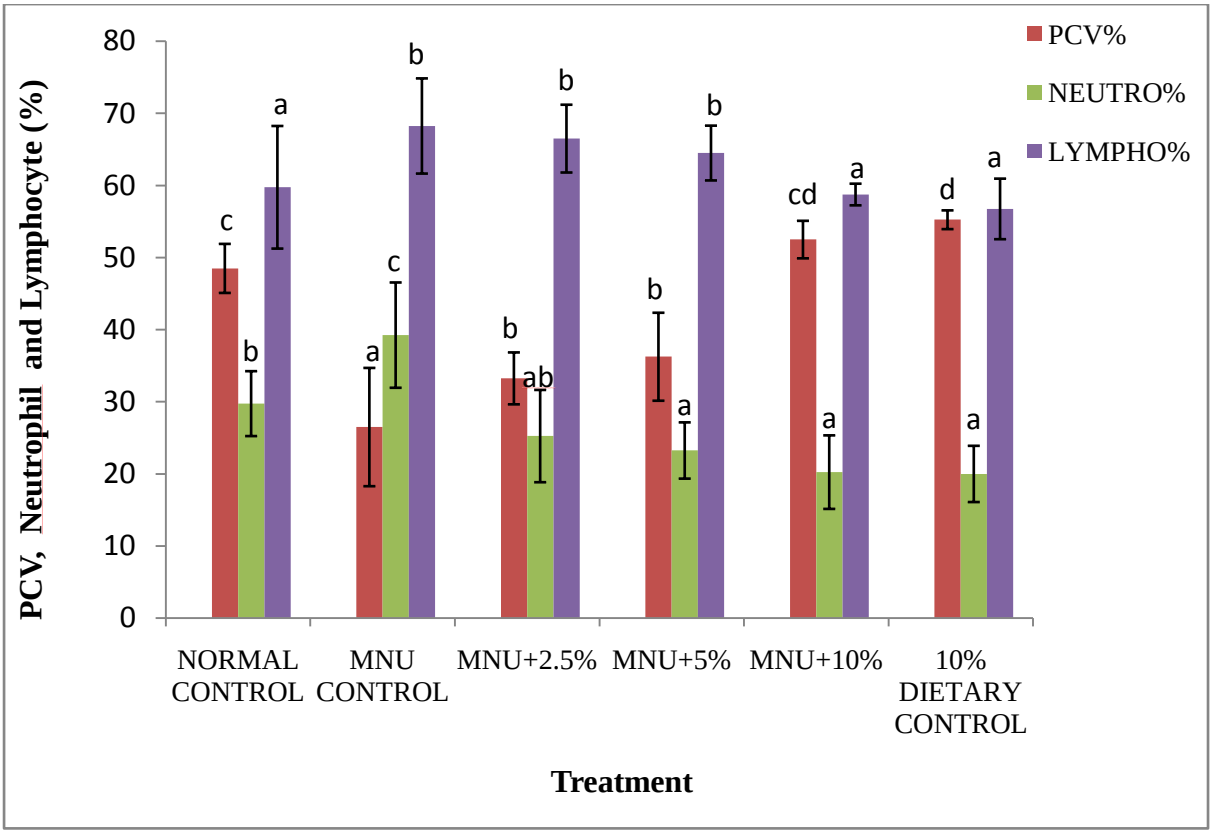


Figure 4.1: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on Percentage Packed Cell Volume (PCV), Neutrophil and Lymphocyte in the Blood following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

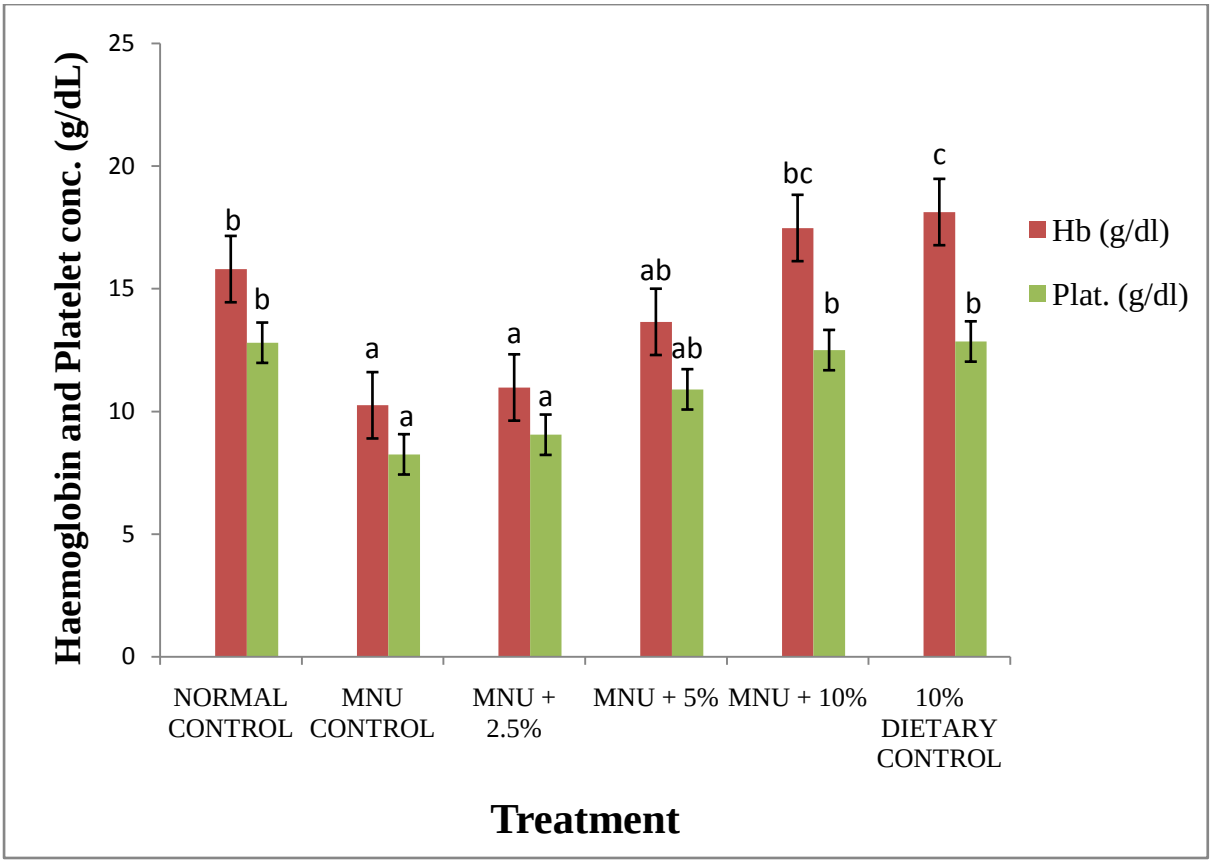


Figure 4.2: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on Haemoglobin and Platelet Concentration in the Blood following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Effect of *H. thebaica* fruit on white blood cells and red blood cells of different experimental group revealed that the MNU control group showed higher level of white blood cell concentration which was significantly ($p \leq 0.05$) different from the normal control group and the groups that received 2.5%, 5% and 10% of the dietary inclusion of *H. thebaica* fruit. The MNU control group also showed lower level of red blood cells which was significantly ($p \leq 0.05$) different from the normal control group and the group that received 2.5%, 5%, and 10% of dietary inclusion of *H. thebaica* fruit with MNU or normal saline respectively (Figure 4.3).

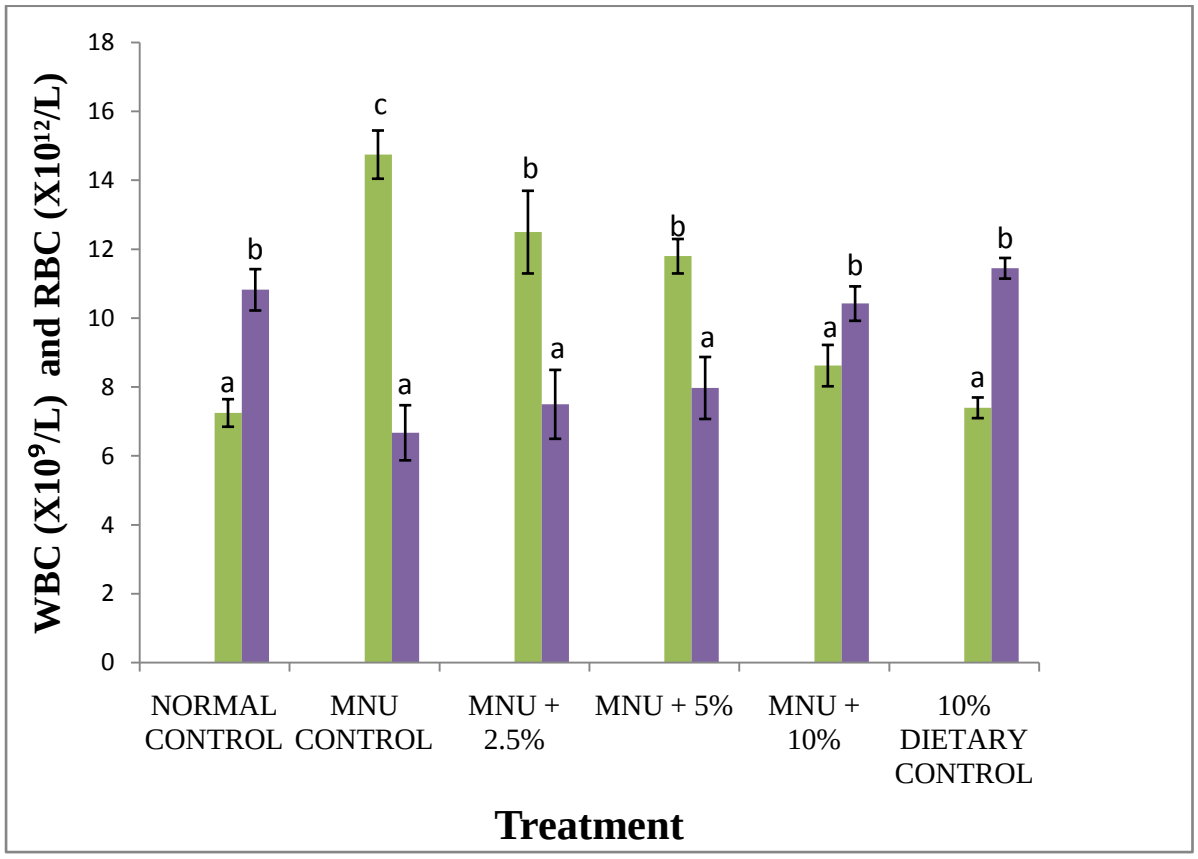


Figure 4.3: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on White Blood Cell (WBC) and Red Blood Cell (RBC) in the Blood following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Assessment of the effect of *H. thebaica* fruit on lipid peroxidation levels in the liver and colon homogenate showed that there was increase in the level of malondialdehyde (MDA) in the liver and colon of rats in the MNU control group which was significantly ($p \leq 0.05$) higher than the normal control groups. However, dietary inclusion with *H. thebaica* fruit brought the MDA content to a level that there were no significantly ($p \geq 0.05$) different between the group that received various dietary inclusion of *H. thebaica* fruit respectively in which lipid peroxidation level was low (Table 4.1).

Assessment of the effect of *H. thebaica* fruit on activities of superoxide dismutase in liver and colon homogenate showed that the MNU control group had a significantly ($p \leq 0.05$) lower activities of superoxide dismutase in the liver and colon compared to the normal control group, also there were no significantly ($p \geq 0.05$) differences between groups that received various dietary inclusion of *H. thebaica* fruit (Table 4.2).

Assessment of the effect of *H. thebaica* fruit on activities of catalase activities in liver and colon homogenate showed that the MNU control group had a significantly ($p \leq 0.05$) lower activities of catalase in the liver and colon compared with the normal control group, but there were no significantly ($p \geq 0.05$) differences between the groups that received different level of dietary inclusion of *H. thebaica* fruit (Table 4.3).

Table 4.1: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on MDA Level in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Group	Treatment	MDA levels (nmol/mg protein)	
		Liver	Colon
(i)	Normal Control	50.25±4.1^{c}	15.01±2.6^{b}
(ii)	MNU Control	94.39±5.0^{d}	55.01±6.2^{e}
(iii)	MNU+2.5% of *H. thebaica* fruit	31.00±2.3^{b}	44.99±7.6^{d}
(iv)	MNU+5% of *H. thebaica* fruit	29.91±6.8^{ab}	33.93±2.4^{c}
(v)	MNU+10% of *H. thebaica* fruit	25.82±6.6^{a}	17.98±3.4^{b}
(vi)	10% of *H. thebaica* fruit Control	23.38±2.4^{a}	10.11±2.3^{a}

N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).

Table 4.2: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on SOD Activities in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Group Treatment		SOD Activity (U/min/mg protein)	
		Liver	**Colon**
(i)	Normal Control	20.07 ± 09.8^{b}	84.50 ± 11.3^{b}
(ii)	MNU Control	07.65 ± 2.0^{a}	18.66 ± 12.2^{a}
(iii)	MNU+2.5% of *H. thebaica* fruit	25.69 ± 10.3^{b}	70.99 ± 22.8^{b}
(iv)	MNU+5% of *H. thebaica* fruit	26.59 ± 12.1^{b}	108.39 ± 11.3^{c}
(v)	MNU+10% of *H. thebaica* fruit	31.83 ± 29.8^{bc}	122.73 ± 13.7^{d}
(vi)	10% of *H. thebaica* fruit Control	36.29 ± 20.4^{c}	125.68 ± 13.9^{d}

N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).

Table 4.3: Effect of Dietary Inclusion of *Hyphaene thebaica* Fruit on Catalase Activities in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

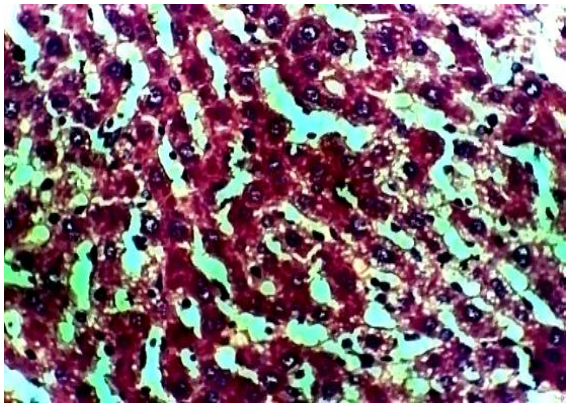
	Group Treatment	Catalase Activity (U/min/mg protein)	
		Liver	**Colon**
(i)	Normal Control	48.47 ± 3.3^{b}	34.19 ± 3.4^{b}
(ii)	MNU Control	36.23 ± 2.1^{a}	18.33 ± 9.2^{a}
(iii)	MNU+2.5% of *H. thebaica* fruit	79.78 ± 3.8^{c}	83.61 ± 7.2^{c}
(iv)	MNU+5% of *H. thebaica* fruit	84.99 ± 6.9^{c}	89.27 ± 3.9^{cd}
(v)	MNU+10% of *H. thebaica* fruit	93.37 ± 6.7^{cd}	88.78 ± 4.8^{cd}
(vi)	10% of *H. thebaica* fruit Control	96.33 ± 3.4^{d}	92.37 ± 3.3^{d}

N=6: Values are Mean± S.D; Values having different letters across the column are significantly different ($p \leq 0.05$).

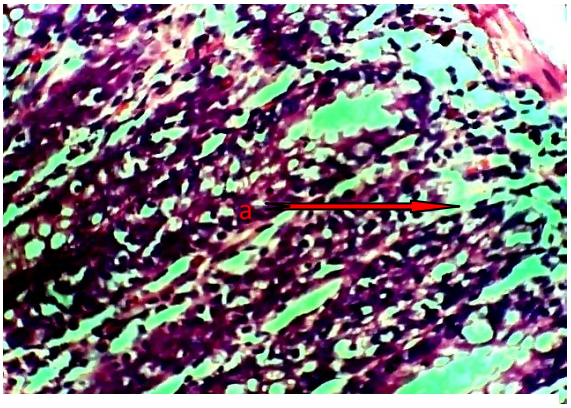
The photomicrogragh of histopathological section of the liver following 12 weeks MNU administration intra-rectally, fed with dietary inclusion of *H. thebaica* fruit in rats; revealed that the MNU control group showed focal interglandular inflammation when compared to the normal control group and other groups that received 5% and 10% dietary inclusion of *H. thebaica* fruit respectively, which showed essentially normal liver, except the group that received lowest dietary inclusion of *H. thebaica* fruit (2.5% of dietary inclusion of *H. thebaica* fruit) which showed focal inflammation (Plate 4.1).

The Photomicrogragh of histopathological section colon following 12 weeks MNU administration intra-rectally fed with dietary inclusion of *H. thebaica* fruit in rats; showed that the MNU control group showing a colon with severe damage to the mucosa cells when compared to the colon tissue of the normal control group. A better colon cells was seen in the groups that were treated with the 5% and 10% of dietary inclusion of *H. thebaica* fruit, but mild surface ulceration was observed in the group that received lowest percentage (2.5%) of dietary inclusion of *H. thebaica* fruit (Plate 4.2).

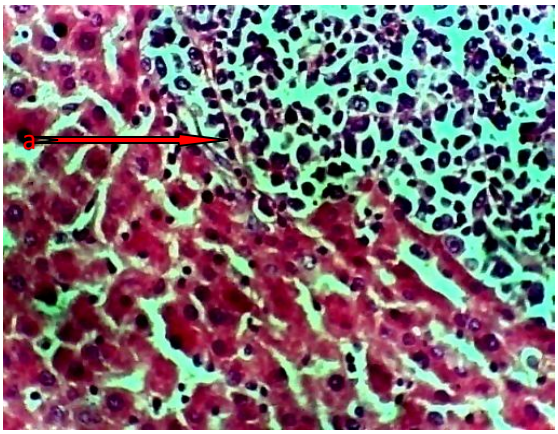
Effect of *Hyphaene thebaica* fruit on carcinoembryonic antigen (CEA) in the serum of different experimental group following 12 weeks MNU administration intra-rectally, fed with dietary inclusion of *H. thebaica* fruit in rats; revealed that the MNU control group showed higher level of CEA concentration which was significantly ($p \leq 0.05$) different from the normal control group. However, there is dose-dependent decrease in the groups that received 2.5%, 5% and 10% dietary inclusion of *H. thebaica* fruit respectively (Plate 4.4).



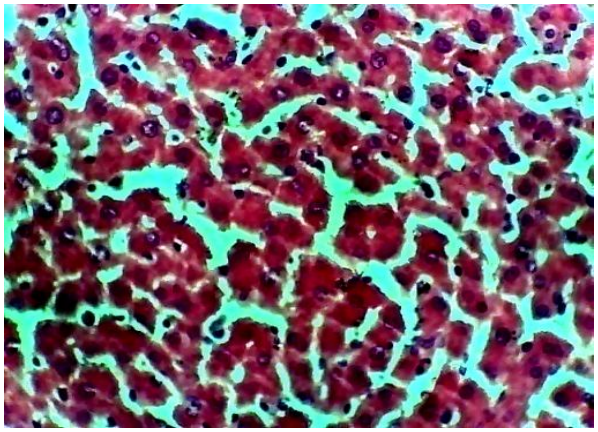
1a: Normal control group: essentially normal liver (X40).



1b: MNU control group: essentially normal liver with (a) focal interglanduloar inflammation (X40).



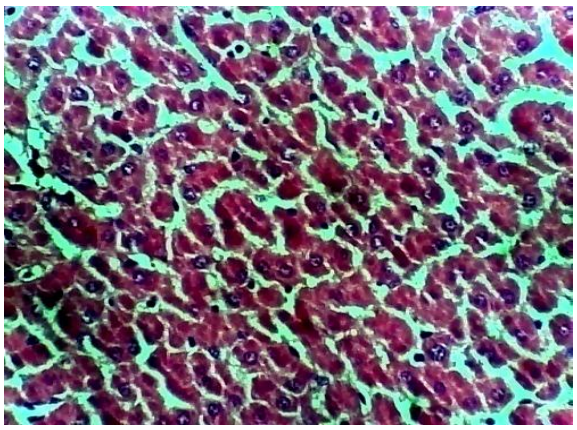
1c: MNU+2.5% *H. thebaica* fruit: (a) focal inflammation (X40).



1d: MNU+5% *H. thebaica* fruit: essentially normal liver (x40).



1e: 10% + MNU *H. thebaica* fruit: essentially normal liver (X40).

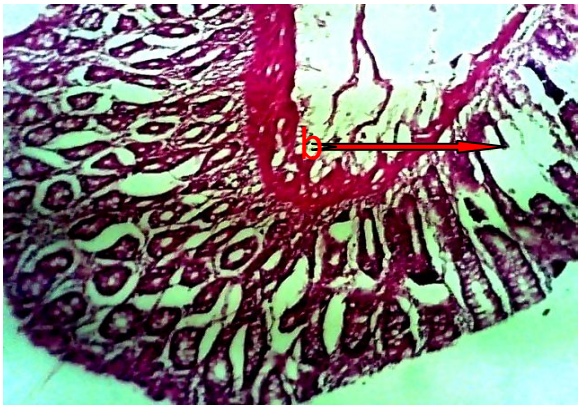


1f: 10% *H. thebaica* fruit control:essentially normal liver (x40).

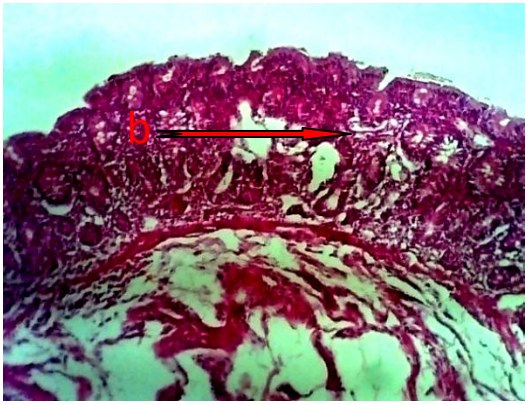
Plate 4.1: **Photomicrograph of Histopathological Section of the Liver Using Eosin and Haematoxylin Stain following Twelve (12) weeks Intrarectal Administration of MNU in Rats fed with Different Level of Dietary inclusion of *Hyphaene thebaica* fruit.**



2a: Normal control group: essentially normal colon (X10).



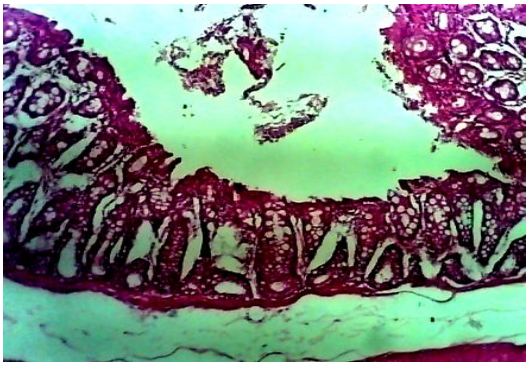
2b: MNU control group: colon with (b) deep mucosal ulceration (X10).



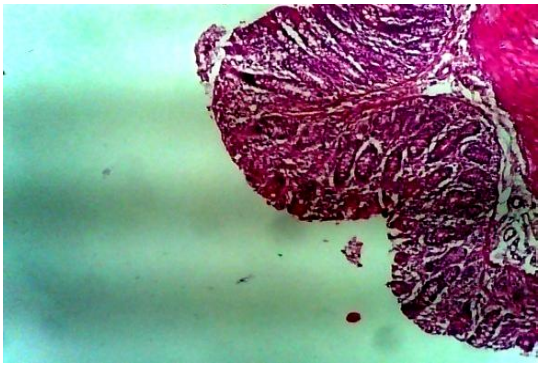
2c: MNU+2.5% *H. thebaica* fruit:(b) mild surface ulceration (X10).



2d: MNU+5%*H. thebaica* fruit: predominantly normal colon (X10).



2e: MNU+10%*H. thebaica* fruit: essentially normal colon with non-ulceration mucosal, normal muscular and serosa (X10).



2f: 10% *H. thebaica* fruit control: essentially normal colon with non-ulceration mucosal, normal muscular and serosa (X10).

Plates 4.2: Photomicrograph of Histopathological Section of the Colon Using Eosin and Haematoxylin Stain following Twelve (12) weeks Intrarectal Administration of MNU in Rats fed with Different Level of Dietary inclusion of *Hyphaene thebaica* fruit.

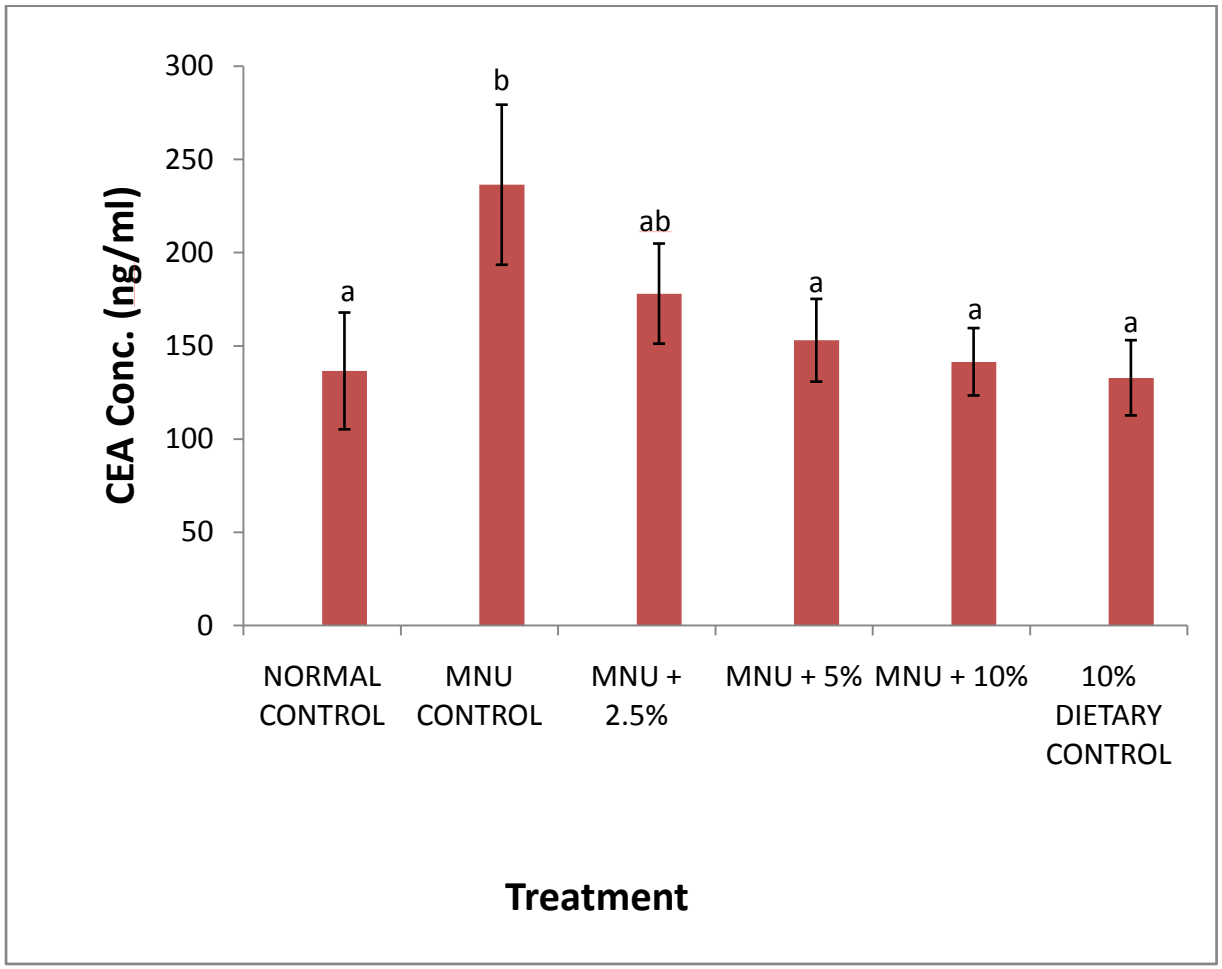
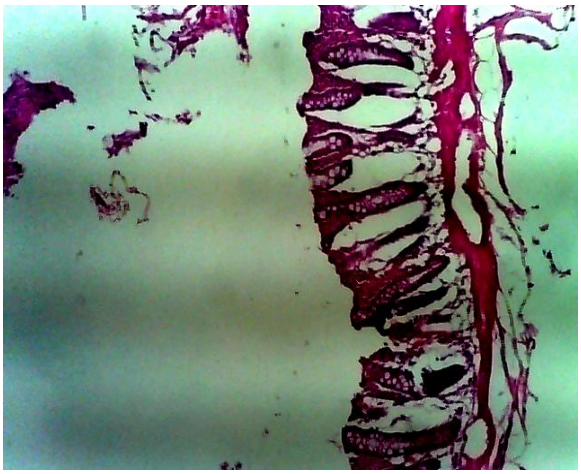


Figure 4.4: Carcinoembryonic Antigen (CEA) Concentration of the Serum following Twelve (12) weeks Intrarectal Administration of MNU in Rats Fed with Different Level of Dietary inclusion of *Hyphaene thebaica* Fruit.

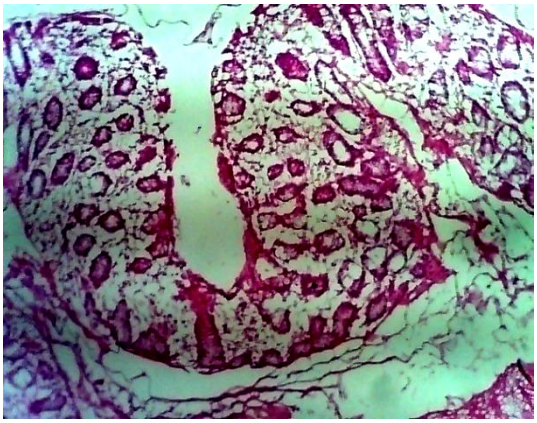
The Photomicrograph of immunohistochemistry section of the colon following 12 weeks MNU administration intra-rectally, fed with dietary inclusion of *H. thebaica* fruit in rats showed that there was mild to moderate expression (brown colouration on mucosa cells) of MLH1 antibody in the normal control group and the groups treated with 10% dietary inclusion of *H. thebaica* fruit, but there was no expression of MLH1 antibody in the MNU control group and the groups that received 2.5% and 5% dietary inclusion of *H. thebaica* fruit (Plate 4.3).



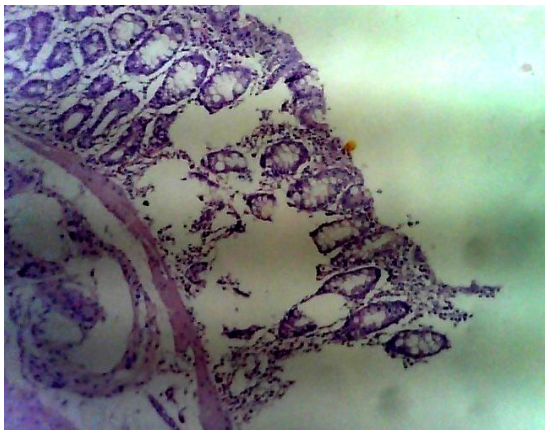
3a: Normal control group: moderate expression of the antibody (MLH1) (X40).



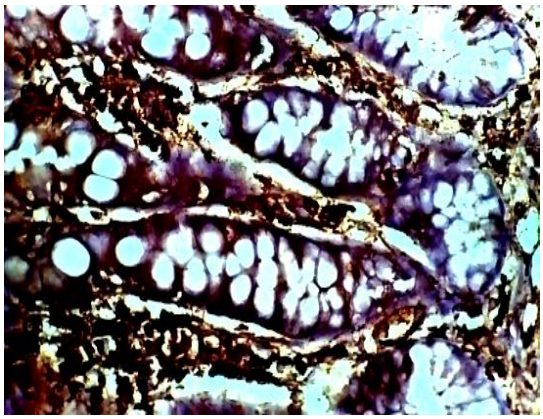
3b: MNU control group: no expression of the antibody (MLH1) (X40).



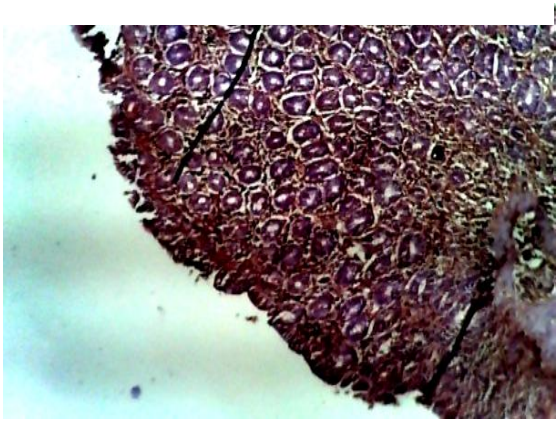
3c: MNU +2.5%*H. thebaica* fruit: showing no mucosal expression of the antibody (MLH1) (X40).



3d: MNU +5% *H. thebaica* fruit: no expression of the antibody (MLH1) (X40).



3e: MNU +10% *H. thebaica* fruit: moderate expression of the antibody (MLH1) (X40).



3f: 10% *H. thebaica* fruit control: predominantly normal mucosal with mild expression of the antibody (MLH1) (X40).

Plates 4.3: Photomicrogragh of Immunohistochemistry Section of the Colon following Twelve (12) weeks Intrarectal Administration of MNU in Rats Fed with Different Level of Dietary inclusion of *Hyphaene thebaica* fruit.

CHAPTER FIVE

5.0 DISCUSSION

Haematological parameters and its knowledge can be used to assess the health as well as the physiological status of farm animals under consideration. Changes of these parameters have been studied in rats (Oyawoye and Ogunkunle, 2004). The significant ($p \leq 0.05$) decrease in the packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) concentration of the MNU control group indicating anaemia, which may be due to red blood cell destruction (Collen *et al*., 2003), which in this experiment, is connected with MNU toxicity. According to Isaac *et al.* (2013), packed cell volume is involved in the transport of oxygen and absorbed nutrients. According to Oyawoye and Ogunkunle (2004), haemoglobin concentrations are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals. Thus, low packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) concentration might suggest oxidative stress-related to red blood cell destruction in the MNU control group, while the higher level of packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) concentration in the group that received various percentage of dietary inclusion of *Hyphaene thebaica* fruit indicate ability of *Hyphaene thebaica* fruit to prevent oxidative-stress related damage to red blood cell membrane (Asuku *et al.,* 2012), which may be due to the presence of the substantial amount of their water-soluble phenolic content (Fig. 4.1, Fig. 4.2 and Fig. 4.3). This is in agreement with work of Katalinic *et al*., (2006) and Eldahshan *et al*., (2008), (2009), that the presence of water-soluble phenolic content in *Hyphaene thebaica* fruit prevents damage to red blood cell membrane.

The significant ($p \leq 0.05$) increase in platelet concentration of the MNU control group may be due to red blood cell destruction (Collen *et al*., 2003), which in this experiment, is connected

with MNU toxicity. According to Hsu *et al.,* (2006), blood platelets are implicated in blood clotting. Therefore, low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged, resulting in excessive loss of blood in the case of injury. Thus, increase in platelet concentration might suggest oxidative stress-related red blood cell destruction in the MNU control group, while decrease in the platelet concentration of the group that received 10% dietary inclusion of *Hyphaene thebaica* fruit in (Table 4.2) suggest the potency of the *Hyphaene thebaica* fruit to prevent oxidative-stress related damage to red blood cell membrane.

The significant ($p \leq 0.05$) increase in the white blood cell (WBC) concentration of the MNU control group may be due to ability of white blood cell (WBC) to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response (Fig. 4.3). According to Isaac *et al.*, (2013); Soetan *et al*., (2013) and Kabir *et al*., (2012), animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions.

Oxidative stress has been suggested to contribute to the pathogenesis of carcinogenicity and lipid peroxidation is one of the characteristic features of cancer (Halliwell, 2007). Malondialdehyde (MDA) is found during oxidative degeneration as a product of free oxygen radicals which is accepted as an indicator of lipid peroxidation.

In the present study, N-Methyl-nitrosourea (MNU) toxicity leads to formation of lipid peroxides, which in turn produce malondialdehyde that cause damage to cell membranes (Siddhartha *et al*., 2011). This lead to the fact that the groups of rats that received dietary

inclusion of *Hyphaene thebaica* fruit showed significantly lower level of malondialdehyde in the colon and liver compared to the MNU control group (Fig.4.1) suggesting that the lipid peroxidation of membranes caused by MNU toxicity was prevented by dietary inclusion of *Hyphaene thebaica* fruit and thus implying that this fruit contains antioxidant compounds such as polyphenols, flavonoids, that were capable of scavenging free radicals from the tissues of the rats that received dietary inclusion of *Hyphaene thebaica* fruit. This is in agreement with the work of Asuku *et al*., (2012), that the presence of flavonoids and polyphenols in fruits prevent lipid peroxidation of cell membranes.

The significant ($p \leq 0.05$) elevation in the activities of superoxide dismutase and catalase in the liver and colon of the groups that received dietary inclusion of *Hyphaene thebaica* fruit compared to the MNU control group strongly suggest high anti-oxidative effect of the *Hyphaene thebaica* fruit, probably due to the substantial amount of their water-soluble phenolic content. According to Kamis *et al.,* (2003), doum fruit is a rich source of fibers and antioxidants, also other valuable substances such as carbohydrates and minerals especially potassium for people suffering from blood pressure problems, also since it has been reported that some antinutrients exhibit protective effects, thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders (Reddy *et al.,* 1991 and Asuku and Atawodi, 2012). According to Rice-Evans *et al.,* (1996), endogenous antioxidant enzymes (superoxide dismutase) are effective in conversion of superoxide anion to hydrogen peroxide and oxygen, while catalase are effective in conversion of hydrogen peroxide to water and oxygen, which is the primary defense mechanism against oxidative stress since superoxide is such a strong initiator of chain reactions.

Hyphaene thebaica fruit has a strong antioxidant capacity which is in agreement with earlier work by Atawodi *et al.,* (2010a), that other African foods of Nigerian origin, including *Moringa oleifera*, Palm oil, *Canarium schweinfurthii* oil, *Syzygium aromaticum*, *Hibiscus esculentum* and *Dacryodes edulis* have been demonstrated to contain appreciable levels of antioxidant polyphenols, and flavonoids, that have proven to be of therapeutic potential.

The histopathological findings of the colon and liver following 12 weeks MNU administration in rats in (Plate 4.1 and Plate 4.2) showed essentially normal colon and liver in the normal control group and the groups that were treated with 5% and 10% dietary inclusion of *H. thebaica* fruit, strongly suggest high anti-oxidative effect of the *Hyphaene thebaica* fruit , which may be as a result of it's high contents of antioxidant compounds such as polyphenols and antioxidant vitamins, inflammation observed in the MNU control group and the groups treated with 2.5% dietary inclusion of *H. thebaica* fruit, revealed that MNU caused severe damage to the mucosal cells, which is connected with MNU toxicity. This is in agreement to the works of Bafeel and Ibrahim, (2008); Yao and Vieira (2011), which suggest that MNU toxicity can lead to inflammation in experimental animal.

In vitro experiments showed that carcinoembryonic antigen (CEA) was capable of both homophilic (CEA binding to CEA) and heterophilic (CEA binding to non-CEA molecules) interactions. Because alterations in cell adhesion are causally involved in cancer invasion and metastasis, it was further suggested that CEA may play a role in these processes *(*Jessup *et al.,* 1989). The significant ($p \leq 0.05$) increase in carcinoembryonic antigen (CEA) concentration in N-methyl-N-nitrosourea (MNU) control group compared to normal control group shows that MNU caused severe damage to the vital organs, which in this study is connected with MNU toxicity. However administration of dietary inclusion of *Hyphaene thebaica* fruit brought the carcinoembryonic antigen (CEA) concentration to a lower level

where there is no significant ($p \leq 0.05$) difference between the group that were fed with different percentage of *Hyphaene thebaica* fruit strongly, suggest the potency of *Hyphaene thebaica* fruit to prevent oxidative-stress related damage to liver and colon. This may be as a result of their substantial amount of water-soluble phenolic content. This is in agreement with works of Katalinic *et al*., (2006) and Eldahshan *et al*., (2009), that the presence of antioxidant in *Hyphaene thebaica* fruit can prevent oxidative-stress related damage to some vital organs.

Immunohistochemistry has been widely used in diagnosing carcinogenesis because it takes advantage of the principle of the antigen-antibody affinity reaction through its ability to identify and localize proteins of interest via detection with labelled conjugates (Lee *et al.,* 2003). This antigens produced in diseased condition have corresponding antibodies produced by the body to form a complex compounds which are usually picked up by immunochemical dyes or stains giving a distinctive colour (brownish colour in the case of the *Mut L-homoloq* 1 gene) as "expression" under the microscope. The distinct moderate to mild expression of *Mut L-homoloq* 1 antibody in the normal control group and the groups treated with 10% dietary inclusion of *H. thebaica* fruit in (Plate 4.3) strongly suggest high anti-oxidative effect of the *Hyphaene thebaica* fruit, which may be as a result of it's high contents of antioxidant compounds including polyphenols and antioxidant vitamins. According to Yao and Vieira, (2011), the presence of high contents of antioxidant compounds including polyphenols and antioxidant vitamins in some plants and fruits prevent oxidative related damage to organs. However, no expression of *Mut L-homoloq* 1 antibody in the MNU control group and the groups treated with 2.5% and 5% dietary inclusion of *H. thebaica* fruit may be due to damaged or mutated *Mut L-homoloq* 1. According to Kaur *et al.*, (2011), defect in the DNA mismatch repair gene like the *Mut L-homoloq* 1 gene can lead to its inability to recognize and repair errors that occur during DNA replication. This loss of DNA mismatch repair gene activity accelerates the rate of accumulation of mutations in other genes leading to excessive

generation of free radicals during aerobic metabolism which play an important role in the genesis of structural and functional damage to organs, that could be responsible for the beginning or aggravation of the basic disease (Smith *et al.*, 1991; Abdulla *et al*., 1986; and Halliwell, 1997) also leading to carcinogenesis (Boland *et al*., 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This study established that;

i. *Hyphaene thebaica* fruit can significantly ($P \leq 0.05$) protect haematological indices (Packed cell volume from 26.50±8.2 to 55.25±1.3, Haemoglobin from 10.25±1.8 to 18.13±0.4, Red blood cell from 6.68±0.8 to 11.45±0.3, White blood cell from 14.75±4.1 to 7.40±1.8) from MNU-induced toxicity.

ii. *Hyphaene thebaica* fruit can significantly ($P \leq 0.05$) prevent protein damage in the organs (liver and colon) from MNU-induced damage and damage to the DNA mismatch repair gene (*Mut l homoloq* 1) by the MNU carcinogen.

iii. *Hyphaene thebaica* fruit prevented oxidative stress in colon carcinogenesis by increasing the activities of catalase and SOD, and also by decreasing the concentration of MDA in the organs (liver and colon).

6.2 CONCLUSION

Plants secondary metabolites such as isoprenoids,phenolics and alkaloids and other natural products have been demonstrated to offer substantial preventive benefits and also serve as leading sources of cancer preventing agents whose potential have been demonstrated by both *in vitro* methods and use of animal models. This study has demonstrated the positive potential of dietary inclusion of *H. thebaica* fruit to prevent colon carcinogenesis against MNU-induced oxidative stress and organs damage.

6.3 RECOMMENDATION

From this study it is recommended that elaborate and further experimental design to evaluate the preventive effect of dietary inclusion with *Hyphaene thebaica* fruit on other types of cancers should be carry out and people should be encouraged to increase the consumption of *Hyphaene thebaica* fruit because it has a positive potential to prevent colon carcinogenesis.

REFERENCES

Abas, F., Lajis, N., Israf, D., Khozirah, Y., Umi, K.Y. (2006). Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chemistry*, 95: 566-573.

Abdulkareem, F.B., Abudu, E.K., Awolola, N.A., Elesha, S.O., Rotimi, O., Akinde, O.R. (2008). Colorectal Carcinoma in Lagos and Sagamu, Southwest Nigeria: *A Histopathological Review. World Journal Gastroenterology,* 14:6531-5. Medline: 19030207 doi:10.3748/wjg.14.6531.

Abdulla, D.S.P., Monteiro, I.I.P., Oliveira, J.A.C. and Bechara, E.J.H. (1986). Activities of Superoxide dismutase and Glutathione peroxidase in Schizophrenic and Manic Depressive Patients. *Clinical Chemistry,* 32 (5), 805-807.

Aebi, H. (1984). Catalase *In vivo: Method of Enzymatic Analysis*, pp: 673-684.

Aebi, H. (1984). Catalase *In vitro: Methods of Enzymology,* 105:121-126.

Adams, M., Jasani, B., Fiander, A. (2007). Human papilloma virus (HPV) prophylactic vaccination: Challenges for public health and implications for screening. *Vaccine*, 25 : (16):3007-3013.

Aderemi, F. A. (2004). Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. *Tropical Journal of Animal Science, 7,* 147-153.

Afolabi, K. D., Akinsoyinu, A. O., Olajide, R., and Akinleye, S. B. (2010). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production* (p.247).

Ahmad, N., Zia-ur-Rahman, Akhtar N., and Ali, S. (2012). Effects of aqueous methanolic extract of flax seeds (*Linum usitatissimum*) on serum estradiol, progesterone, kidney and liver functions and some serum biochemical metabolites in immature female rats. *Pakistan Veterinary Journal*, 32: 211_215.

American Cancer Society (2011). Cancer in Africa, American Cancer Society, Atlanta, Ga, USA, (p.187).

American Cancer Society, Colorectal Cancer Facts and Figures, (2017). Exit Disclaimer Exit Disclaimer. Atlanta: *American Cancer Society*; 2017. Accessed June 22, 2017.

American Cancer Society (2015). *Cancer Facts and Figures* Atlanta,GA: Accessed at www.cancer.gov/types/colorectal/hp/colon-treatment-pdq.

American Cancer Society, (2010).*Cancer Prevention and Early Detection Facts and Figures* 2010.Atlanta: American Cancer Society. Atlanta.

American Cancer Society, (2014). *Colorectal Cancer Facts and Figures* 2014-2016. Atlanta, Ga: American Cancer Society.

American Cancer Society, "Cancer Facts and Figures (2015). Reported by the North American Association of Central Cancer Registries (NAACCR). Available at http:// www.cancer.org/acs/groups/content/@editorial/documents/document/acspc 044552.pdf.

American cancer society. (n.d.). Colorectal cancer. Retrieved June 28, (2015), from http://www.cancer.org/acs/groups/cid/documents/webcontent/003096-pdf.pdf

American Cancer Society. Colorectal Cancer Facts and Figures 2014–2016. Exit Disclaimer Exit Disclaimer. Atlanta: *American Cancer Society*; 2014. Accessed May 20, 2016.

Anand, P., Kunnumakkara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S., Sung, B. and Aggarwal, B.B. (2008). "Cancer is a preventable disease that requires major lifestyle changes". *Pharmaceutical Research. 25 (9): 2097–116.* doi*:*10.1007/s11095-008-9661-9. PMC 2515569.PMID 18626751.

Anwar, F. and R. Przybylski, (2012). Effect of solvent extraction on total phenolics and antioxidant activity of extracts form flaxseed (*Linum usitatissimum* L.).*Acta Science Polish, Technology Alimentary,* 11: 293-301.

Aster, J. C., Kumar V., Abbas A. K., Fausto N., Robbins S. L., and Cotran R. S. (2004). Anaemia of diminished erythropoiesis. *Robbins and Cotran Pathologic Basis of Disease* Saunders Co. Philadelphia. 7th edition, page 638-649.

Asuku, O., Atawodi, S.E. and Onyike, E. (2012). Antioxidant, hepatoprotective and ameliorative effects of methanolic extract of,leaves of *Grewia mollis* Juss on carbon tetrachloride-treated albino Rats. *Journal Medline Food*, 15: 83-88.

Atawodi, S.E., Atawodi, J.C., Idakwo, G.A., Pfundstein, B., Haubner, R., Wurtele, G., Bartsch, H. and Owen, R.W. (2010a). Evaluation of the Polyphenols contents and antioxidants properties of methanol extracts of leaves, stem and root barks of *Moringa oleifera* Lam. *Journal of Medicinal Food*s, 13: 710-716

Atawodi, S.E. (2011a). Evaluation of the hypoglycemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-ofa" polyherbal tea (A-Polyherbal) in alloxan-induced diabetic rats.*Drug Invent. Today,* 3: 270-276.

Atawodi, S.E. (2011b). Polyphenol Content and *In Vitro* Antioxidant Activityof methanol extract of Seeds of *Irvingia gabonensis* Baill of Nigerian Origin. *Electronic Journal Environmental Agriculture Food Chemistry,* 10:2314-2321.

Atawodi, S.E. (2011c). Nigerian foodstuffs with prostate cancerchemopreventive polyphenols.Proceedings, science of global prostate cancer disparities in black men conference. .*Infect Agent Cancer,* 6: S2-S9.

Atawodi, S.E. and Onaolapo, G.S. (2010). Comparative *in vitro* antioxidant potential of different parts of *Ipomoea asarifolia,* Roemer and Schultes, *Guiera senegalensis* J.F.Gmel and *Anisopus mannii* N.E. Brown. *Brazilian Journal of Pharmaceutical Research,* 46: 245-250.

Atawodi, S.E., (2010). Polyphenol Composition and *in vitro* Antioxidant Potential of Nigerian *Canarium schweinfurthii* England Oil. *Advance Biology Research,* 4: 314-322.

Atawodi, S.E., Atawodi, J.C., Idakwo, G.A., Pfundstein, B., Haubner, R., Wurtele, G., Spiegelhalder, B., Bartsch, H., and Owen, R.W. (2009a). Polyphenol composition and antioxidant potential of *Hibiscus esculentus* L. Fruit Cultivated in Nigeria. *Journal Medline Food,* 12:1316-1320.

Atkin, W.S., Edwards, R. and Kralj-Hans, I. (2010). Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: *A Multicentre Randomised Controlled Trial. Lancet*. 375(9726):1624-1633.

Awodi, S., Ayo, J. O., Atodo, A. D., and Dzende, T. (2005). Some haematological parameters and the erythrocyte osomotic fragility in the Laughing dove *(Streptopella senegalensis)* and the Village weaner bird (*Ploceus cucullatus*). *Proceedings of the 10th Annual Conference of Animal Science Association of Nigeria.*p.384-387.

Bafeel, S.O. and Ibrahim, M.M. (2008). Antioxidants and Accumulation of alpha tocopherol Induce Chilling Tolerance in *Medicago sativa. Internet Journal of Agriculture Biol*ogy, 10: 593–598.

Balaguer, F., Link, A., Lozano, J. J., Cuatrecasas, M., Nagasaka, T., Boland, C. R. and Goel, A. (2010). "Epigenetic Silencing of miR-137 is an Early Event in Colorectal Carcinogenesis". *Cancer Research*. 70(16): 6609–6618. doi:10.1158/0008-5472.CAN-10-0622. PMC 2922409 .PMID 20682795.

Bamishaiye E. I., Muhammad N. O., and Bamishaiye O. M. (2009). Haematological parameters of albino rats fed on tiger nuts (*Cyperus esculentus)* tuber oil meal-based diet. *The International Journal of Nutrition and Wellness,* 10 (1).Retrieved from http://ispub.com /IJNW/10/1/9293.

Boland, C.R., Koi M., Chang, D.K. and Carethers, J.M. (2008). The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. *Familial Cancer,* (7): 41-52.

Boni C., Andre T., Navarro, M.,Tabernero, J., Hickish T., Topham, C., Bonetti, A., Clingan, P., Bridgewater, J., Rivera F., and Aimery de Gramont J. (2009). Improved Overall Survival With Oxaliplatin, Fluorouracil, and Leucovorin as Adjuvant Treatment in Stage II or III Colon Cancer in the MOSAIC Trial. *Clinical Oncology,* 27: 309-36.

Boucher, D., Cournoyer, D., Stanners, C.P., Fuks, A. (2006). Studies on the control of gene expression of the carcinoembryonic antigen family in human tissue. *Cancer Res*earch, 49:847-852.

Brothman, A. R., Dolan, M.M., Goodman, B.K., Park, J.P., Persons, D.L., Saxe, D.F., Theil, K.S. (2011). College of American Pathologist/ American College of Medical Genetics proficiency testing for constitutional cytogenomic microarray analysis. Genetics in Medicine, 13(9), 765-769. DOI: 10.1097/ GIM.0b013e31821d3165.

Brunken, U., Schmidt, M., Dressler, S., Janssen, T., Thombiano, A. and Zizka, G. (2014). West African plants - A Photo Guide. Forschungs institute Senckenberg, Frankfurt/Main.

Brunken, U., Schmidt, M., Dressler, S., Janssen, T., Thombiano, A. and Zizka, G. (2008). West African plants - A Photo Guide. Forschungsinstitut Senckenberg, Frankfurt/Main.

Carter, J., (1993). Dietary fiber guide. *Cereal Foods World*, 38 (10): 755-759.

Center, M.M., Jemal, A., Smith, R.A., Ward, E. (2009). Worldwide variations in colorectal cancer.*CA A Cancer Journal for Clinicians*, 59(6):366-378.

Center, M.M., Jemal, A., Ward, E. (2011). International trends in colorectal cancer incidence rates. *Cancer Epidemiologiest Biomarkers*, 18 (6):1688-1694.

Chineke, C. A., Ologun, A. G., and Ikeobi, C. O. N. (2006). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences*, 9(11), 2102-2106.

Collen, S.M., Allan, D. and Micheal, L. (2003). Marks Basic Medical Biochemistry: *A Clinical Approach*. Second Edition.

Crebs, C. (1928). Experimenteller Alkohol krebs bei weissen Ma¨usen. Z. *Immunohistological Expression Therapeutic*, 50, 203–218.

Croce, C.M. (2008). "Oncogenes and cancer". *The New England Journal of Medicine*, 358 (5): 502–511.doi:10.1056/NEJMra072367. PMID 18234754.

Daniel, F. I., Cherubini, K., Yurgel, L. S., De Figueiredo, A. Z., Salum K., Fernanda, G. (2011). *The role of epigenetic transcription repression and DNA methyltransferases in cancer. Cancer. 117 doi:10.1002/cncr.25482. PMID 20945317. Review*.(4): 677–687.

Das, N.P., Pereira T.A. (1990). Effects of flavonoids on thermal autooxidation of Palm oil: structure- activity relationship. *Journal of American Oil Chemists Society,* 67,255-258.

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

Doren, E.T. (1999). Vegetable ivory and other palm nuts/seeds as an art/craft medium. *Journal of the International Palm Society,* 41 (4), 18-25.

Dosumu, O.O., Nwosu, F.O., Nwogu, C.J. (2006). Phytochemical screening and anti-microbial studies of extracts of *Hyphaene thebaica* linn (Mart) Palmae. *International Journal of Tropical Medicine* 1(4), 186-189.

Doyle, D. (2006). William Hewson (1739-74). The father of haematology. *British Journal of Haematology, 133*(4), 375-381.

Dreosti, I.E. (1991). Trace elements, micronutrients, and free radicals. Totowa: *Human Press*, page 149-199.

Duh, P.D., Tu, Y.Y., Yen, G.C. (1999). Antioxidant activity of water extract of Harng jyur (*Chrysanthemum morifolium* Ramat). *Lebensm Wiss Technology,* 32,269-277.

Dusica, P. and Vesna, T. (2002).Oxidative stress as marker of positive symptoms in schizophrenia. *Facta Universitatis. Medicine & Biology,* 9 (2), 157-161.

Dwivedi, V., Shrivastava, R., Hassan, S., Ganguly, C., Bharadwaj, M., Ganguly, C. (2011). Flavoring agents used in Indian cooking and their anticarcinogenic properties. *Asian Pacific Journal of Cancer Prevention,* 11, 25-28.

East, J. E., Saunders, B. P., and Jass, J. R. (2008). Sporadic and Syndromic Hyperplastic Polyps and Serrated Adenomas of the Colon: Classification, Molecular Genetics, Natural History, and Clinical Management. *Gastroenterology Clinics of North America*. http://doi.org/10.1016/j.gtc.2007.12.014.

Echendu, A.O., Akarolo-Anthony, S.N., Ogundiran, T.O., Adebamowo, C.A. (2015). Emerging breast cancer epidemic: evidence from Africa. *Breast Cancer Research,* 4: 8-11.

Eldahshan, O., Ayoub, N., Singab, A., Al-Azizi, M. (2008). Potential Superoxide Anion Radical Scavenging Activity of Doum Palm (*Hyphaene thebaica* L.) Leaves Extract *Records of Natural Products Journal* 2: 83-93.

Eldahshan, O., Ayoub, N., Singab, A., Al-Azizi, M. (2009). Potential antioxidant phenolic metabolites from doum palm leaves. *Africa Journal Pharmacy Pharmacology,* 3(4): 158-164.

El-Gendy, A., El-Mileegy, A., Ghyaty, E., Malek, H., El-Hamid Mousa, A. (2008). The Beneficial Dietary Hypotensive and Hypolipidaemic Effects of *Hyphaene thebaica* (Doum). *Internet Journal of Alternative Medicine,* 7 (1) p8.

Facciola, S. (2014). *Cornucopia, A Source Book of Edible Plants.* Califonia USA. Kampong Publication.

Faten, M. A. E. (2009). Antioxidant and anticancer activities of Doum fruit extract (*Hyphaene thebaica*). *African Journal of Pure and Applied Chemistry,* 3 (10), 197-201.

Fearon, E.R. and Vogelstein, B. (1990). *A genetic model for colorectal tumorigenesis. Cell. 61 (5): 759–67. doi:10.1016/0092-8674(90)90186-I. PMID 2188735.*

Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Bray, F. (2015). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase. No. 11. http://doi.org/10.1016/j.ucl.2013.01.011

Ferri, F. (2015). Ferri's Clinical Advisor, 1st edition, St. Louis, MO: *Elsevier Mosby*. 2014.

Fletcher, R.H. (1986). Carcinoembryonic antigen. *Annual Internet Medline*, 104:66-73.

Foley, K.M., Wagner, J.L., Joranson, D.E., Gelband, H. (2006). Pain Control for People with Cancer and AIDS. In: New York: *Oxford University Press,* 981-994.

Fraga, C. G., Leibovitz, B. E., and Tappel, A. L. (1988). Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine, 4*(3), 155–161. *http://doi.org/10.1016/0891-5849(88)90023-8.*

Fuchs, C.S., Giovannucci, E.L., Colditz, G.A. (1999). Dietary fiber and risk of colorectal cancer and adenoma in women . *New England Journal of Medicine,* (340):169-176.

Ghanbari, R.F., Anwar, K.M., Alkharfy, A.H., Gilani and Saari, N. (2012). Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea L.)-A Review. Internet Journal of Molecular Science,* 13: 3291_3340.

GLOBOCAN (2008). Cancer Incidence and Mortality Worldwide: IARC CancerBase No.10 (Internet). Lyon, France: International Agency for Research on Cancer. 2010; Available from: http://globocan.iarc.fr.

GLOBOCAN (2012). Cancer Incidence and Mortality. Accessed at www.cancer.gov/types/colorectal/hp/colon-treatment-pdq.

GLOBOCAN (2012). Estimated Cancer Incidence, Mortality and Preventive. World wide.

Gold, P., Freedman, S.O. (1965). Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *Journal Express Medline,* 121:439-462.

Goldstein M., Hinneburg, I., Dorman, D. (2007). Neural substrate of the interaction of emotional stimulus processing and motor inhibitory control: An emotional linguistic go/no-go fMRI study. *Neurolmage*, 36, 1026-1040.

Graczyk, S., Pliszczak-Król, A., Kotonski, B., Wilczek, J., and Chmielak, Z. (2003). Examination of haematological and metabolic changes mechanism of acute stress in turkeys. *Electronic Journal of Polish Agricultural Universities: Veterinary Medicine,* 6(1), 1-10.

Graham, R.A., Wang, S., Catalano, P.J. and Haller, D.G. (1998). Post surgical surveillance of colon cancer: preliminary cost analysis of physician examination, CEA testing, chest X-ray and colonoscopy. *Annual Surgery,* 228:59-63.

Halliwell, B. (1989). Oxidants and central nervous system. Some fundamental questions. Acta. Neurol. Scand. 126, 23-33.

Halliwell, B. (1997). Reactive oxygen species in living systems: source, biochemistry and role in human disease. *American Journal of Medicine,* 91,14-22.

Halliwell, B. (2007). Reactive oxygen species and the central nervous system. *Journal of Neurochemistry,* 59, 1609-1623.

Hammarstrom, S. (1999). The carcinoembryonic antigen (CEA) family: structure, suggested functions and expression in normal and malignant tissue. *Seminal Cancer Biology,* 9:67-81.

Hammond, K.L., van Eeden, F.J., and Whitfield, T.T. (2010). Repression of Hedgehog signaling is required for the acquisition of dorsoliteral cell fates in the zebrafish otic vesicle . Development (Cambridge, England). 137 (8): 1361-1371.

Hanna, T.P. and Kangolle, A.C. (2010). Cancer control in developing countries: using health data and health services research to measure and improve access, quality and efficiency. *BMC International Health Human Rights.* 10:24. Medline: 20942937 doi:10.1186/1472-698X-10-24.

Hinneburg, I., Dorman, D., Hiltunen, R. (2006a). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry,* 97: 122–129.

Hostetter, R.B., Augustus, L.B., Mankarious, R., Chi, K., Fan, D., Toth, C. (1990). Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis. *Journal National Cancer Institute,* 82:380-385.

Hsu, B., Coupar, I.M., Ng K. (2006b). Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. *Food Chemistry,* 98,317-328.

Hsu, D.K., Yang, R.Y., Liu, F.T. (2006). Galectins in apoptosis. *Methods Enzymology,* 417:256–273.

IARC Cancer Base No. 10. Lyon, France: International Agency for Research on Cancer, 2010. Available at: http://globocan.iarc.fr. Accessed: 12 April 2012.

International Agency for Research on Cancer.Cancer in Africa, Epidemiology and Prevention. IARC Science Publication 2003;153:1-414. Medline:14570050.

International Agency for Research on Cancer.GLOBOCAN 2008. Lyon, France: IARC, 2010. Available at: http://globocan.iarc.fr/. Accessed: 16 April 2012.

International Association of Cancer Registries. Membership list: Africa. Lyon, France: IACR, 2012. Available at: http://www.iacr.com.fr/africact-frame.htm. Accessed: 17 April 2016.

Isaac, L. J., Abah, G., Akpan, B., and Ekaette, I. U. (2013). Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria. p.24-27.

Islam, M. S., Lucky, N. S., Islam, M. R., Ahad, A., Das, B. R., Rahman, M. M. and Siddiui, M. S. I. (2004). Haematological Parameters of Fayoumi, Assil and Local Chickens Reared in Sylhet Region in Bangladesh. *International Journal of Poultry Science, 3*(2), 144-147.

Iwuji, T. C., and Herbert, U. (2012).Haematological and serum biochemical characteristics of rabbit bucks fed diets containing garcimia kola seed meal. *Proceedings of 37th Annual Conference of Nigerian Society for Animal Production,* (p.87-89).

Janero, D.R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free radical Biology Medline,* 9, 515-540.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D. (2011). Global cancer statistics.*CA Cancer Journal of Clinical Medline*.21296855 doi:10.3322/caac.20107, 61:69- 90.

Jemal, A., Cente,r M.M., Desantis, C., Ward, E.M.(2010). Global Patterns of Cancer Incidence and Mortality Rates and Trends. *Cancer Epidemiologies Biomarkers Preview,* 19(8):1893-1907.

Jessup, M., Thomas, P. (1989). Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. *Cancer Metastasis Review* 8:263-280.

Johnston, J. K., & Morris, D. D. (1996). Alterations in blood proteins.In B. P. Smith (Edition), *International Animal Medicine* (2nd edition). USA: *Mosby Publishers*.

Kabir, M., Akpa, G. N., Nwagu, B. I., Adeyinka, I. A., and Bello, U. I. (2011). Sexual dimorphism, breed and age characteristics of rabbits in Zaria, Nigeria. *Proceedings of the 16th Annual Conference of Animal Science Association of Nigeria,* p.133-137.

Kamis, A.B., Modu, S., Zanna, H., Oniyangi, T.A. (2003). Preliminary biochemical and haematological effects of aqueous suspension of pulp of *Hyphaene thebaica* (*L*.) mart in rats. *Journal of Biochemistry,* 13, 1-7.

Katalinic, V., Milos, M., Kulisic, T., Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, 94: 550–557.

Kaur J., Stuart, N., Carney, J.M. (2011). Truncation of the Mr p20 protein reveal new ribosome-assembly subcomplex in mitochondria. LID-10.1038/embor. 133[doi]. EMBO Rep. *Journal of Biological Chemistry.* 80(1):228-32.

Khan, T. A., and Zafar, F. (2005). Haematological Study in Response to Varying Doses of Estrogen in Broiler Chicken. *International Journal of Poultry Science, 4*(10), 748-751.

Kilani, S., Sghaier, M., Limem, L., Bouhlel, I., Boubaker, J. Bhouri, W., Skandrani, L., Neffatti, A., Ammarb, R., Dijoux- Franca, M., Ghedira, K., Chekir-Ghedira, L. (2008). *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic

activities of the tubers infusion and extracts of *Cyperus rotundus. Bioresource Technologist*, 99: 9004–9008.

Kitts, D., Yuan, Y., Wijewickreme, A.N., Hu, C. (2000). Antioxidant properties of a North American ginseng extract. *Molecular Cellular Biochemistry*, 203: 1–10.

Langley-Evans, S.C. (2000). Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. *International Journal of Food Science Nutrition*, 51: 181–188.

Lee, S.E., Hyun, J.H., Ha, J., Jeong, H., Kim, J.H. (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Science*, 73: 167–179.

Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., Johnson, J.M. (2006). "Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs". *Nature*.433(7027): 769–773. doi:10.1038/nature03315. PMID 15685193.

Lisco, H., Jeong, H., Hyun, J.H., Kim, J.H.(1947). Carcinoma of the colon in rats following the feeding of radioactive *yttrium Cancer Research*, 7, 721–725.

Lopez-Lazaro, M., (2015). The migration ability of stem cells can explain the existence of cancer of unknown primary site. Rethinking metastasis. *Oncoscience. 2 (5): 467–75. doi:10.18632/oncoscience.159. PMC 4468332 .PMID 26097879.*

Lopez-Lazaro, M., (2015a). Stem cell division theory of cancer. *Cell Cycle. 14 (16): 1–2. doi:10.1080/15384101.2015.1062330. PMID 26090957.*

Lorenz, E., Kitts, D., Yuan, Y., (1941). Intestinal Carcinoma and other lesions in mice following oral administration of 1,2,5,6-dibenzanthracene and 20-methyleholanthrene. *Journal of National Cancer Institute.* 1941;1:17-40.

Lynch, H. T., Grimson, A., Schelter, J.M., de la Chapelle, A. (2008). Hereditary colorectal cancer. *The New England Journal of Medicine*, *348*(10), 919–32. http://doi.org/10.1056/NEJMra012242.

Madara, J.L., Sghaier, M., Limem, L., Bouhlel, I. (1983). Evidence for an adenoma-carcinoma sequence in dimethylhydrazine-induced neoplasms of rat intestinal epithelium. *America Journal of Pathology*, 110, 230–235.

Mann, R.K., Grunstein M., Grimson, A., Schelter, J.M. (1902). Histone H3N- terminal mutations allow hyper action of the yeast GAL 1 gene *in vivo EMBO Journal* 11(9): 3297-306.

Manzoor, M., Anwar, F., Mahmood, Z., Rashid, U., Ashraf, M. (2012).Variation in minerals, phenolics and antioxidant activity of peel and pulp of different varieties of peach (*Prunus persica L.*) fruit from Pakistan. *Journal of Molecular Science*, 17: 6491-6506.

Martin, C., Rouel, J., Jouany, J.P. and Doreau, M. (2008). Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or

linseed oil. *Journal of Animal Science*, (10): 2642-2650.

Martin, J. P., Dailey, M. and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics, 255*(2), 329–336. http://doi.org/10.1016/0003-9861(87)90400-0.

Maton, A., Hopkins, R. L. J., McLaughlin, C. W., Johnson, S., Warner, C. W., LaHart, D., Wright, J. D. (1993). *Human Biology and Health.* Englewood Cliffs, New Jersey, USA: Prentice Hall.

Merck Manual (2012). Haematologic reference ranges. *Mareck Veterinary Manual*. Retrieved from http://www.merckmanuals.com/.

Mmereole, F. U. C. (2008). The Effects of Replacing Groundnut Cake with Rubber Seed Meal on the Haematological and Serological Indices of Broilers. *International Journal of Poultry Science, 7*(6), 622-624.

Nambiar, V.S., Bhadalkar, K. and Daxini, M. (2003). Drumstick leaves as source of vitamin A in ICDS-SFP. *Indian Journal of Pediatric*, 70: 383–387.

National Cancer Institute (2011). DevCan: Probability of Developing or Dying of Cancer Software, Version 6.7.0; Statistical Research and Applications Branch. National Cancer Institute.

National Institute for Occupational Health.Incidence of Histologically Diagnosed Cancer in South Africa, 2003.

National Institute for Occupational Health.Incidence of Histologically Diagnosed Cancer in South Africa, 2004.

Ogunbajo, S. O., Alemede, I. C., Adama, J. Y., Abdullahi, J. (2009). Haematological parameters of Savannah brown does fed varying dietary levels of flamboyant tree seed meal. *Proceedings of 34th Annual Conference of Nigerian Society for Animal production.* p. 88-91.

Okunlola, D. O., Olorunisomo, A. O., Aderinola, A. O., Agboola, A. S., and Omole, O. G. (2012). Haematology and serum quality of red Sokoto goats fed Baobab (*Adansonia digitata*) fruit meal as supplement to guinea grass *(Panicum maximum). Proceedings of the 17th Annual Conference of Animal Science Association of Nigeria.*p.427-433.

Olafedehan, C. O., Obun, A. M., Yusuf, M. K., Adewumi, O. O., Oladefedehan, A. O., Awofolaji, A. O., and Adeniji, A. A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits. *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production*, p.212.

Omiyale, C. A., Yisa, A. G., and Ali-Dunkrah, L. A. (2012). Haematological characteristics of Yankasa sheep fed Fonio (*Digitaria iburua*) straw based diets.

Proceedings of 37th Annual Conference of Nigerian Society for Animal Production, p. 87-89.

Onyeyili, P. A., Egwu, G. O., Jibike, G. I., Pepple, D. J., and Ohaegbulam, J. O. (1992). Seasonal variation in haematological indices in the grey-breasted guinea fowl (*Numida mealagris Gallata pallas*). *Nigerian Journal of Animal Production*, 18(2), 108-110.

Orwa, C., Mutua, A., Kindt,R., Jamnadass, R., Simons, A. (2011). "Agroforestree database: a tree reference and selection guide version 4.0". Url: http://www.Worldagroforestry.org/af/treedb/ (Accessed on 15 February, 2011).

Ovuru, S. S., and Ekweozor, I. K. E. (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. *African Journal of Biotechnology, 3*(6), 346-348.

Oyawoye, B. M., and Ogunkunle, H. N. (2004). Biochemical and haematological reference values in normal experimental animals, *New York: Masson*, (p. 212-218).

Parkin D.M. (2008). The role of cancer registries in cancer control. *Internet Journal of Clinical Oncol*ogy, 13(2):102-111.

Peters, S. O., Gunn, H. H., Imumorin, I. G., Agaviezor, B. O., and Ikeobi, C. O. (2011). Haematological studies on frizzled and naked neck genotypes of Nigerian native chickens. *Tropical Animal Health Production, 43*(3), 631-638.

Piao, M., Kang, k., Dong, R., Okko, D., Wang, Z., You, H., Kim, H., Kim, J., Kang, S., Hyun, J. (2008). Hyperoside prevents oxidative damage induced by hydrogen peroxide in lung fibroblast cells via an antioxidant effect. *Biochimical Biophysical Acta*, 1780 : 1448–1457.

Popoola, A.O., Omodele, F.O., Oludara, M.A., Ibrahim, N.A., Igwilo, A.I., Makanjuola, S.B.L. (2013). Prevalence and pattern of cancers among Adults attending a Tertiary Health Institution in Lagos, Nigeria.

Purves, W. K., Sadava, D., Orians, G. H., and Heller, H. C. (2003). Life*: The Science of Biology*, (7th edition, page 954).

Reddy, R., Mahadik, P.S., Sukdeb, M. and Jayasimha, N.M. (1991).Enzymes of the Antioxidant Defense System in Chronic Schizophrenic Patients. *Biology of Psychiatry*. 30, 409-412.

Rice-Evans, C., Miller, N., Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical, Biology Medline*, 20: 933–956.

Richie, J.P., Black R.J., Purves, W. K., Sadava, D. (2007). Induction of colon tumorigenesis by glutathione depletion in knock-out mice. *Internet Journal of Oncology*, 30, 1539–1543, p53.

Sankaranarayanan R. and Boffetta P. (2010). Research on cancer prevention, detection and management in low- and medium-income countries. *Annual Oncology,* 72.

Sankaranarayanan, R. (2009). HPV vaccination: the promise and problems. *Indian Journal Medline Research,* 130(3):322-326.

Sankaranarayanan, R., Swaminathan, R., Brenner, H. (2010). Cancer survival in Africa, Asia, and Central America: a population-based study. *Lancet Oncology* 11:165-173.

Seiser, P. E., Duffy, L. K., David, M. A., Roby, D. D., Golet, G. H., and Litzow, M. A. (2000).Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the Exxon Valdez oil spill. *Marine Pollution Bulletin, 40* (2), 152-164.

Siddhartha, S., Mehta, A. and Mehta, P. (2011). Hepatoprotective activity of *Cajanus cajan* against Carbon tetrachloride Induced Liver Damage. *Internet Journal Pharmacy Pharmacological Science,* 3: 0975-1491.

Sidell, B. D., and O' Brien, K. M. (2006). When bad things happen to good fish: the loss of haemoglobin and myoglobin expression in Antarctic icefishes. *The Journal of Experimental Biology,* 209, 1791-1802.

Sinet, P.M., Heikkila, R.E. and Cohen, G. (1980). Hydrogen peroxide production from rat brain *in vivo*. *Journal of Neurochemistry*, 34, 1421-1428.

Singh, A. S., Pal, D. T., Mandal, B. C., Singh, P., and Pathak, N. N. (2002). Studies on Changes in Some of Blood Constituents of Adult Cross-bred Cattle Fed Different Levels of Extracted Rice Bran. *Paksitan Journal of Nutrition,* 1(2), 95-98.

Smith, C.D., Carney, J.M., Sturke Reed, P.E., Oliver, C.N., Stadman, E.R., Floyd, R.A. and Markesbery, W.R. (1991). Excess brain protein oxidation and enzymes dysfunction in normal aging and in Alzheimer disease. Proc. *National Academic Science.* USA. 88, 10540-10543.

Smith, RA, Cokkinides V., Brawley O.W. (2009). Cancer screening in the United States. A review of current American Cancer Society guidelines and issues in cancer screening. *CA Cancer Journal of Clinical*; 59: 27-41.

Soetan, K. O., Akinrinde, A. S., and Ajibade, T. O. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production,* p. 49-52.

Souri, E., Amin, G., Farsam, H. and Barazandeh, T.M. (2008). Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU,* 16: 83-87.

Srijita-Dutta (2015). Natural sources as potential anti-cancer agents: *A review. Department of Pharmacology, NSHM College of Pharmaceutical Technology, Kolkata.* ISSN: 2348-8948. Volume: 3; Issue: 5.

Sturgeon, M., Allemani, C., Santaquilani, M., Knijn, A., Marchesi, F., Capocaccia, R. (2008). EUROCARE-4. Survival of cancer patients diagnosed. Results and commentary. *European Journal of Cancer*, 45(6):931-991.

Swanson, M. J. (1996). Physiological properties, cellular and chemical constituents of blood.*Cornstock Publishing Associates, Cornell University Press, Ithaca, London,* 8th edition, p.75-83).

Taylor, R.J., Patterson, T.F., Harrod, R.J. (1994). Systematic of Mexican Spruce-Revisited. *Systematic Botany*, 19: 47-59.

Thomas, P., Toth, C.A., Saini, K.S., Jessup, J.M., Steele, G. (1990).The structure, metabolism and function of the carcinoembryonic antigen gene family. *Biochimical Biophysical Acta,* 1032:177-189.

Thompson, J.A., Grunert, F., Zimmermann, W. (1991). Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. *Journal Clinical Laboratory Analytical,* 5:344-366.

Tiwari, A.K. (2001). Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy. *Current Science,* 8: 1179–1187.

Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonijebu, D. C., Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbits - a preliminary study, *Proceedings of the 41st Conferences of the Agricultural Society of Nigeria,* (p. 341).

Ugwuene, M. C. (2011). Effect of Dietary Palm Kernel Meal for Maize on the Haematological and Serum Chemistry of Broiler Turkey. *Nigerian Journal of Animal Science,* 13, 93-103.

Ullah, M.F. and Khan, M.W. (2008). Food as Medicine: Potential therapeutic tendencies of plant derived polyphenolic compounds. *Asian Pacific Journal Cancer Prevalence,* 9: 187-196.

Verma, Sheetal, D., Jasani, B., Fiander, A. (2013). Current and future status of herbal medicines. *Veterinary World*, 1:347-350.

Walpole, A.L., Saini, K.S., Jessup, J.M., Steele, G. (1952). The carcinogenic action of 4-aminodiphenyl and 3:2-dimethyl-4-amino-diphenyl. *British Journal of Industrial Medicine,* 9, 255–263.

Wang, H., Khor, T. O., Shu, L., Su, Z. Y., Fuentes, F., Lee, J. H. and Kong, A. N. T. (2009). Plants vs. cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. *Anti-Cancer Agents in Medicinal Chemistry*, *12*(10), 1281–305.

Wasowicz, W., Neve, J and Peretz, A. (1993). Optimised Steps in Flourimetric Determination of Thiobarbituric Acid reactive substances in serum: Importance of extraction pH and influence of sample preservation and storage. *Clinical Chemistry*, 39 (12), 2522-2526.

Waugh, A., Grant, A. W., and Ross, J. S. (2001). Ross and Wilson Anatomy and Physiology in Health and Illness, *Churchill Livingston, an imprint of Elsevier Science Limited.* 9th edition, p. 59-71.

Wolff E.C., Kang K.R., Kim Y.S., Park, M.H. (2007). Post translational synthesis of hypusine: evolutionary progression and specificity of the hypusine modification. *Amino Acids,* 33(2): 341-50.

Wood D, Jenne J (2007). The conservation of agrobiodiversity on-farm: Questioning emerging paradigm. *Biodiversity Conservation*, 6: 109-129.

Wood, L.D., Turner P.C., Sylla A., Gong Y.Y. (2007). The genomic landscapes of human breast and colorectal cancers. *Journal of Science,* (318): 1108–1113.

World Health Organization.Framework convention for tobacco control. Geneva: WHO, 2012. Available at: http://www.who.int/fctc/en/. Accessed: 15 April 2016.

Yao, Y. and VieiraA. (2011). Comparative antioxidant properties of citrus species: Evidence for potent, non-vitamin, antioxidants in *C. aurantifolia. International Journal of Food Nutrition Public Health,* 1: 1-9.

Yeatman, T.J. (2001). Colon Cancer. *Encyclopedia of Life Sciences*. Macmillan Publishers Ltd, Nature Publishing Group.

Zia-Ul-Haq, M., Ahmad, S., Calani, L., Mazzeo, T., Rio, D.D., Pellegrini N. and DeFeo, V. (2012a). Compositional study and antioxidant potential of *Ipomoea hederacea* Jacq.and*Lepidium sativum* L. seeds. *Molecules,* 17: 10306_10321.

Zia-Ul-Haq, M., Shahid, S.A., Ahmad, S., Qayum M. and Khan, I. (2012b). Antioxidant potential of various parts of *Ferula assafoetida* L. *Journal of Medicinal Plant Res*earch, 6: 3254-3258.

APPENDIX 1.0

Effect of *Hyphaene thebaica* fruit on Percentage Packed Cell Volume (PCV), Neutrophil and Lymphocyte in the Blood following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Group	Treatment	PCV(%)	NEUTRO(%)	LYMPHO(%)
(i)	Normal Control	48.50 ± 3.4^{c}	29.75 ± 9.0^{b}	59.75 ± 8.5^{a}
(ii)	MNU Control	26.50 ± 8.2^{a}	39.25 ± 7.3^{c}	68.25 ± 6.6^{b}
(iii)	MNU+2.5% of *H. thebaica* fruit	33.25 ± 3.6^{b}	25.25 ± 6.4^{ab}	66.50 ± 4.7^{b}
(iv)	MNU+5% of *H. thebaica* fruit	36.25 ± 6.1^{b}	23.25 ± 3.9^{a}	64.50 ± 3.8^{b}
(v)	MNU+10% of *H. thebaica* fruit	52.50 ± 2.6^{cd}	23.00 ± 5.1^{a}	58.75 ± 1.5^{a}
(vi)	10% of *H. thebaica* fruit Control	55.25 ± 1.3^{d}	20.00 ± 5.9^{a}	56.75 ± 4.2^{a}

N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).

APPENDIX 2.0

Effect of *Hyphaene thebaica* fruit on Haemoglobin and Platelet Concentration in the Blood Serum following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Group	Treatment	Haemoglobin(g/dL)	Platelet (g/dL)
(i)	Normal Control	15.80±1.2^{b}	12.80±0.9^{b}
(ii)	MNU Control	10.25±1.8^{a}	8.10±1.4^{a}
(iii)	MNU+2.5% of *H. thebaica* fruit	10.98±1.2^{a}	9.05±0.2^{a}
(iv)	MNU+5% of *H. thebaica* fruit	13.65±2.0^{ab}	10.90±1.0^{ab}
(v)	MNU+10% of *H. thebaica* fruit	17.48±0.9^{bc}	12.50±0.4^{b}
(vi)	10% of *H. thebaica* fruit Control	18.13±0.4^{c}	12.85±0.4^{b}

N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).

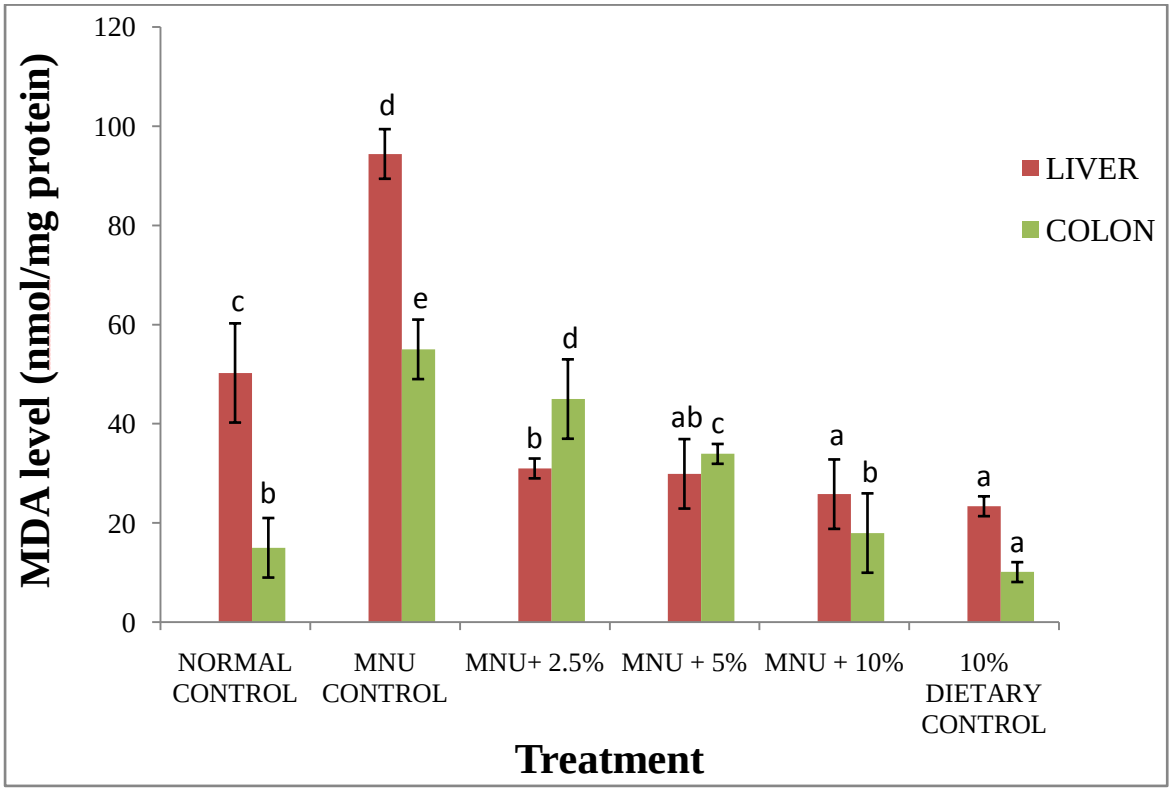
APPENDIX 3.0

Effect of *Hyphaene thebaica* fruit on White Blood Cell and Red Blood Cell in the Blood following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

Group Treatment	WBC (10^9/L)	RBC (10^{12}/L)
(i) Normal Control	7.25±4.6[a]	10.83±0.6[b]
(ii) MNU Control	14.75±4.1[c]	6.68±0.8[a]
(iii) MNU+2.5% of *H. thebaica* fruit	12.50±4.8[b]	7.50±1.0[a]
(iv) MNU+5% of *H. thebaica* fruit	11.80±3.8[b]	7.98±0.9[a]
(v) MNU+10% of *H. thebaica* fruit	8.63±0.1[a]	10.43±0.5[b]
(iv) 10% of *H. thebaica* fruit Control	7.40±1.8[a]	11.45±0.3[b]

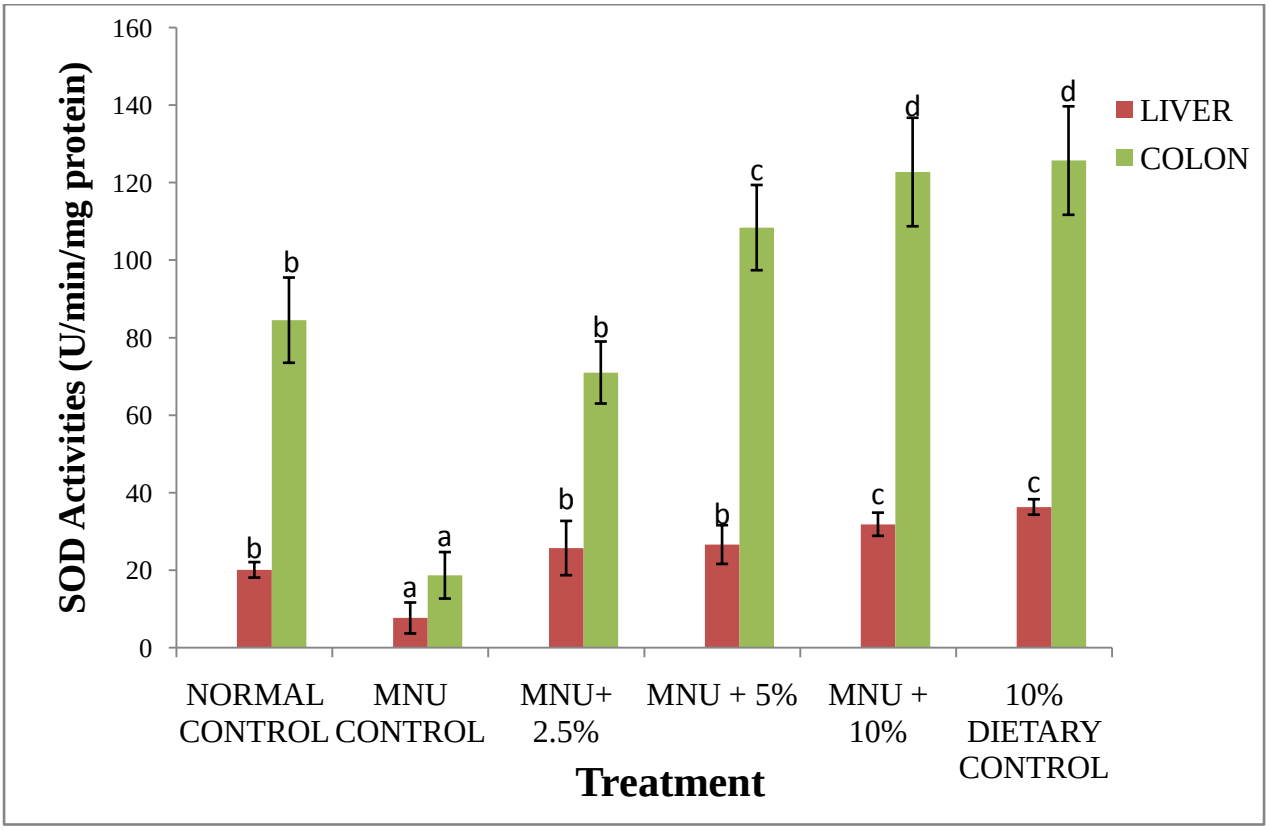
N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).

APPENDIX 4.0



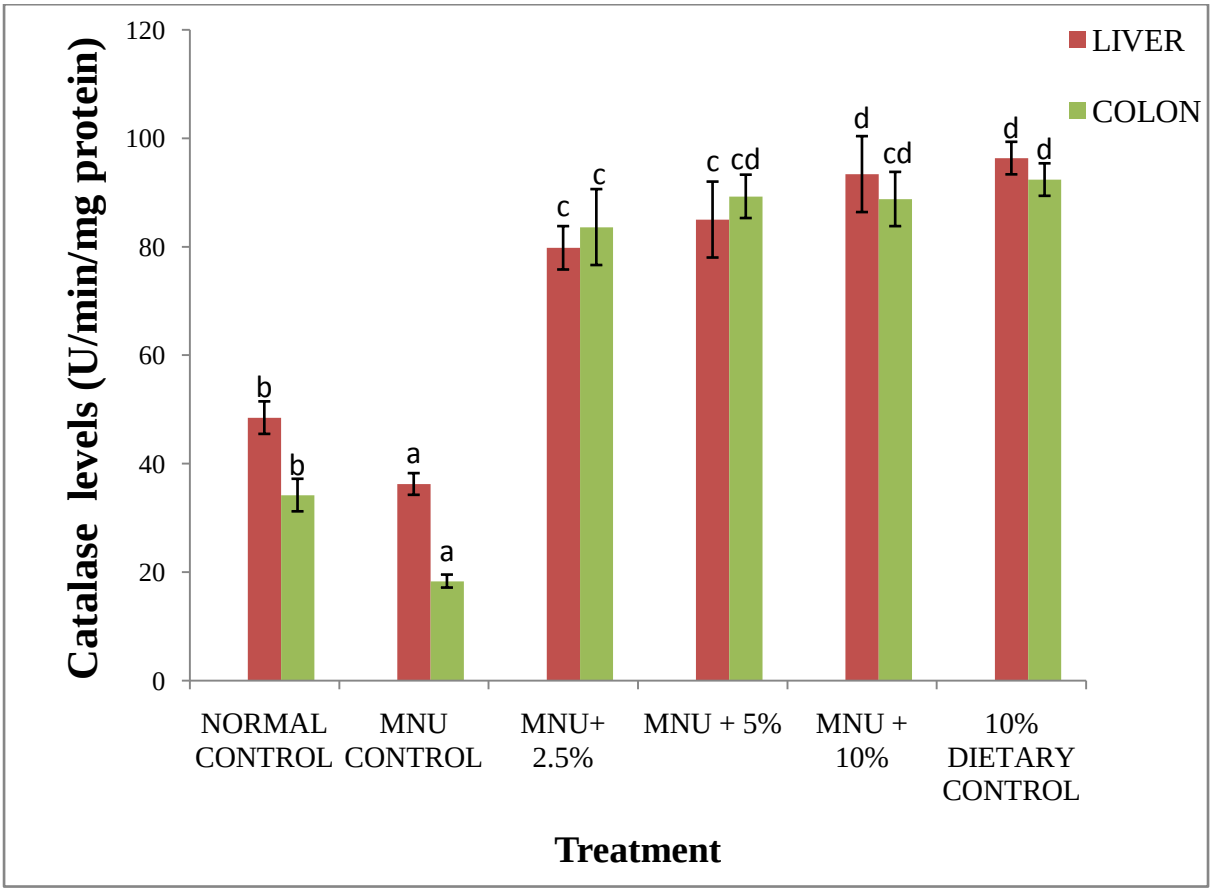
Effect of *Hyphaene thebaica* fruit on MDA Level in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

APPENDIX 5.0



Effect of *Hyphaene thebaica* fruit in SOD Activities in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

APPENDIX 6.0



Effect of *Hyphaene thebaica* fruit on Catalase Activities in Liver and Colon Homogenate following Twelve (12) weeks Intrarectal Administration of MNU in Rats.

APPENDIX 7.0

CEA Concentration of the Serum following 12 weeks Intrarectal Administration of MNU in Rats fed with Different Level of Dietary inclusion of *Hyphaene thebaica* fruit.

Group	Treatment	CEA Concentration (ng/ml)
(i)	NORMAL Control	136.50±41.34[a]
(ii)	MNU Control	236.42± 67.95[b]
(iii)	MNU+2.5% of *H. thebaica* fruit	177.99±46.87[ab]
(iv)	MNU+5% of *H. thebaica* fruit	152.95±22.21[a]
(v)	MNU+10% of *H. thebaica* fruit	141.41± 45.08[a]
(vi)	10% of *H. thebaica* fruit Control	132.82±42.19[a]

N=6: Values are Mean±SD; Values having different letters across the column are significantly different ($p \leq 0.05$).