PREVENTIVE EFFECT OF DIFFERENT LEVELS OF JEW'S MALLOW (*CORCHORUS OLITORIUS* LINN*)* AND SCENT LEAF (*OCIMUM GRATISSIMUM* LINN*)* DIET SUPPLEMENTATION ON MNU-INDUCED COLON CANCER IN WISTAR RATS

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SEPTEMBER, 2015

DECLARATION

I declare that the work in this Dissertation entitled 'PREVENTIVE EFFECT OF DIFFERENT LEVELS OF *CORCHORUS OLITORIUS* LINN AND *OCIMUM GRATISSIMUM* LINN DIET SUPPLEMENTATION ON MNU INDUCED COLON CANCER IN WISTAR 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 thesis was previously presented for another degree at this or any other institution.

Ogungbemi Kunle _____ _____

MSc/Sci/22990/2012-2013 Signature Date

CERTIFICATION

I declare that the work in this Dissertation entitled PREVENTIVE EFFECT OF DIFFERENT LEVELS OF *CORCHORUS OLITORIUS* LINN AND *OCIMUM GRATISSIMUM* LINN DIET SUPPLEMENTATION ON MNU INDUCED COLON CANCER IN WISTAR RATS by Ogungbemi Kunle (MSc/Sci/22990/2012-2013), meets the regulations governing the award of the degree of Masters in Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to the Family of Elder B.K Ogungbemi whom God used in the entire period of my Masters programme.

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ABSTRACT

The preventive effect of different levels of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn leaf supplementation on MNU induced colon cancer in wistar rats was investigated using standard method. Weaning rats of 70-90g of weight were fed with vital feed (growers mash) for 2 weeks for acclimatization before being separated into ten (10) groups. All animals except those in the vehicle (normal saline)-treated groups were administered MNU intrarectally thrice a week for 10 weeks and four (4) control groups received normal saline 3 times weekly and for ten (10) weeks. All groups we were given different levels of the supplementation for ten (10) weeks prior to induction and continued on their respective dietary regimen until the termination of the experiment after which the animals were sacrifices for further analysis. Carcinoembryonic antigen assay (CEA) showed that there was a significant increase ($P<0.05$) with MNU control group (3.47 ± 0.29) when compared with the normal control (1.17 ± 0.3) and the varying supplemented diet both for *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn. Level of CEA also decreases with increasing supplementation. The histopathology of the colon of the MNU control group showed a distorted colonic architecture with distorted blood vessels, hyperchromaticity, and cellular pleomorphism generating anisocytosis and poikilocytosis, with atrophy in the gland, also the section showed necrosis of the lamina propria when compared to the normal control while, normalcy increases with increasing level of dietary supplementation. Thiobarbituric acid reactive substance (TBARS) increased significantly ($P<0.05$) in both kidney and liver of MNU control group when compared to the normal control group whereas there was significant decrease ($p<0.05$) in superoxide dismutase (SOD) and catalase (CAT) in MNU control and MNU induced group when compared to the normal control group. Effect of dietary supplementation with *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn leaf supplemented diets on haematological parameters showed a significant difference ($P<0.05$) in the white blood cell (WBC), lymph, hemoglobin (HGB), red

blood cell (RBC), hematocrit (HCT) , Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH) in the MNU control group when compared with that of the normal control group whereas there was no significant difference ($P>0.05$) between the PLT and MCV of the normal control group and the MNU control group. Therefore, it can be concluded that both *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn leave supplementation posses anticancer potentials with the highest activity in the 10% supplementation.

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ABBREVIATIONS

ANOVA: Analysis of Variance

APC: Adenomatous Polyposis Coli

CAT: Catalase

CEA: Carcinoembryonic Antigen

CIN: Chromosomal Instability

CRC: Colorectal Cancer

DNA: Deoxyribonucleic Acid

EDTA: Ethylene Diamine Tetraacetic Acid

ELISA: Enzyme-Linked Immunosorbent Assay

HB: Haemoglobin

HCT: Hematocrict

HRP: Horseradish Peroxidase

MCH: Mean Corpuscular Haemoglobin

MCHC: Mean Corpuscular Haemoglobin Concentration

MCV: Mean Corpuscular Volume

MDA: Malon Dialdehyde

MMR: Mismatch Repair

MNU: N-methyl-N-nitrosourea

NSAID: Non Steroidal Anti-Inflammatory Drugs

OD: Optical Density

PLT: Platelets

RBC: Red Blood Cell

SOD: Superoxide Dismutase

TBARS: Thiobarbituric Acid Reactive Substance

TCA: Trichloroacetic Acid

WBC: White Blood Cell

CHAPTER ONE

1.1 INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells, that starts in one organ or tissue called the primary site; and if undetected or cannot be controlled through treatment, spreads (metastasizes) to other organs throughout the body. It causes 10% of all deaths worldwide and is second only to cardiovascular disease as the main cause of death in developed countries after heart disease Jemal *et al.,* (2011). It was also estimated that nearly 1.5 million men and women had been diagnosed in 2009 with cancer in the United States, American Cancer Society (2009). To maintain good health, the body must effectively eliminate food and bodily waste. The colon together with the lungs, skin and kidneys are designed to accomplish this essential task by eliminating toxins in the intestines, blood and lymph systems.

The colon (large intestine, rectum, and anus) is the end portion of the human gastrointestinal (GI) tract which extends from the mouth to the anus. It is a muscular tube approximately 152.4cm in length and has an average diameter of approximately 6.35cm. The colon starts on the lower right side of the abdomen, where the small intestine empties the contents of digestion (chyme) into the first portion of the colon (cecum). The ascending colon goes up from the cecum to the level of the liver; it then bends sharply to the left and crosses the abdomen as the transverse colon. At the level of the spleen the descending colon goes down the left side of the abdomen to the pelvis, where it becomes the *sigmoid region*. The sigmoid colon empties into the rectum, from which waste material is ultimately eliminated from your body. The contents of digestion (chyme) are

moved along by muscular movements called peristalsis which is initiated by the nerve supply to the colon.

The main functions of the colon are absorption of water and minerals, and the formation and elimination of feces. The colon contains nearly 60 varieties of microflora or bacteria to aid digestion, promote vital nutrient production, to maintain pH (acid-base) balance, and to prevent proliferation of harmful bacteria. These bacteria provide important functions such as the synthesis of folic acid and valuable nutrients from foods, including vitamins 'K' and portions of the 'B' complex. Bacillus coli and acidophilus comprise the majority of healthy bacteria in the colon along with other disease producing bacteria in lesser numbers. The process of digestion from ingestion of food to defecation, normally takes between 12 to 24 hours assuming that the colon is fully functional and non-toxic. Irregular or infrequent bowel movements can allow toxic residues, from the by-products of undigested foods, to remain in the colon. A person with a healthy colon will have 2 to 3 bowel movements per day, shortly after each meal taken. Elimination should be complete and easy. The stool should be light brown in color, long and large in diameter. There should be no offensive odor and it should break apart with toilet flushing. This describes the normal function of a healthy colon.

Colorectal cancer is a disease originating from the epithelial cells lining the colon or rectum of the gastrointestinal tract, most frequently as a result of mutations in the Wnt signaling pathway. The Wnt signaling pathways are a group of signal transduction pathways made of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell that artificially increase signaling activity. The mutations can be inherited or are acquired, and most probably occur in the intestinal crypt stem cell Srikumar *et al.,* (1999). The most commonly

mutated gene in all colorectal cancer is the adenomatous polyposis coli (APC) gene Markowitz, and Bertagnolli, (2009), which produces the APC protein. The APC protein is a "brake" on the accumulation of β-catenin protein (cadherin-associated protein); without APC, β-catenin accumulates to high levels and translocates into the nucleus, binds to DNA, and activates the transcription of genes that are normally important for stem cell renewal and differentiation but when inappropriately expressed at high levels can cause cancer Kraus *et al.,* (1994). While APC is mutated in most colon cancers, some cancers have increased β-catenin because of mutations in β-catenin (CTNNB1) that block its degradation, or they have mutation(s) in other genes with function analogous to APC such as AXIN1, AXIN2, TCF7L2, or NKD1 Markowitz *et al.,* (2009). Beyond the defects in the Wnt-APC-beta-catenin signaling pathway, other mutations must occur for the cell to become cancerous. The p53 protein, produced by the *TP53* gene, normally monitors cell division and kills cells if they have Wnt pathway defects. Eventually, a cell line acquires a mutation in the *TP53* gene and transforms the tissue from an adenoma into an invasive carcinoma. Sometimes the gene encoding p53 is not mutated, but another protective protein named BAX is Markowitz *et al.,* (2009).

Currently, surgery is the principal method of treatment of colon cancer but the surgical success rate of patients treated for colon cancer is less than 40% Mandel *et al.,* (1993). Besides, almost all the present cancer treatment methods have invasive/toxic effects on the patients and they are also very expensive. It is, therefore, imperative to provide an effective herbal intervention to supply chemo preventive agents for controlling colon cancer since herbal products may act in a pathway similar to pharmaceuticals yet without side effects Wargovich, (2001). Some plants derived chemicals have been proven to have chemopreventive potentials on intestinal cancer Yoshimi, (1992). Along this line, much has since been done by the researchers whose interests

domicile in cancer interventions to unravel the essential ingredients with anticancer properties that are hidden in plants and other food sources, among which are the phytochemicals, vitamins and minerals Higdon *et al*., (2007). The use of pharmacological agents such as plant components to inhibit, delay or reverse the multi-step process of carcinogenesis Ghadi *et al*., (2009) is what is known as chemoprevention.

Corchorus olitorius Linn is widely spread all over the tropics, and it probably occurs in all countries of tropical Africa. Jew's mallow is used as a leafy mucilaginous vegetable. The cooked leaves form a slimy sticky sauce, comparable to okra. In Nigeria this sauce is found suitable for easy consumption of starchy balls made from cassava, yam or millet. Wild plant of *Corchorus olitorius* Linn grow in grassland and fallow or abandoned fields, often close to marshes, rivers and lakes, at up to 1250(–1750)m altitude. Jew's mallow thrives best under hot and humid conditions.

According to Li *et al.*, (2012) *Corchorus olitorius* Linn is a culinary and medicinal herb, widely used as a vegetable in several countries. Many studies have shown that *C. olitorius* Linn contains several antioxidants and exhibits anti-inflammatory and anti-proliferative activities in various in-vitro and in vivo settings. *C. olitorius* Linn has been approved for its antitumor activity Li *et al.*, (2012), Similarly, Previous studies have shown that *O. gratissimum* Linn posses antifungal antibacterial and anticancer activity Lin *et al.*, (2002). It is widespread over Asia, Africa, central and southern America with its centre of diversity in Africa Matasyoh, (2012). Thus it can be use as a food supplement for the management/prevention of colon cancer.

1.2 STATEMENT OF PROBLEM

Every year, about 140,000 people are diagnosed with colorectal cancer (colon cancer). More than 50,000 people die from it, making it the third most commonly diagnosed cancer and the second most common cause of cancer deaths in the United States, Center of disease control and prevention, USA (1997).

In 2013, an estimated new cases of colon cancer were observed with a total number of 102,480 with the male estimated value of 50,090 and female estimated value of 52,390, the estimated deaths for male is 26,300 and female 24,530 making a total of 50,830 in United State Siegel *et al.*, (2013). From this finding it means over half of colon cancer patients lose their life

In Nigeria, there is yet to be a precise statistical data on the prevalence of colon cancer, but the male/female ratio is averagely equal and the peak age remains around 44 years Irabor and Adedeji, (2009).

Some of the non-steroidal anti-inflammatory drugs (NSAIDs) used in the treatment of cancer (e.g colon cancer) can cause acute hypertension (increase in blood pressure). These drugs cause the body to retain fluid and can also lead to decreased kidney function. Excess fluid puts increased pressure on the blood vessels and can cause blood pressure to increase. If this is not well monitored it could progress from the acute stage to chronic stage. Examples of NSAIDs include naproxen, aspirin and ibuprofen Leigh, (2013). In addition, other chemotherapy and radiotherapy preventive measures may present some side effects such as an increased risk for infections, hair loss, fatigue, lip sores, nausea, vomiting, diarrhea, and bloody stools. Moreover the menace involved in the conventional treatment of colon cancer, the cost is quite exorbitant Ladabaum, (2003) and too expensive to most poor patients

1.3 JUSTIFICATION

Due to the vast effect of cancer (example colon cancer) in the world especially in developing countries such as Nigeria, different means on how to reverse the colon cancer (if detected earlier and administration at early stage, though within the convertible limit) has been the interest of several researchers. thus, there is need to search for a cheaper substitute, interference which when supplemented with diet will avert or diminish the risk of development of colon cancer using plants such as *Corchorus olitorius* and *Ocimum gratissimum* which are very much available and accessible by everyone whether poor or rich instead of drugs which could have other adverse effect.

1.4 NULL HYPOTHESIS

Dietary supplementation with the *Corchorus olitorius* and *Ocimum gratissimum* can neither treat colon cancer nor help to extend survival

1.5 AIM

The aim of this work is to investigate the effect of dietary supplementation of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on MNU-induced colon cancer in wistar rats.

1.6 OBJECTIVES

I. To establish the preventive effect of *C. olitorius* Linn *and O. gratissimum* Linn supplementation on MNU induced colon cancer in rats.

II. To assess the effect of *C. olitorius* Linn *and O. gratissimum* Linn supplementation on haematological parameters in MNU-induced colon cancer in rats

III. To determine the effect of *C. olitorius* Linn *and O. gratissimum* Linn supplementation on the levels of endogenous antioxidant activity activities such as superoxide dismutase, catalase and malondialdyhyde activity in MNU-induced colon cancer wistar rats

CHAPTER TWO

LITERATURE REVIEW

2.1 COLON CANCER

2.1.1 Definition

Colon cancer is cancer of the large intestine (colon), the lower part of your digestive system. Rectal cancer is cancer of the last several inches of the colon. Together, they're often referred to as colorectal cancers. Most cases of colon cancer begin as small, noncancerous (benign) clumps of cells called adenomatous polyps. Over time some of these polyps become colon cancers. Polyps may be small and produce few, if any, symptoms. For this reason, doctors recommend regular screening tests to help prevent colon cancer by identifying polyps before they become colon cancer. It was estimated that nearly 1.5 million men and women had been diagnosed in 2009 with cancer in the United States, American Cancer Society (2009). Also in 2009, the Center for Disease Control listed cancer as the second leading cause of death behind heart disease. The loss of cellular regulation such as the mechanisms governing the control of growth and proliferation of cells often gives rise to cancer. Causes of cancer can be genetically inherited such as genetic mutations and immune deficiencies, but also involve external factors such as tobacco use, alcohol consumption and exposure to radiation.

The colon (large intestine, rectum, and anus) is the end portion of the human gastrointestinal (GI) tract which extends from the mouth to the anus. It is a muscular tube approximately 5 to 5 ½ feet in length and has an average diameter of approximately 2 ½ inches. The colon starts on the lower right side of the abdomen, where the small intestine empties the contents of digestion (chyme) into the first portion of the colon (cecum). The ascending colon goes up from the cecum to the level of the liver; it then bends sharply to the left and crosses the abdomen as the transverse

colon. At the level of the spleen the descending colon goes down the left side of the abdomen to the pelvis, where it becomes the *sigmoid region*. The sigmoid colon empties into the rectum, from which waste material is ultimately eliminated from your body. The contents of digestion (chyme) are moved along by muscular movements called peristalsis which is initiated by the nerve supply to the colon.

The main functions of the colon are absorption of water and minerals, and the formation and elimination of feces. The colon contains nearly 60 varieties of micro-flora or bacteria to aid digestion, promote vital nutrient production, to maintain pH (acid-base) balance, and to prevent proliferation of harmful bacteria. These bacteria provide important functions such as the synthesis of folic acid and valuable nutrients from foods, including vitamins 'K' and portions of the 'B' complex. Bacillus coli and acidophilus comprise the majority of healthy bacteria in the colon along with other disease producing bacteria in lesser numbers. The process of digestion from ingestion of food to defecation, normally takes between 12 to 24 hours assuming that the colon is fully functional and non-toxic. Irregular or infrequent bowel movements can allow toxic residues, from the by-products of undigested foods, to remain in the colon. A person with a healthy colon will have 2 to 3 bowel movements per day, shortly after each meal taken. Elimination should be complete and easy. The stool should be light brown in color, long and large diameter. There should be no offensive odor and it should break apart with toilet flushing. This describes the normal function of a healthy colon

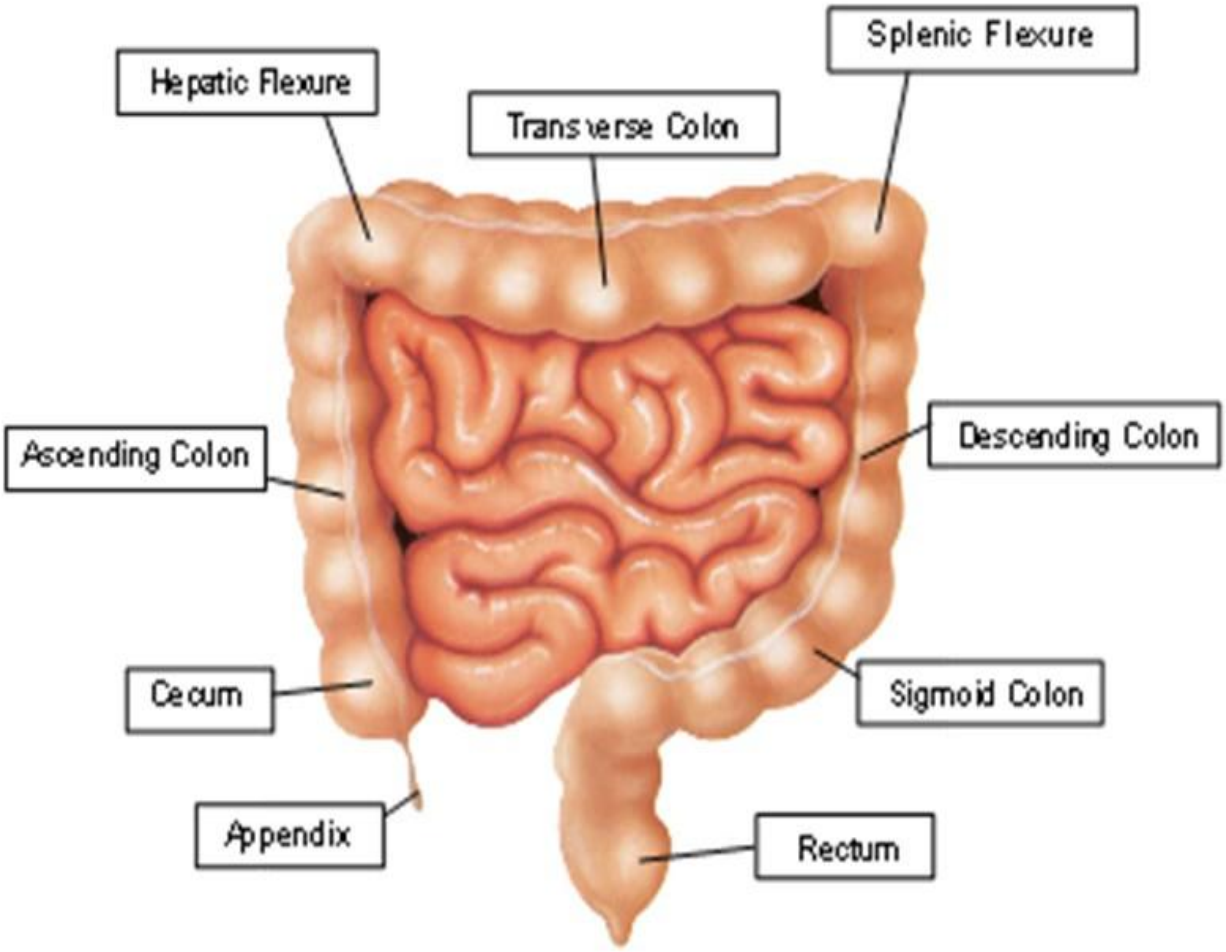


Figure 1.0: Structure of the Colon

2.1.2 Origin of Colon Cancer

Colorectal cancers begin with epithelial cells that line the surface of the colon along finger-like projections called *villi*. The spaces between the villi are called *crypts*, and at the base of each crypt are immature stem cells that give rise to ever-renewing cells that migrate up the crypt and toward the tips of the villi. This normal cellular process is strictly governed by a balance of cellular renewal (normal proliferation) and cellular death (apoptosis), as well as elegantly choreographed expression of various genes along the path from immature stem cells to mature epithelial cells, (Boman and Huang, 2008; D'Errico and Moschetta, 2008).

Early in the course of colon cancer development, however, the normal renewal of cells is disturbed. Cellular maturation (differentiation) is blocked and apoptosis is impaired leading to an accumulation of immature cells in the crypts. This is called an "aberrant crypt" and it is the first step in the carcinogenic process of colorectal cancers, (Boman and Huang, 2008; D'Errico and Moschetta, 2008). Once the aberrant crypt forms, it may go on to become a polyp, a growth along the lining of the colon.

Colon cancer usually develops slowly over a period of 10 to 15 years Kelloff *et al.,* (2004). The tumour typically begins as a noncancerous polyp; a polyp is a growth of tissue that develops on the lining of the colon or rectum that can become cancerous. Polyps are benign, but they can progress to adenomas, which are considered precancerous. If further mutations occur, an adenoma can then progress to cancer over years or decades Levin *et al.,* (2008).

Polyps may be flat, or they may be raised. Raised polyps may grow on the inner surface of the colon or rectum like mushrooms without a stalk (sessile polyps), or they may grow like a mushroom with a stalk (pedunculated polyps). Adenomatous polyps or adenomas are the most common type of polyp and make up about 70% of the polyps found in the colon. Early detection

and removal of these polyps prevents colorectal cancer. About 96% of colorectal cancers are adenocarcinomas, which evolve from glandular tissue1 Stewart *et al.*, (2006).

2.1.3 Epidemiology and Description

The American cancer society estimated that, in 2012, colorectal cancer would be diagnosed in more than 103,000 Americans and would account for nearly 52,000 deaths. This ranks colorectal cancer as the third most common type of cancer and the third leading cause of cancer death in the United States. General risk factors for colorectal cancer include age older than 50 years, history of hereditary intestinal polyposis and nonpolyposis conditions, personal or family history of colorectal cancer, history of inflammatory bowel disease (i.e., ulcerative colitis or Crohn's disease), history of *Streptococcus bovis* bacteremia, use of ureterosigmoidostomy, and presence of type 2 diabetes. Lifestyle factors that may contribute to the development of colorectal cancer include a diet high in animal fat, tobacco use, physical inactivity, obesity, and heavy alcohol use. Night-shift work for 3 or more days per week for at least 15 years has been shown to have a weak link to colorectal cancer. Overall, the lifetime risk of developing colorectal cancer is estimated to be 1 in 18 Steinberg *et al.*, (2009).

2.1.4 Molecular Events Associated with Colon Carcinogenesis

The transition from normal epithelium to adenoma to carcinoma is associated with acquired molecular events Lengauer *et al.*, (1998).This tumor progression model was deduced from comparison of genetic alterations seen in normal colon epithelium, adenomas of progressively larger size, and malignancies Vogelstein *et al*., (1988). At least five to seven major deleterious molecular alterations may occur when a normal epithelial cell progresses in a clonal fashion to

carcinoma. There are at least two major pathways by which these molecular events can lead to CRC. While the majority of CRCs are due to events that result in chromosomal instability (CIN), 20% to 30% of CRCs display characteristic patterns of gene hypermethylation, termed CpG island methylator phenotype (CIMP), of which a portion display microsatellite instability (15% of CRCs) Leggett and Whitehall, (2010).

The spectrum of somatic mutations contributing to the pathogenesis of CRC is likely to be far more extensive than previously appreciated. A comprehensive study that sequenced more than 13,000 genes in a series of CRCs found that tumors accumulate an average of approximately 90 mutant genes. Sixty-nine genes were highlighted as relevant to the pathogenesis of CRC, and individual CRCs harbored an average of nine mutant genes per tumor. In addition, each tumor studied had a distinct mutational gene signature Sjöblom *et al*., (2006)

Key changes in CIN cancers include widespread alterations in chromosome number (aneuploidy) and frequent detectable losses at the molecular level of portions of chromosome 5q, chromosome 18q, and chromosome 17p; and mutation of the *KRAS* oncogene. The important genes involved in these chromosome losses are *APC* (5q), *DCC/MADH2/MADH4* (18q), and *TP53* (17p) Sjöblom *et al.*, (2002) and chromosome losses are associated with instability at the molecular and chromosomal level Lengauer *et al*., (1998). Among the earliest events in the colorectal tumor progression pathway is loss of the *APC* gene, which appears to be consistent with its important role in predisposing persons with germline APC mutations to colorectal tumors. Acquired or inherited mutations of DNA damage-repair genes also play a role in predisposing colorectal epithelial cells to mutations. Furthermore, the specific genes that undergo somatic mutations and the specific type of mutations the tumor acquires may influence the rate of tumor growth or type

of pathologic change in the tumors. For example, the rate of adenoma-to-carcinoma progression appears to be faster in microsatellite-unstable tumors compared with microsatellite-stable tumors. Characteristic histologic changes such as increased mucin production can be seen in tumors that demonstrate microsatellite instability (MSI), suggesting that at least some molecular events contribute to the histologic features of the tumors Rajagopalan *et al.,* (2002).

The key characteristics of MSI cancers are that they are tumors with a largely intact chromosome complement and that, as a result of defects in the DNA mismatch repair (MMR) system, they more readily acquire mutations in important cancer-associated genes compared with cells that have an effective DNA MMR system. These types of cancers are detectable at the molecular level by alterations in repeating units of DNA that occur normally throughout the genome, known as DNA microsatellites Rajagopalan *et al.,* (2002).

The knowledge derived from the study of inherited CRC syndromes has provided important clues regarding the molecular events that mediate tumor initiation and tumor progression in people without germline abnormalities. Among the earliest events in the colorectal tumor progression pathway (both MSI and CIN) is loss of function of the *APC* gene product, which appears to be consistent with its important role in predisposing persons with germline *APC* mutations to colorectal tumors.

2.1.5 Pathology and Tumorigenesis

The earliest phases of colorectal tumorigenesis initiate in the normal mucosa, with a generalised disorder of cell replication, and with the appearance of clusters of enlarged crypts (aberrant crypts) showing proliferative, biochemical and biomolecular abnormalities. The large majority of colorectal malignancies develop from adenomatous polyps. Although several lines of evidence

indicate that carcinomas usually originate from pre-existing adenomas, besides, adenomas, other types of polypoid lesions include hyperplastic polyps, serrated adenomas, flat adenomas, hamartomatous polyps, and inflammatory polyps De Leon and Di Gregorio, (2001).

In the development of colon cancinogenesis, the normal cells are being distressed thus creating aberrant crypts with the cellular maturation being blocked and apoptosis is impaired leading to an accumulation of immature cells in the crypts Boman and Huang, (2008). These aberrant crypts almost always involve a genetic pathway that both embryos and colon cancer have in common, a pathway called *Wnt* Abdul-Ghani *et al*., (2011). Many natural agents exert protective action through influencing this Wnt pathway. Once the aberrant crypt forms, it may go on to become a polyp, which is a growth along the lining of the colon that can be seen during a colonoscopy examination. Polyps are benign, but they can progress to adenomas, which are considered precancerous. If further mutations occur, an adenoma can then progress to cancer over years or decades. This is the primary reason that screening colonoscopies are recommended, to remove the polyps or adenomas before they have a chance to become cancer.

2.1.6 Risk Factors of Colon Cancer

There are many known factors that increase or decrease the risk of colorectal cancer; some of these factors are modifiable and others are not. Non-modifiable risk factors include a personal or family history of colorectal cancer or adenomatous polyps, and a personal history of chronic inflammatory bowel disease Levin *et al*., (2008). Modifiable risk factors that have been associated with an increased risk of colorectal cancer in epidemiologic studies include physical inactivity, obesity, and high consumption of red or processed meats, smoking, and moderate-to-heavy alcohol consumption. A recent study found that about one-quarter of colorectal cancer cases could be avoided by following a healthy lifestyle, i.e., maintaining a healthy abdominal weight, being physically active at least 30 minutes per day, eating a healthy diet, not smoking, and not drinking excessive amounts of alcohol Kirkegaard *et al*., (2010).

2.1.6.1 Heredity and Family History

People with a first-degree relative (parent, sibling, or offspring) who has had colorectal cancer have 2 to 3 times the risk of developing the disease compared to individuals with no family history; if the relative was diagnosed at a young age or if there is more than one affected relative, risk increases to 3 to 6 times that of the general population (Butterworth *et al*., 2006; Johns and Houlston, 2001). About 20% of all colorectal cancer patients have a close relative who was diagnosed with the disease Lynch and Chapelle, (2003).

2.1.6.2 Personal Medical History

People who have had colorectal cancer are more likely to develop new cancers in other areas of the colon and rectum, even if the first cancer was completely removed. The risk of a second cancer is much greater if the first cancer was diagnosed at age 60 or younger.

People who have had one or more adenomatous polyps have an increased risk of colorectal cancer. This is especially true if the polyps were large or if there was more than one Schatzkin *et al*., (1994).

People who have a chronic inflammatory bowel disease have an increased risk of developing colorectal cancer which increases with extent and duration of the disease, Bernstein *et al*., (2001). This includes conditions such as ulcerative colitis and Crohn disease, in which the colon is inflamed over a long period of time. It is estimated that 18% of patients with a 30-year history of ulcerative colitis will develop colorectal cancer Eaden *et al*., (2001).

Many studies have found an association between diabetes and increased risk of colorectal cancer (Larsson *et al*., 2005; Campbell *et al*., 2010). Though adult onset (Type 2) diabetes (the most common type) and colorectal cancer share similar risk factors, including physical inactivity and obesity, a positive association between diabetes and colorectal cancer has been found after accounting for physical activity, body mass index, and waist circumference Larsson *et al*., (2005). A recent study suggests that the association may be stronger in men than in women, Campbell *et al*., (2010).

2.1.6.3 Age

Incidence and death rates for colorectal cancer increase with age. Overall, 90% of new cases and 94% of deaths occur in individuals 50 years. The incidence rate of colorectal cancer is more than

15 times higher in adults 50 years and older than in those between 20 to 49 years of age Jemal *et al*., (2010).

2.1.6.4 Sex

Overall, colorectal cancer incidence and mortality rates are about 35% to 40% higher in men than in women. The reasons for this are not completely understood, but likely reflect complex interactions between gender-related differences in the exposure to hormones and risk factors Murphy *et al*., (2010). Gender differences in risk patterns may also help explain why the proportion of colorectal tumors occurring in the rectum is higher in men (31%) than in women (24%).

2.1.6.5 Physical Inactivity

One of the most consistently reported relationships between colon cancer risk and behaviour is the protective effect of physical activity Wolin *et al.,* (2009). Based on these findings, as well as the numerous other health benefits of regular physical activity, the American Cancer Society recommends engaging in at least moderate activity for 30 minutes or more on 5 or more days per week. Forty-five to 60 minutes of intentional physical activity is preferable. Epidemiologic studies find that:

High levels of physical activity decrease the risk of colon cancer among men and women by possibly as much as 50% Chan and Giovannucci, (2010).

Overweight and obesity

Being overweight or obese is associated with a higher risk of colorectal cancer, with stronger associations more consistently observed in men than in women. Overweight and obesity increase risk of colorectal cancer independent of physical activity Larsson and Wolk, (2007). Abdominal obesity (measured by waist size) may be a more important risk factor for colon cancer than overall obesity in both men and women Wang *et al*., (2008).

2.1.6.6 Diet

Geographic differences in colorectal cancer rates suggest that diet and lifestyle strongly influence colorectal cancer risk. Studies suggest consuming a healthy diet with emphasis on plant sources; namely: limit consumption of red and processed meats, eat a variety of vegetables and fruits, and choose whole grains in preference to processed grains and consuming the recommended levels of calcium will help reduce the risk of developing colorectal cancer Huxley *et al*., (2009).

Several studies, including one by the American Cancer Society, have found that high consumption of red and/or processed meat increases the risk of both colon and rectal cancer (Huxley *et al*., 2009; Cross *et al*., 2010).

2.1.6.7 Smoking

In November 2009, the International Agency for Research on Cancer reported that there is now sufficient evidence to conclude that tobacco smoking causes colorectal cancer Secretan *et al*., (2009). The association appears to be stronger for rectal than for colon cancer Liang *et al*., (2009).

2.1.6.7 Alcohol

Colorectal cancer has been linked to even moderate alcohol use. Individuals who have a lifetime average of 2 to 4 alcoholic drinks per day have a 23% higher risk of colorectal cancer than those who consume less than one drink per day Ferrari *et al*., (2007).

2.1.7 Signs and Symptoms of Colon Cancer

Colon cancer tends not to produce signs until advanced Cappell and Goldberg, (1992). Early colon cancer often has no symptoms as it begins as a small polyp, a small growth in the wall of the colon. Common symptoms include abdominal pain, rectal bleeding, altered bowel habits, and

involuntary weight loss Peppercorn *et al.*, (2011). Although colon cancer can present with either diarrhea or constipation, a recent change in bowel habits is such more likely to be from colon cancer than chronically abnormal bowel habits. Less common symptoms include nausea and vomiting, malaise, anorexia, and abdominal distention Cappell and Goldberg, (1992). Symptoms depend on cancer location, cancer size, and presence of metastases. Left colonic cancers are more likely than right colon cancers to cause partial or complete intestinal obstruction because the left colonic lumen is narrower and the stool in the left colon tends to be better formed because of reabsorption of water in the proximal colon Posner *et al*., (2002). Large exophytic cancers are also more likely to obstruct the colonic lumen. Partial obstruction produces constipation, nausea, abdominal distention, and abdominal pain. Partial obstruction occasionally paradoxically produces intermittent diarrhea as stool moves beyond the obstruction. Distal cancers sometimes cause gross rectal bleeding, but proximal cancers rarely produce this symptom because the blood becomes mixed with stool and chemically degraded during colonic transit Cappell, (1998). Bleeding from proximal cancers tends to be occult, and the patient may present with iron deficiency anemia without gross rectal bleeding Harewood and Ahlquist, (2000). The anemia may produce weakness, fatigue, dyspnea, or palpitations. Advanced cancer, particularly with metastasis, can cause cancer cachexia, characterized by a symptomatic tetrad of involuntary weight loss, anorexia, muscle weakness, and a feeling of poor health Theologides, (1979).

2.1.8 Preventive Agents of Colon Cancer

2.1.8.1 Antioxidants

Antioxidants were the first agents evaluated in the chemoprevention of colorectal cancer, following the observation that colorectal cancer seemed to be less common in populations whose diets were rich in fruits and vegetables Steinmetz and Potter, (1991). The antioxidant vitamins, especially β-carotene, vitamins E and C and α-tocopherol, appeared to be perfect candidates to prevent colorectal cancer, given their minimal toxicity and their repeated association with a lower risk of colorectal cancer in several studies examining dietary intake or serum levels (Longnecker *et al*., 1992; Bostick *et al*., 1993; van Poppel 1993). Experimental studies have shown that antioxidants may have effects on cell proliferation and DNA mutation, mechanisms that are directly implicated in the formation of colorectal neoplasia Paganelli *et al*., (1992).

Selenium, although not an antioxidant, is often considered in this group because of its important role in glutathione peroxidase, an antioxidant enzyme system. In the Skin Cancer Prevention Trial Clark *et al*., (1996), selenium unexpectedly showed protective effects against cancers of the colorectum, lung and prostate.

2.1.8.2 Dietary Fibre

Research has also focused on fiber as a possible alternative to explain the inverse association between risk of colorectal neoplasia and intake of vegetables and fruits Ogimoto *et al*., (2000). Fiber increases the motility of the large bowel and may dilute carcinogenic metabolites, actions that potentially might inhibit chemically induced carcinogenesis Reddy, (1999).

2.1.8.3 Calcium and Vitamin D

The suggestion that calcium intake might lower the risk of colorectal neoplasia has generated considerable investigation Shaukat *et al*., (2005). The proposed mechanism involves calcium's ability to bind and precipitate soluble fatty acids and bile acids in the bowel lumen, inhibiting their well known proliferative and carcinogenic effects Lamprecht and Lipkin, (2003). In support of this hypothesis, animal studies have shown a protective effect of dietary calcium on bile-induced mucosal damage or experimental bowel carcinogenesis. Recently, it has been postulated that calcium affects cellular proliferation and differentiation by acting directly on the calcium-sensing receptor, a cell surface receptor expressed in normal and cancer cell lines.

2.1.8.4 Folate

Several cohort and case–control studies have suggested that increased consumption of vegetables and fruits reduces the risk of colorectal cancer (Trock *et al*., 1990; Thun *et al*., 1991).

Folate is a micronutrient found abundantly in vegetables and fruits. Epidemiologic studies have found a lower incidence of colorectal cancer among those with the highest dietary folate intake (Benito *et al*., 1991: Ferraroni *et al.,* 1994) whereas those with diets low in folate (and often with high alcohol intake) appear to have an increased risk of colorectal adenomas and carcinomas, (Giovannucci *et al*., 1993; Giovannucci *et al*., 1995 and Baron *et al*., 1998). Although large amounts of folate in the diet appear to be protective against the development of colorectal adenomas (relative risk, 0.91 in women and 0.78 in men), the degree of benefit is greater among those who take folate supplements (relative risk, 0.66 for women and 0.63 for men). In the

Nurses' Health Study, supplementation with folate (usually as part of multivitamin supplementation) was protective against colorectal cancer, with the greatest risk reduction among women taking high daily doses of folate (more than 400μg); this reduction (relative risk, 0.25) became statistically significant only after 15 years Giovannucci *et al*., (1998). The long time needed for a clinical benefit to become evident suggests that folate acts early in colon carcinogenesis.

2.1.9 Carcinoembryonic Antigen (CEA)

Carcinoembryonic antigen (CEA) is a member of a family of cell surface glycoproteins that are produced in excess in essentially all human colon carcinomas and in a high proportion of carcinomas at many other sites Benchimol *et al*., (1989), therefore CEA is usually present only at very low levels in the blood of healthy adults. However, the serum levels are raised in some types of cancer, which means that it can be used as a tumor marker in clinical tests Boehm and Peakins, (2000). The function of this widely used tumor marker and its relevance to malignant transformation is therefore of considerable interest. CEA mediates Ca^{2+}-independent, homotypic aggregation of cultured human colon adenocarcinoma cells (LS-180) and rodent cells transfected with functional CEA cDNA. Furthermore, CEA can affect the homotypic sorting of cells in heterogeneous populations of aggregating cells. CEA can thus be considered a new addition to the family of intercellular adhesion molecules. CEA is localized mainly to epithelial cell membranes facing the lumen in normal adult intestine; it is found on adjacent cell membranes in both embryonic intestine and colonic tumors. A model for the role of CEA in the tissue architecture of adult, embryonic, and aberrant tumor intestinal epithelium is presented Benchimol *et al*., (1989) Carcinoembryonic antigen (CEA) is one of the most widely used tumor markers worldwide. Its main application is mostly in gastrointestinal cancers, especially in

colorectal malignancy. Although in use for almost 30 years, the clinical value of CEA in colorectal cancer is still not clear Duffy, (2001).

2.2 Description, Distribution, Classification, Uses and Ecology of *Corchorus olitorius* Linn

2.2.1 Description of *Corchorus olitorius* Linn

Erect annual herb up to 2(–4) m tall, usually strongly branched; stems reddish, fibrous and tough. Leaves alternate, simple; stipules narrowly triangular with long point; petiole (0.5–)1–7 cm long; blade narrowly ovate, ovate or elliptical, 4–15(–20) cm × 2–5(–11) cm, cuneate or obtuse and with setaceous appendages up to 2.5 cm long at base, acuminate to acute at apex, margin serrate or crenate, almost glabrous, usually shiny dark green, 3–7-veined from the base. Inflorescence a 1–4-flowered axillary fascicle, bracteate. Flowers bisexual, regular, usually 5-merous, shortly stalked; sepals free, narrowly obovate, 5–7 mm long; petals free, obovate, 5–7 mm long, yellow, caducous; stamens numerous; ovary superior, usually 5-celled, style short from a cylindrical capsule up to 7(–10) cm long, ribbed, with a short beak, usually dehiscing by 5 valves, many-seeded. Seeds angular, 1–3 mm long, dark grey. Seedling with epigeal germination; hypocotyl 1–2 cm long; cotyledons foliaceous, broadly elliptical to circular, 3–8 mm long Kirtikar and Basu, (1975).

2.2.2 Geographical Distribution of *Corchorus olitorius* Linn

The geographical origin of *Corchorus olitorius* is often disputed, because it has been cultivated since centuries both in Asia and in Africa, and it occurs in the wild in both continents. Some authors consider India or the Indo-Burmese area as the origin of *Corchorus olitorius* and several other *Corchorus* species. However, the presence in Africa of more wild *Corchorus* species and the larger genetic diversity within *Corchorus olitorius* point to Africa as the first centre of origin

of the genus, with a secondary centre of diversity in the Indo-Burmese region. At present *Corchorus olitorius* is widely spread all over the tropics and it probably occurs in all countries of tropical Africa. In tropical Africa it is reported as a wild or cultivated vegetable in many countries. It is a leading leaf vegetable in Côte d'Ivoire, Benin, Nigeria, Cameroon, Sudan, Kenya, Uganda and Zimbabwe. Jew's mallow is also cultivated as a leaf vegetable in the Caribbean, Brazil, India, Bangladesh, China, Japan, Egypt and the Middle East. It is cultivated for jute production in Asia (India, Bangladesh, China) together with *Corchorus capsularis* L., but in Africa it is of no importance as a fibre crop, although the fibre may be used domestically Holm *et al.*, (1979).

2.2.3 Classification of *Corchorus olitorius* Linn

Kingdom	Plantae
Phyllum	Angiosperms
Subphyllum	Eudicots
Class	Rosids
Order	Malvales
Family	Malvaceae
Genus	Corchorus
Species	olitorius



Plate I: leaves of *Corchorus olitorius* Linn **(Photo: plant production system group)**

2.2.4 Uses of *Corchorus olitorius* Linn

Corchorus olitorius is used as a leafy mucilaginous vegetable. The cooked leaves form a slimy sticky sauce, comparable to okra. In Nigeria this sauce is found suitable for easy consumption of starchy balls made from cassava, yam or millet. A powder prepared from dried leaves is used to prepare this sauce during the dry season. The immature fruits, called bush okra, are also dried and ground to a powder for the preparation of this slimy sauce. In East Africa several recipes exist, e.g. Jew's mallow may be cooked with cowpeas, pumpkin, cocoyam leaves, sweet potato, milk and butter, meat, and flavored with pepper and lemon. Jute has been the most widely used packaging fiber for more than 100 years because of its strength and durability, low production costs, ease of manufacturing and availability in large and uniform quantities. However, jute production is insignificant in Africa. The types of *Corchorus olitorius* that are used as a leaf vegetable are quite distinct from the types used for jute production. Whole jute stems are suitable as raw material for paper pulp. However, when jute is used for pulping, it is usually in the form of cuttings from burlap manufacture, old sugar bags and wrappings. The resulting pulp is made into hard, thick paper, suitable for cards and labels. The woody central core ('stick') remaining after removal of the bark can also be processed into paper, board and cellulose. Root scrapings of Jew's mallow are used in Kenya to treat toothache; a root decoction as a tonic, leafy twigs in Congo against heart troubles, an infusion from the leaves is taken in Tanzania against constipation, and seeds in Nigeria as a purgative and febrifuge Danny, (2010).

2.2.5 Ecology OF *Corchorus olitorius* Linn

Wild plant of *Corchorus olitorius* grow in grassland and fallow or abandoned fields, often close to marshes, rivers and lakes, at up to 1250(–1750) m altitude. Jew's mallow thrives best under

hot and humid conditions. In the savanna and Sahel zone, it performs best during the hot rainy season. It is cultivated where annual rainfall averages 600–2000 mm. The optimal temperature is 25–32°C. Growth stops below 15°C. Jew's mallow is a short-day species. In Nigeria a day length of 12.5 hours caused a much stronger vegetative growth expressed in weight of roots, stems and leaves than a day length of 11.5 hours, but the fruit and seed production was higher at a photoperiod of 11.5 hours. Jew's mallow prefers sandy loam soils rich in organic matter and grows poorly on heavy clay (Duke, 1978; Duke, 1979).

2.3.1 Botanical Description of *Ocimum gratissimum* Linn

Ocimum gratissimum is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite; petiole 2-4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5-16 cm x 1-8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate-serrate, apex acute, puberulent or pubescent. Inflorescence a verticillaster, arranged in a terminal, simple or branched raceme 5-30 cm long; rachis lax, softly pubescent; bracts sessile, ovate, 3-12 mm x 1-7 mm, acuminate, caducous; pedicel 1-4 mm long, spreading or ascending, slightly curved; flowers in 6-10-flowered verticillasters, small, hermaphrodite; calyx 2-lipped, 2-3 mm long, in fruit 5-6 mm, pubescent, upper lip rounded and recurved, reflexed in fruit, lower lip with 4, narrow, pointed teeth, central pair of teeth minute and much shorter than the upper lip; corolla campanulate, 3.5-5 mm long, 2-lipped, greenishwhite, pubescent outside, upper lip truncate, 4-fid, lower lip longer, declinate, flat, entire; stamens 4, declinate, in 2 pairs, inserted on the corolla tube, filaments distinctly exserted, upper pair with a bearded tooth at the base; ovary superior, consisting of 2 carpels, each 2-celled, style 2-fid. Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx (the lower lip closing the

mouth of the fruiting calyx); nutlet subglobose, 1.5 mm long, rugose, brown; outer pericarp not becoming mucilaginous in water. *O. gratissimum* is a variable polymorphic complex species, often subdivided into subspecies, varieties and formas, mainly based on differences in chemical content, the morphology of the fruiting calyx, and on different degrees of hairiness, but the variation forms a continuum. Sometimes *O. gratissimum* (existing chromosome counts: 2n = 40, 48, 64), O. suave (2n = 32, 48, 64) and O. viride (2n = 38, 40) (here treated as one complex species *O. gratissimum*) are considered as three different species. Although more research is needed it seems certain that those three taxa are closely related and have 10 homologous chromosomes in common. Crosses between *O. gratissimum* and *O. viride* resulted in partially fertile F1 hybrids. Variability is greatest in Africa and India. In Java, 2 chemotypes exist, the eugenol and the thymol type, respectively described as *O. gratissimum*. forma caryophyllatum Backer and forma graveolens Backer. Forma caryophyllatum is characterized by: leaves clove-scented when bruised, upper side short-haired, lower side densely gland-dotted, bracts 4-6 mm long, much longer than wide, lower lip of corolla not flushed with violet; and forma graveolens by: leaves strongly odoriferous but not clove-scented when bruised, upper surface covered with minute hairs, bracts 2-4 mm long, about as long as wide, lower lip of corolla flushed violet inside. Most *Ocimum* species contain essential oil but are primarily used as vegetable (e.g. hoary basil, O. americanum L.), as spice (e.g. sweet basil, *O. basilicum*), or as vegetable and medicine (e.g. sacred or holy basil, *O. tenuiflorum*), Orwa *et al.*, (*2009).*

2.3.2 Ecology of *Ocimum gratissimum*

In its native area *O. gratissimum* occurs from sea-level up to 1500 m altitude in coastal scrub, along lake shores, in savanna vegetation, in submontane forest, and disturbed land. In South-East

Asia it is not frequently found in open locations like roadsides and clearings, but more often cultivated as a hedge plant, up to about 300 m altitude.

Flowering started after 136 days and continued until 195 days. Seed matured after 259 days. Flowering and seed set were much poorer than in *O. basilicum* or *O. minimum*. In South-East Asia flowers can be found year-round. In northern India, oil content of young plants was low (2.3%) until the seed setting stage, then remained constant at 2.8% until the seed maturation stage, Orwa *et al.*, (2009).

2.3.3 Distribution of *Ocimum gratissimum* Linn

The plant grows widely in tropical Africa. It is also grown in South-East Asia, largely in India and Hawaii. The native areas of the plant are mostly found to be regions that are 1500 m above sea level. The plant also grows well in lake shores, coastal bush lands and in sub-montane regions.

2.3.4 Scientific Classification of *Ocimum gratissimum* Linn

Kingdom: Plantae

Phylum: Angiosperms

Class: Eudicots

Order: Asterids

Family:Lamiaceae

Genus: *Ocimum*

Species: *O. graatissimum*



Plate II: *Ocimum gratissimum* Linn **plant (Photo: Agwan Jeba farm, Zaria)**

2.3.5 Uses of *Ocimum gratissimum* Linn

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion Rabelo *et al*., (2003). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea Effraim *et al.,* (2003). In the Savannah areas decoctions of the leaves are used to treat mental illness Akinmoladun *et al*., (2007). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh Ijeh *et al.,* (2005). Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children**.** People of Kenyan and sub Saharan African communities' use this plant for various purposes like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum Matasyoh *et al.,* (2008). In India, the whole plant has been used for the treatment of sunstroke, headache, influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity Prajapati (2003).

The tribes of Nigeria use the leaf extract in treatment of diarrhea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis Adebolu and Oladimeji, (2005). Studies suggest that *Ocimum gratissimum* effectively combats several types of invasive bacteria. These range from Shigella and Salmonella to Escherichia and Proteus

strains. The oils of the plant also were effective in fighting strains of *E. coli*, dysentery and typhoid. Some research also confirms that clove basil is effective in treating various veterinary problems, from killing worms in goats to increasing libido in laboratory mice. Some even use the oil as an aromatic, yet deadly, mosquito repellant.

Other uses of *Ocimum gratissimum* are still being studied in 2011, particularly the use of the essential oil as an analgesic, or pain reliever. Though the plant is not used alone in providing this relief, it has shown success when administered in unison with other proven antibacterial and anti-inflammatory herbal agents from Africa.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagent

The carcinogen (MNU) was procured from Sigma Chemical Co, or BDH Chemical Ltd, Poole, England. Rat CEA ELISA kit was procured from Wkea Med Supplies Corp, China. Formal-saline, graded alcohol, xylene, paraffin, eosin, hematoxylin, were obtained from the Department of Veterinary Pathology, Ahmadu Bello University, Zaria. All other reagents and chemicals used were of analytical grade.

3.1.2 Equipment

The equipment used include mortar and pestle, dissecting set, centrifuge, homogenizer, syringes, cannula, spectrophotometer (Jenway 6305), microscope, automated analyzer, (Sysmex Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan) hand gloves, electrical weighing balance, water trough, cages, feeders, microtome machine.

3.1.3 Plant Material

Fresh leaves of *Cochorus olitorius and Ocimum gratisimu*m were collected from Angwan Jeba farm, Zaria. The leaves were taken to the herbarium of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University Zaria and were identified.

3.1.4 Plant Preparation

The leaves were destalked, washed and dried at room temperature. The dried samples were be pulverized to fine powder using an automated grinding machine (ken wood) at Institute for

Agricultural Research (IAR) Ahmadu Bello University, Zaria then powdered samples were stored in glass containers at room temperature until needed for use in the experimental diet.

3.1.5 Experimental Diet

Vital feed formulated to contain appropriate nutrient was be bought at Samaru market and used in the entire study. The diet (vital feed) was mixed with *Corchorus olitorius* and *Ocimum gratisimum* according to the percentage of supplementation (2.5%, 5% and 10%), Yan *et al.,* (1998).

3.1.6 Animals' Procurement and Groupings

A total of fifty (50) Wistar rats weighing 70-90g were purchased and housed in the Faculty of Pharmaceutical Science Animal house, Ahmadu Bello University Zaria and allowed free access to water and standard diet for three weeks for acclimatization. The study was conducted in accordance with the US guideline as contained in the National Institute of Health guide for the care and use of laboratory animals, NIH publication No. 18-23, (1985).

At the end of the acclimation period, all animals were weighed and randomly divided into ten (10) groups of five animals each (n= 5). The experimental animals were fed according to the group's percentage of supplementation or basal diet for eight (8) weeks, subsequently induced with MNU or normal saline and given water *ad libitum*.

The groupings are as follows:

Group 1: Normal Control (Normal Saline + Normal feed)

Group 2: NMU Control (NMU + Normal feed)

Group 3: NMU induced + 2.5% diet supplementation with *Corchorus olitorius* Linn

Group 4: NMU induced + 5% of diet supplementation with *Corchorus olitorius* Linn

Group 5: NMU induced + 10% of diet supplementation with *Corchorus olitorius* Linn

Group 6: NMU induced + 2.5% diet supplementation with *Ocimum gratissimum* Linn

Group 7: NMU induced + 5% of diet supplementation with *Ocimum gratissimum* Linn

Group 8: NMU induced + 10% of diet supplementation with *Ocimum gratissimum* Linn

Group 9: Diet Control 1 (Normal Saline + 10% *Corchorus olitorius* Linn)

Group 10: Diet Control 2 (Normal Saline + 10% *Ocimum gratissimum* Linn)

3.2.1 Experimental Design

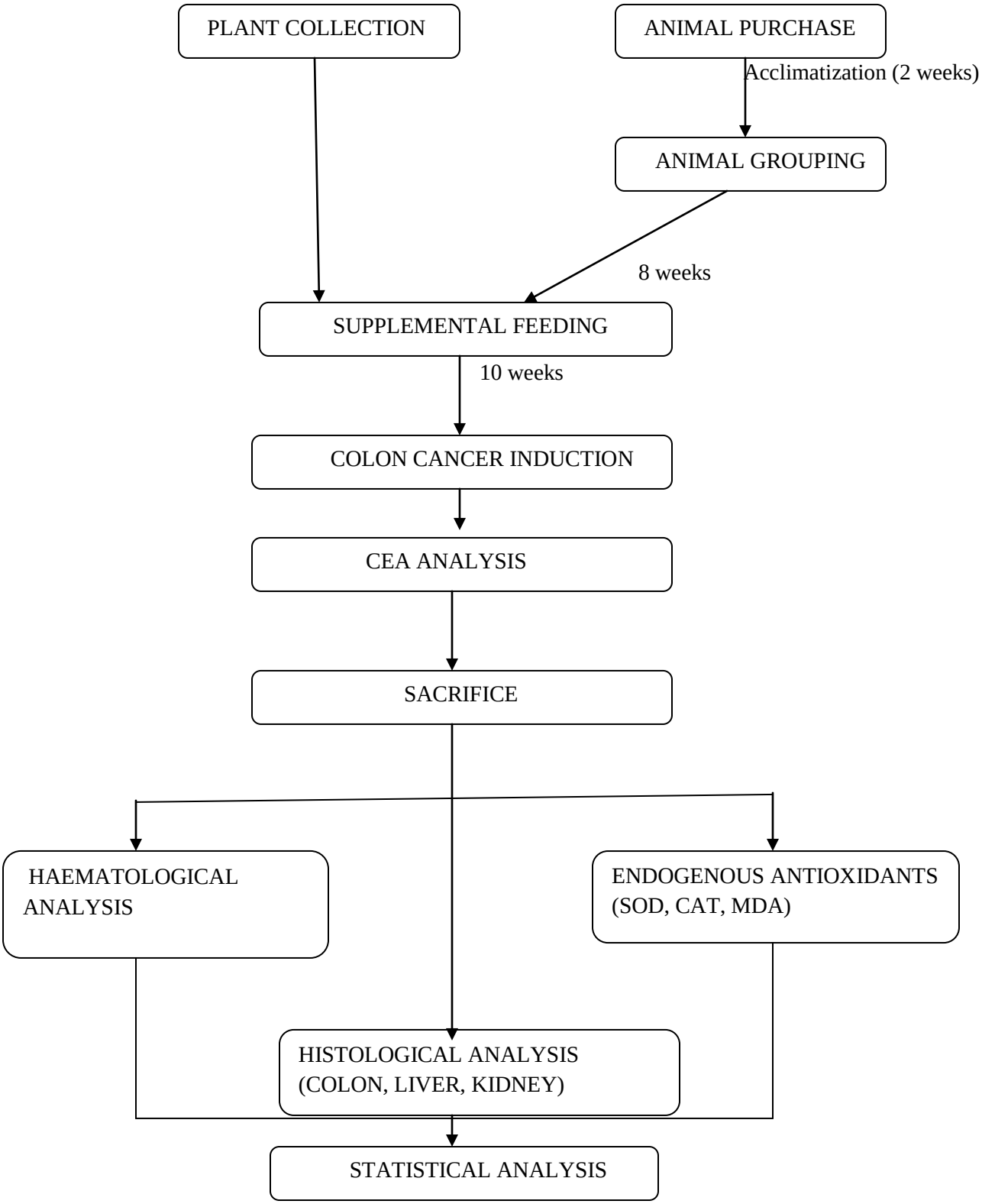


Figure 2.0: A flow chart of the experimental design

3.2.2 Induction of Rat Colon Cancer

MNU Sigma Chemical Co., St. Louise Mo, Catalogue No. N4766-25G was purchased from Sigma Aldrich and used to initiate the carcinogenesis process. Induction of the colon cancer was carried out after eight (8) weeks of supplemented feeding. The rats in the treatment groups received an intra-rectal instillation of MNU solution for 10 weeks while the control groups received normal saline for 10 weeks.

3.2.3 Records of Food Consumption and Body Weight

The food consumption of each group of animals was maintained weekly. The diet was weighed prior to the feeding; the left over feed was also weighed and subtracted from the known mass of feed given to the rats in each cage. This was to obtain accurate weight of the food consumed.

The animals were weighed prior to the commencement of the study and weekly changes in body weight of the rats were noted after which the quarterly measurement was done on individually to know the difference between the control and experimental groups.

3.2.4 Animal Sacrifice

Induction of the animals was terminated at the completion of ten weeks; the animals were fed for an extra one week. Animals were fasted overnight, anesthetized with chloroform and then sacrificed by decapitation. Whole blood, sera, colon, liver and kidney were collected from all the animals.

3.2.5 Collection of Organs

The rats' colon, liver and the kidney were dissected out quickly, rinsed immediately with ice-cold saline to remove blood, after which the organ was placed on a filter paper to mop up water

and weighed (absolute weight) using a digital weighing scale, Halim *et al.,* (2011) and refrigerated.

The relative organ weight of each organ was calculated according to the following equation:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight (g) of rat on the termination Day}} \times 100$$

3.2.6 Preparation of Homogenate

A 10% homogenate of each organ (liver and kidney) was prepared by homogenizing the organs with 50mM phosphate buffer pH 7.4. The organs (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 10464.48g for 10 minutes and the supernatant collected with pasteur pipette was used for analyses. Serum and homogenates were preserved frozen at -4^{0}c until required for use.

3.2.7 Estimation of Endogenous antioxidants

The endogenous enzymatic antioxidants analyzed in the liver and kidney homogenates are superoxide dismutase and catalase.

3.2.8 Assay of Superoxide dismutase (SOD)

SOD was assayed according to the method of Martin *et al.,* (1987).

Principle

The enzyme superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at a high reaction rate.SOD activity assay was based on the rate of auto-

oxidation of haematoxylin in aqueous alkaline solution, which yields a chromophore with maximum spectrophotometric absorbance at 560nm.

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.

$$\text{Haematoxylin} \xrightarrow{\text{Auto-oxidation}} H_2O + \text{Haematin(active form of haematoxylin)}$$

Procedure

Exactly 920µL of phosphate buffer (0.05M, pH7.8) was dispensed into clean test-tubes; 40µL of sample homogenates were added. A reagent test was also prepared by replacing the sample with 40µL of sample dilution buffer. The mixture was incubated for 2 minutes at 25°C before the addition of 40µL of heamatoxylin. Following the addition of 40µL of heamatoxylin, absorbance of the sample test and reagent test were read at 560nm in a spectrophotometer immediately against the sample blank which is distilled water.

The SOD concentration was calculated as percentage inhibition of the rate of auto-oxidation of haematoxylin as follows:

The rate of auto-oxidation (r) is the ratio of average oxidation rate of samples ($Rate_i$) to the average auto-oxidation rate of blank solution ($Rate_{blank}$).

$$\text{Percentage of SOD inhibition of reaction rate} = 100\% - r_i$$

$$\text{The SOD concentration in the sample I in the reaction mixture } C_i \left(\text{SOD}\frac{U}{ml}\right) = 125 \times (100\% - r_i)$$

3.2.9 Assay of Catalase (cat)

Catalase activity was assayed according to Aebi, (1974).

Principle

The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated.

Procedure

Exactly 10µL of sample homogenate was added to test tubes containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm at 5 minutes on a spectrophometer. A molar extinction coefficient (E) of 0.041 mM^{-1}-cm^{-1} was used to calculate the catalase activity.

$$\text{Catalase}(\mu\text{mol/min}) = \frac{\text{Absorbance}}{E}$$

3.2.10 Estimation of Lipid Peroxidation

Endogenous lipid peroxidation levels was determined by monitoring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa *et al.* (1979) with slight modification by Atawodi *et al.*, (2009), Atawodi *et al.*, (2010).

Principle

Lipid peroxidation generates peroxide intermediates which upon cleavage releases malondialdehyde, a product which reacts with thiobarbituric acid. The product of this reaction is a pink coloured complex which absorbs maximally at 532nm wavelength.

Procedure

In the presence of 1ml of 15% (w/v) trichloroacetic acid (TCA), 1ml of thiobarbituric acid and 50µL of sample homogenate were added. The mixture was incubated at 80^{o}C for 30 minutes in a water bath. After cooling, the solution was centrifuged at 940g for 10 minutes and the precipitate obtained was removed. The absorbance of the supernatant was taken at 532nm wavelength against the reagent blank.

TBARS concentration was expressed in nmol/mg protein and calculated as follows:

$$\text{Conc. nmols/mgprotein} = \frac{\text{Absorbance of sample}}{1.56 \times 10^{-5} \times \text{Protein conc. (mg)}}$$

3.2.11 Histological Analysis

The colon, liver and kidney tissues were rapidly fixed for 18-24 hours in 10 % formal saline and dehydrated in ascending grades of alcohol (70%, 80%, 90%, 95%, and absolute 100 %) for 2 hours each. The tissue was cleared in xylene and subsequently transferred through a pure paraffin in an oven at 65^{o}c for 1 hour and then into a second pot of melted paraffin for additional 2 hours (infiltration). The tissue was 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 were be prepared according to standard micro techniques onto glass slide and stained with hematoxylin and eosin. Photomicrographs under

light microscope at high power magnifications (× 100) were examined for histopathological changes.

3.2.12 Haematological Analysis

On the day of sacrifice, approximately 2 ml of blood samples were collected from the rat's jugular vein into vacutainer tubes coated with Ethylene diamine tetraacetic acid (EDTA) after the animals were sacrificed by decapitation. Red blood cells (RBC), total white blood cells (WBC) and differential white blood cells, granulocytes, Platelets (PLT), Mean Corpuscular volume (MCV), Haemoglobin Concentration (HB), Haematocrit count (HCT) Mean Haemoglobin Concentration (MHC) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined using an automated haematology analyzer, Halim *et al.,* (2011) at Ahmadu Bello University Teaching Hospital (ABUTH), Shika and values were recorded.

3.2.13 CEA Assay

CEA is one of the most widely used tumor markers worldwide and certainly the most frequently used marker in colorectal cancer. The concentration of carcinoembryonic antigen (CEA) was determined by enzyme-linked immunosorbent assay (ELISA) test.

Principle

CEA assay is 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 is 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.

Procedure

The test sample is allowed to react simultaneously with the two antibodies, resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 30 minutes incubation at room temperature, the wells were washed with wash solution to remove unbound labelled antibodies. A solution of tetramethylbenzidine reagent was added and incubated for 30 minutes, resulting in the development of a blue colour. The colour development was stopped with the addition of Stop Solution changing the colour to yellow. The concentration of CEA was directly proportional to the colour intensity of the test sample. Absorbance was measured spectrophotometrically at 450nm using a microplate reader. The concentration of CEA in the sample was determined by comparing the optical density (OD) of the samples to the standard curve.

3.2.14 Statistical Analysis

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 ≤ 0.05 level of significance. The results were given as mean ± SD.

CHAPTER FOUR

4.1.1 The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *C. olitorius* Linn on MNU Induced Colon Cancer Wistar Rats

The result obtained from the weight change in percentage of diet supplementation with leaves of *Corchorus olitorius* on MNU induced colon cancers rats as seen in **Table 4.1**. There was an observable percentage increase from the first quarter to the fourth quarter of the groups fed with the basal diet (control group), 10% diet control and 10% supplemented diet with the second quarters having the highest weight gain. While, there was decrease in the third quarter weight change of the MNU control group (-05.638), fourth quarter of the lowest supplementation diet group (-33.561) and third quarter of 5% supplementation diet group (-05.560) when compared to other supplemented diet treated groups which had increase in their weight percentage.

Table 4.1: Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *Corchorus olitorius* Linn on MNU Induced Colon Cancer Wistar Rats

TREATMENT	1st quarter (%)	2nd quarter (%)	3rd quarter (%)	4th quarter (%)
Control feed	21.165	36.014	23.225	19.596
MNU Control	40.7201	57.717	-05.638	07.201
10% supplemented control	25.434	55.720	01.819	20.594
2.5% Supplemented diet	43.410	72.841	17.311	-33.561
5% Supplemented diet	17.449	60.890	-05.560	27.220
10% Supplemented diet	26.58554	39.995	10.976	22.443

MNU control (MNU induction without treatment)

Supplemented Control (10% *C. olitorius* without MNU induction)

4.1.2 The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *Ocimum gratissimum* Linn on MNU Induced Colon Cancer Wistar Rats

The result obtained from the weight change in percentage of diet supplementation with leaves of *O. gratissimum* on MNU induced colon cancers rats as seen in **Table 4.2**. A steady increase in the weight gain(%) were observed in the first to fourth quarter of the groups fed with the basal diet (control group) and the 10% diet control group with the highest weight gain at the second quater. While, there were decrease in the third quarter weight gain of the MNU control group (-05.638), fourth quarter of the lowest supplementation diet group (-16.008) and third quarter of 5% (04.644) and 10% supplementation diet group (-17.000) when compared to other supplemented diet treated groups which had increase in their weight percentage.

Table 4.2: Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *Ocimum gratissimum* Linn on MNU Induced Colon Cancer Wistar Rats

TREATMENT	1st quarter (%)	2nd quarter (%)	3rd quarter (%)	4th quarter (%)
Control feed	21.165	36.014	23.225	19.596
MNU control	40.720	57.717	-05.638	07.201
10% Supplemented control	18.176	72.190	05.845	15.478
2.5% Supplemented diet	30.154	84.538	01.317	-16.008
5% Supplemented diet	22.954	58.779	-04.644	22.910
10% Supplemented diet	09.232	83.836	-17.000	23.932

MNU control (MNU induction without treatment)

Supplemented Control (10% *O. gratissimum* without MNU induction)

4.2 The Animal average weekly Feed Intake (g/kgbodyweight/week) of Diet Supplemented with *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on MNU Induced Colon Cancer Rats.

The result obtained from the effect of leaf supplementation of *Corchorus olitorius* on feed intake on MNU induced colon cancer rats shows there was a significant decrease ($P<0.05$) on MNU control group (363.293 ± 24.81) when compared to 10% C. olitorius supplementation control (569.213 ± 57.34) but shows no significant difference ($P>0.05$) when compared with group treated with 2.5% C. olitorius supplementation (365.629 ± 39.83). Also group treated with 10% *C. olitorius* supplementation (491.039 ± 31.14), 5% *C. olitorius* supplementation (475.592 ± 27.52) and the control group (510.595 ± 52.02) shows no significant ($p>0.05$) difference

Also, there was a significant decrease ($p<0.05$) in the feed intake of animals in the MNU control group (363.293 ± 24.81) and the lowest supplementation diet group (389.627 ± 33.78) while group treated with 5% *O. gratissimum* supplementation (491.169 ± 6.721) and 10% *O. gratissimum* supplementation (504.107 ± 41.87) and the normal control group (510.595 ± 52.02) shows a significant increase in their feed intake when compared with the MNU control group.

Table 4.3: The Average Weekly Feed Intake (g/kgbodyweight/week) of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn Leaf Supplementation carried out for 20 Weeks on Rats induced with MNU.

TREATMENT	Feed intake	Feed intake
	C. olitorius	*O. gratissimum*
Control feed	510.595 ± 52.02^{ab}	510.595 ± 52.02^{a}
MNU + Basal Feed	363.293 ± 24.81^{b}	363.293 ± 24.81^{b}
10% Supplemented Control	569.213 ± 57.34^{a}	468.576 ± 21.66^{ab}
MNU + 2.5% Supplemented diet	365.629 ± 39.83^{b}	389.627 ± 33.78^{b}
MNU + 5% supplemented diet	475.592 ± 27.52^{ab}	491.169 ± 6.721^{a}
MNU + 10% supplemented diet	491.039 ± 31.14^{ab}	504.107 ± 41.87^{a}

Values with different superscript down the column are significantly different ($P<0.05$)

4.3 Level of Carcinoembryonic Antigen (CEA) in rats Treated with *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn Diet Supplementation on N-methyl-N-nitrosourea (MNU) Induction

The result obtained from the level of Carcinoembryonic Antigen (CEA) in rats treated with N-methyl-N-nitrosourea (MNU) and *Corchorus olitorius* and *Ocimum gratissimum* diet supplementation as seen in **Table 4.4** shows there was a significant difference ($P<0.05$) with MNU control (3.47± 0.29) when compared with the normal control (1.17±0.3), 10% *C. olitorius* supplementation control (0.35 ± 0.1), 10% *C. olitorius* supplemented diet (0.42±0.04), 5% *C. olitorius* Supplemented diet (0.42 ± 0.05) and 2.5% *C. olitorius* supplemented diet (1.00 ± 0.000) whereas, there was no significant difference ($p>0.05$) between 10% *C.olitorius* supplemented diet and 5% supplemented diet.

Also, the level of CEA in the MNU control group (3.47±0.29) shows a significant increase ($P<0.05$) when compared to normal control group (1.17±0.3), 10% *O. gratissimum* supplemented control (1.63 ± 0.11), 5% *O. gratissimum* Supplemented diet (2.35 ± 0.37) but shows no significant difference ($p>0.05$) when compared with 2.5% *O. gratissimum* Supplemented diet (2.93 ± 0.29). Also there was no significant difference ($P>0.05$) between the normal control group (1.17± 0.3) and 10% *O. gratissimum* Supplementation control (1.63±0.11).

Table 4.4: Level of Carcinoembryonic Antigen (CEA) in Rats Treated with N-methyl-N-nitrosourea (MNU) given Alongside *C. olitorius* and *O. gratissimum* Linn

TREATMENT	Level of CEA (ng/ml)	Level of CEA (ng/ml)
	C. olitorius	*O. gratissimum*
Control feed	1.17 ± 0.3^{b}	1.17 ± 0.3^{c}
MNU + Basal Feed	3.47 ± 0.29^{a}	3.47 ± 0.29^{a}
10% Supplemented Control	0.8 ± 0.1^{d}	1.63 ± 0.11^{bc}
MNU + 2.5% Supplemented diet	2.00 ± 0.000^{bc}	2.93 ± 0.29^{ab}
MNU + 5% supplemented diet	0.42 ± 0.02^{cd}	2.35 ± 0.37^{bc}
MNU + 10% supplemented diet	0.42 ± 0.04^{cd}	1.92 ± 0.26^{abc}

Values with different superscript down the column are significantly different ($P<0.05$)

4.4.1 Effect of Diet Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Endogenous Antioxidant Enzyme (SOD and Catalase) on the Kidney of MNU Induced Colon Cancer Wistar Rats

From the result obtained of the effect of diet supplementation with leaves of *Corchorus olitorius* and *Ocimum gratissimum* on endogenous antioxidant enzyme (SOD) on the kidney of MNU induced colon cancer wistar rats (**Table 4.5)** , there was no significant difference (P>0.05) between the normal control group (83.33± 7.6), 10% *C. olitorius* supplementation control (96.00±9.3) and 10% *C. olitorius* supplemented diet whereas it shows a significant difference (P<0.05) when compared to the MNU control group (17.25±1.3), 2.5% *C. olitorius* supplemented diet (36.00±1.8), and 5% *C. olitorius* supplemented diet (56.00±5.8).

Also, in the group treated with *Ocimum gratissimum*, MNU control group (17.25±1.3) shows a significant decrease (p<0.05) when compared to the normal control group (83.33±7.6) and 10% *O. gratissimum* supplemented control (94.60 ± 5.8) and other different level of the supplementation. While, group fed with 2.5% *O. gratissimum* supplemented diet (31.00±3.2) showed no significant difference (p>0.05) when compared with group fed with 5% *O. gratissimum* supplemented diet.

The effect of diet supplementation with leaves of *C. olitorius* and *Ocimum gratissimum* on endogenous antioxidant enzyme catalase activity of the kidney of MNU induced colon cancer wistar rats shows there was a significant decrease (p<0.05) in the MNU control group (53.78±13.9) when compared to the normal control group (122.09+7.7), 10% *C. olitorius* supplemented control (137.44±11.2) and group treated with 10% *C. olitorius* supplemented diet (111.10±9.1) whereas there was no significant difference (P>0.05) when compared to group

treated with 2.5% *C. olitorius* supplemented diet (61.13± 10.9) and 5% *C. olitorius* supplemented diet (74.00±11.6)

Also, on treatment with *Ocimum gratissimum,* a significant decrease ($P<0.05$) was observed in group given MNU only (53.738±13.9) when compared with the normal control group(122.09±7.7), 10% *O. gratissimum* supplemented control (120.85±9.2) and group treated with 10% *O. gratissimum* supplemented diet (110.32±3.2) whereas, there was no significant difference ($P>0.05$) when compared with group treated with 2.5% *O. gratissimum* supplemented diet (77.24±18.3) and 5% *O. gratissimum* supplemented diet (80.23±8.7).

Table 4.5: Effect of Diet Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Endogenous Antioxidant Enzyme (SOD and Catalase) on the Kidney of MNU Induced Colon Cancer Wistar Rats

TREATMENT	SOD (U/ml/mg protein)		CAT (µmol/min/mgprotin)	
	C. olitorius	*O.gratissimum*	*C.olitorius*	*O.gratissimum*
Control feed	83.33 ±7.6^{a}	83.33 ± 7.6^{ab}	122.09±7.7^{a}	122.09±7.7^{a}
MNU + Basal Feed	17.25 ±1.3^{d}	17.25 ± 1.3^{c}	53.74±13.9^{b}	53.74±13.9 ^{c}
10% Supplemented Control	96.00 ± 9.3^{a}	94.60 ± 5.8^{a}	137.44±11.2^{a}	120.85±9.2^{a}
MNU+2.5% Supplemented diet	36.00 ± 1.8^{c}	31.00 ± 3.2^{b}	61.13±10.9^{b}	77.24±18.3^{bc}
MNU+5% supplemented diet	56.00 ± 5.8^{b}	71.00±6.4^{b}	74.00 ±11.6^{b}	80.23±8.7^{bc}
MNU+10% supplemented diet	93.50 ± 3.5^{a}	87.50 ± 7.1^{ab}	111.10 ± 9.1^{a}	110.32±3.2^{ab}

Values with different superscript down the column are significantly different ($P<0.05$)

4.4.2 Effect of Diet Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Endogenous Antioxidant Enzymes (SOD and Catalase) on the Liver of MNU Induced Colon Cancer Wistar Rats.

From the result obtained of the effect of diet supplementation with leaves of *Corchorus olitorius* and *Ocimum gratissimum* on endogenous antioxidant enzyme (SOD) in the liver of MNU induced colon cancer wistar rats (**Table 4.6**). It was observed that there was no significant difference ($P>0.05$) in the group treated with 10% *C. olitorius* supplemented diet (96.5 ± 10.63) when compared to the normal control group (98.8 ±2.97) and the 10% supplemented control group (102.00 ±7.5) while, there was a significant difference ($P<0.05$) when compared to the MNU control group (19.67 ±1.3) and group treated with 2.5% *C. olitorius* supplemented diet (36.67 ±9.21), 5% *C. olitorius* supplemented diet (74.25 ±7.27).

Also in group treated with *Ocimum gratissimum*, there was no significant difference ($p>0.05$) between group treated with 10% *O. gratissimum* supplemented diet (87.00 ±3.34) and 5% *O. gratissimum* supplemented diet but a significant increase was observed ($P<0.05$) when compared to the MNU control group(19.67 ± 1.3) and the normal control group (98.8 ± 2.97)

For catalase activities carried out on the liver, it was observed that there was a decrease in the catalase activity of the MNU control group (36.13 ± 10.56) when compared with the Normal control group (137.39±19.9) and 10% *C. olitorius* diet control (114.97±12.1) and group treated with 10% *C. olitorius* supplemented diet but shows no significant difference ($P>0.05$) when compared with group treated with 2.5% *C. olitorius* supplemented diet and 5% *C. olitorius* supplemented diet.

Group treated with *Ocimum gratissimum*, 10% *O. gratissimum* supplemented control (137.46±5.87) shows no significant difference ($p<0.05$) when compared with the normal control group (137.39±19.9) but shows significant increase when compared with group treated with 2.5% supplemented diet (51.27±5.30), 5% *O. gratissimum* supplemented diet (58.45±15.1), 10% *O. gratissimum* supplemented diet (73.37±12.6) and MNU control group (36.13± 10.56).

Table 4.6: Effect of Diet Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Endogenous Antioxidant Enzyme (SOD and Catalase) on the Liver of MNU Induced Colon Cancer wistar Rats

TREATMENT	SOD (U/ml/mgprotein)		CAT (µmol/min/mgprotein)	
	C. olitorius	*O. gratissimum*	*C. olitorius*	*O.gratissimum*
Control feed	98.8±2.97^{a}	98.8±2.97^{ab}	137.39±19.9^{a}	137.39±19.9^{a}
MNU + Basal Feed	19.67±1.3^{c}	19.67±1.3^{e}	36.13±10.56^{c}	36.13±10.56^{b}
10% Supplemented Control	102.00±7.5^{a}	116.33±3.8^{a}	114.97±12.1^{ab}	137.46±5.87^{a}
MNU+2.5% Supplemented diet	36.67±9.21^{c}	61.75±4.38^{d}	76.18±10.7^{bc}	51.27±5.3^{b}
MNU+5% supplemented diet	74.25±7.27^{b}	92.5±2.02^{bc}	71.51±10.6^{c}	58.45±15.1^{b}
MNU+10% supplemented diet	96.5±10.63^{a}	87.00±3.34^{c}	126.5±12.1^{a}	73.37 ± 12.6^{b}

Values with different superscript down the column are significantly different ($P<0.05$)

4.4.3 Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Lipid Peroxidation on the Kidney and Liver of MNU Induced Colon Cans4cer wistar Rats.

The result obtained from the effect of dietary supplementation with leaves of *Corchorus olitorius* and *Ocimum gratissimum* on the level of TBARS on the kidney of MNU induced colon cancer wistar rats shows that a significant Increase ($P<0.05$) was observed in the MNU control group (6.65±0.93) when compared with the normal control group (3.22±0.84) but no significant difference ($p>0.05$) when compared to group treated with 2.5% *C. olitorius* supplemented diet (5.99±0.4) and 5% *C. olitorius* supplemented diet (5.89±0.4). (**Table 4.7**)

In groups treated with *Ocimum gratissimum*, a significant increase ($P<0.05$) was observed in MNU control group (6.65±0.93) when compared to the normal control group (4.03±0.84) and 10% *O. gratissimum* supplemented control (3.99± 0.46) but shows a gradual decrease with increasing level of supplementation with no significant difference ($P>0.05$).

The result obtained from the effect of dietary supplementation with leaves of *Corchorus olitorius* and *Ocimum gratissimum* on lipid peroxidation on the liver of MNU induced colon cancer wistar rats shows that group treated with 10% *C. olitorius* supplemented diet (5.92±0.93) and 10% *C. olitorius* diet control (6.29±1.60) shows no significant difference ($P>0.05$) when compared to the normal control group (6.76±0.96) but a significant decrease ($P<0.05$) when compared to the MNU control group (15.98±2.58) and group treated with 2.5% *C. olitorius* supplemented diet (10.5 ± 1.65) and 5% *C. olitorius* supplemented diet (6.17± 1.43).

In groups treated with *Ocimum gratissimum*, a significant increase ($P<0.05$) was observed with MNU control group (15.98±2.58) when compared with the normal control group(6.76±0.96) and

10% *O. gratissimum* supplemented control (6.58±1.54) but shows a gradual decrease with increase in different level of *O. gratissimum* supplemented diet.

Table 4.7: Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Lipid Peroxidation on the Kidney and Liver of MNU Induced Colon Cancer Wistar Rats

TREATMENT	KIDNEY TBARS (umols/mg protein)		LIVER TBARS (umols/mg protein)	
	C. olitorius	*O. gratissimum*	*C.olitorius*	*O. gratissimum*
Control feed	4.03 ± 0.84^{bc}	4.03 ± 0.84^{b}	6.76 ± 0.96^{b}	6.76 ± 0.96^{b}
MNU + Basal Feed	6.65 ± 0.93^{a}	6.65 ± 0.93^{a}	15.98 ± 2.58^{a}	15.98 ± 2.58^{a}
10% Supplemented Control	3.22 ± 0.84^{c}	3.99 ± 0.46^{b}	6.29 ± 1.60^{b}	6.58 ± 1.54^{b}
MNU + 2.5% Supplemented diet	5.99 ± 0.4^{ab}	5.66 ± 0.44^{ab}	10.5 ± 1.65^{ab}	11.05 ± 0.83^{ab}
MNU + 5% supplemented diet	5.89 ± 0.4^{ab}	5.55 ± 0.19^{ab}	6.17 ± 1.43^{ab}	9.39 ± 1.94^{ab}
MNU + 10% supplemented diet	4.43 ± 0.58^{abc}	5.27 ± 0.21^{ab}	5.92 ± 0.93^{b}	5.92 ± 1.05^{ab}

Values with different superscript down the column are significantly different ($P<0.05$)

4.5 Result of Quantitative Analysis of the Total Polyphenolic and Flavonoid Content of the Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn Supplemented Vital Feed Diet.

The result obtained from the quantitative analysis of the total polyphenolic content of the leaves of *Corchorus olitorius* and *Ocimum gratissimum* supplemented vital feed diet shows that the total polyphenolic contents were both high in the leaves of *Corchorus olitorius* (180.4618±8.34) and *Ocimum gratissimum* (165.452±3.29). While, the total polyphenolic content in others increased with increased level of supplementation when compared to the normal feed which shows the lowest total polyphenolic content.

Similar result was also obtained from the quantitative analysis of the flavonoid content of the leaves of *Corchorus olitorius* and *Ocimum gratissimum* supplemented vital feed diet which shows that the flavonoid contents were both high in the leaves of *Corchorus olitorius* (180.4618±8.34) and *Ocimum gratissimum* (165.452±3.29). While, the flavonoid content in others increased with increased level of supplementation when compared to the normal feed which shows the lowest total flavonoid content. (**Table 4.8**)

Table 4.8: Result of Quantitative Analysis of the Total Polyphenolic Content (mg gallic acid/g of sample) of the Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn Supplemented Vital Feed Diet

Level of Supplementation	*C. olitorius*	*O. gratissimum*
0% Supplementation	59.32504 ± 0.0028	59.32504 ± 0.0028
2.5% Supplementation	71.9751 ± 1.42	62.6998 ± 0.88
5% Supplementation	104.0853 ± 3.55	78.62643± 0.978
10% Supplementation	132.5044 ± 2.48	107.93 ± 5.77
Pure leaf	180.4618±8.34	165.4529±3.29

Values are mean ± SD for the two supplementations

Table 4.9: Result of Quantitative Analysis of the Flavonoid Content of the Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn Supplemented Vital Feed Diet

Level of Supplementation	*C. olitorius*	*O. gratissimum*
0% Supplementation	30.9776 ± 0.01	30.9776 ± 0.01
2.5% Supplementation	35.7868 ± 3.05	34.1671 ± 0.00667
5% Supplementation	61.1331 ± 3.69	59.7172 ± 2.539
10% Supplementation	82.1091 ± 5.54	78.2614 ± 6.638
Pure leaf	125.2759±11.89	124.6218±6.2904

Values are mean ± SD for the two supplementations

4.6 Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* Linn and *Ocimum gratissimum* Linn on Histopathology of MNU Induced Colon Cancer.

The histopathology of the colon of the MNU control group shows a distorted colonic architecture with distorted blood vessels, hyperchromaticity, and cellular pleomorphism generating anisocytosis and poikilocytosis, with atrophy in the gland, also the organ shows necrosis of the lamina propria when compared to the normal control whereas the architecture of the organ looks more organized with increasing level of dietary supplementation.



Plate 1a: Normal Control (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa

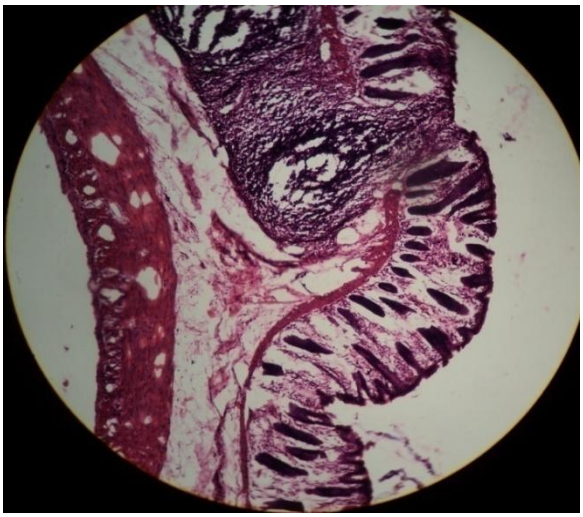


Plate 1b: MNU Control (Mg×200)

A distorted colonic architecture with hyperchromaticity, and with atrophy in the gland, also the organ shows necrosis of the lamina propria.

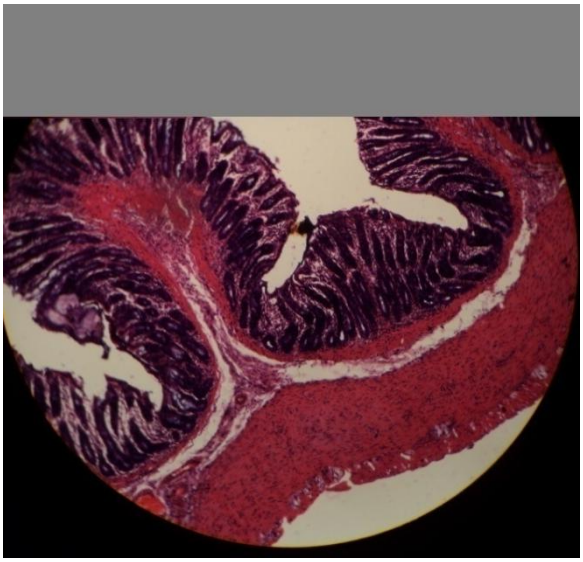


plate 1c: Diet Control of *C. olitorius* (Mg×200)

The diet control group with well arranged architecture of the colon without atrophy of the gland and a living lamina propria.

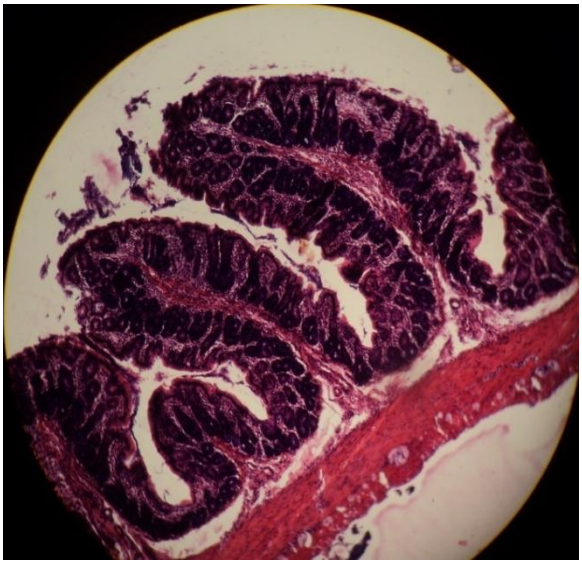


Plate 1d: D Control of *O. gratissimum* (Mg×200)

The diet control group with well arranged architecture of the colon without atrophy of the gland and a living lamina propria.



Plate 1a: Normal control (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa

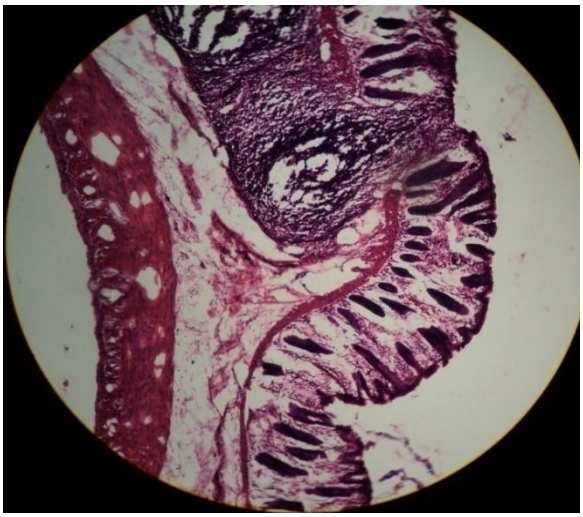


Plate 1b: MNU control (Mg×200)

A distorted colonic architecture with hyperchromaticity, and with atrophy in the gland, also the organ shows necrosis of the lamina propria.

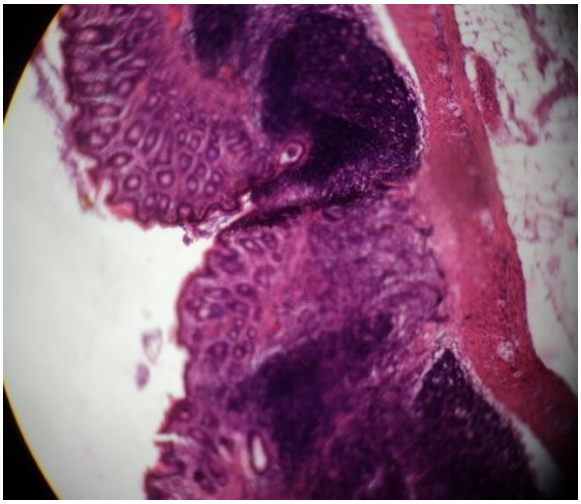


Plate 1e: MNU + 2.5% C.olitorius (Mg×250)

An intense hyperchromaticity in some areas of the organ, and distortion of the gland

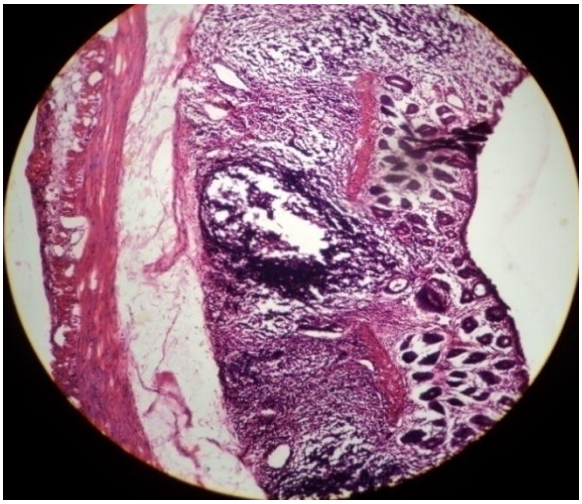


Plate 1f: D Control of *O. gratissimum* (Mg×200)

The diet control group with well arranged architecture of the colon without atrophy of the gland and a living lamina propria.



Plate 1a: Normal control (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa

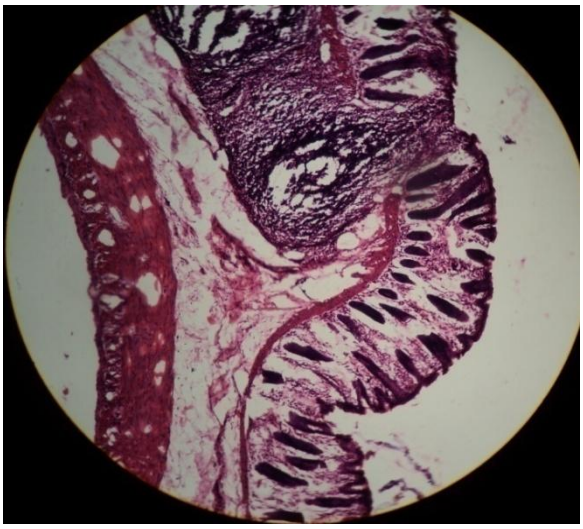


Plate 1b: MNU Control (Mg×200)

A distorted colonic architecture with hyperchromaticity, and with atrophy in the gland, also the organ shows necrosis of the lamina propria.

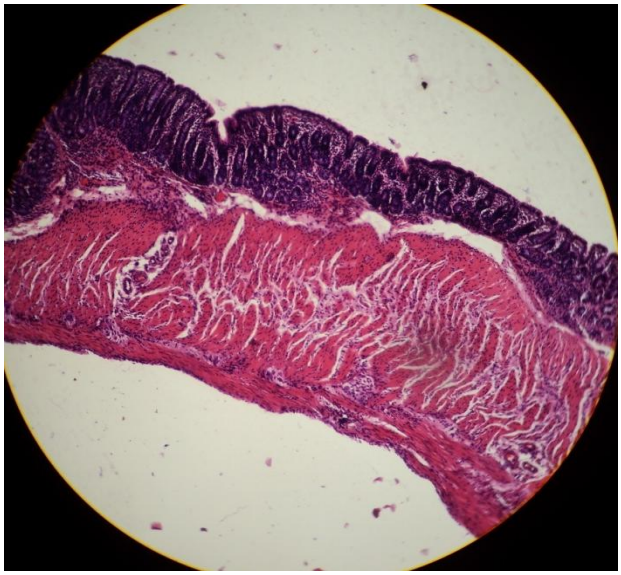


Plate 1g: MNU + 5% C. olitorius(Mg×200)

A better architecture of the colon though with little Atrophy of the gland.

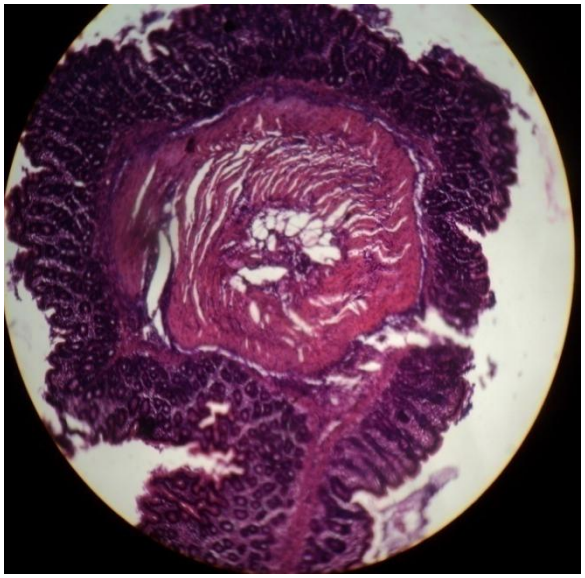


Plate 1h: MNU + 5% O. gratissimum (Mg×200)

Observable distortion of the colon with hyperchromacity.



Plate 1a: Normal Control (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa

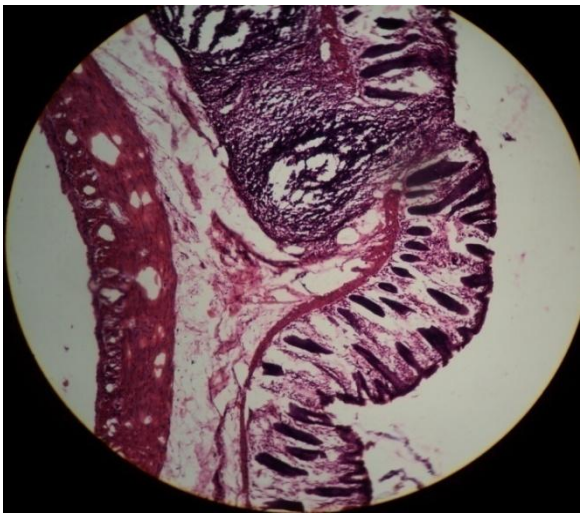


Plate 1b: MNU control (Mg×200)

A distorted colonic architecture with hyperchromaticity, and with atrophy in the gland, also the organ shows necrosis of the lamina propria.

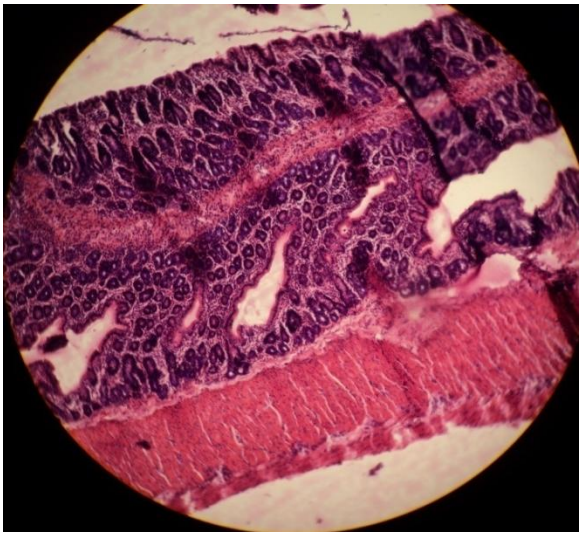


Plate 1i: MNU +10% C. olitorius (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa



Plate 1j: MNU + 10% O. gratissimum (Mg×200)

Section of colon showing normal general histo-architecture of intestinal glands with lamina propria and muscularis mucosa.

4.7 Effect of Dietary Supplementation with *Corchorus olitorius* and *Ocimum gratissimum* Leaf Supplemented Diets on Haematological Parameters.

The result obtained from the effect of dietary supplementation with *Corchorus olitorius* and *Ocimum gratissimum* leaf supplemented diets on haematological parameters shown on **tables 4.10** and **4.11** show that there was a significant difference ($P<0.05$) in the WBC, LYMPH, HGB, RBC, HCT,MCHC, MCH of the MNU control group when compared with that of the normal control group whereas there was no significant difference ($P>0.05$) between the PLT and MCV of the normal control group and the MNU control group.

Furthermore, there was no significant difference ($P>0.05$) in the 10% supplemented control of *Corchorus olitorius* in the WBC, lymph, RBC, HGB, PLT when compared to the normal control group but significantly different in the HCT, MCH, MCHC whereas in *Ocimum gratissimum*, the significant difference ($P<0.05$) was only observed in MCH and MCHC

Table 4.10: Effect of dietary supplementation with *Corchorus olitorius* leaf supplementation on haematological parameters

GROUP	WBC(×10^9/L	Lymph(%)	RBC(×10^{12}/L	HGB(×g/L	PLT(×10^9/L	HCT(%)	MCV(fl)
Control feed	11.66±1.53^{ab}	76.77±12.23 ^{a}	7.99±0.19^{abc}	117.75±22.1^{b}	680.5±64.38^{ab}	36.77±1.37^{c}	46.1±1.39
MNU Control	6.32±1.57^{c}	58.76±10.67^{c}	9.34±0.073^{a}	147.6±17.73^{a}	745±80.73^{abc}	43.76±0.498^{a}	47.53±0.2
10% D. control	14.02±2.53 ^{a}	62.92±14.82^{ab}	9.082±0.26^{ab}	143.3±5.51^{ab}	658.75±90.01^{ab}	40.1±1.23^{ab}	45.4±0.85
MNU + 2.5% supplemention	10.14±2.25 ^{b}	60.96±5.89^{ab}	8.67±0.33^{abc}	142.2±8.79^{ab}	966.67±62.0	42.467±1.65^{ab}	46.0±3.28
MNU + 5% supplemention	10.66±2.2^{b}	63.82±10.24^{ab}	8.51±0.411^{abc}	140.2±7.19^{ab}	840.5±76.02^{bc}	38.47±1.79^{bc}	45.3±1.08
MNU + 10% Supplementation	11.98±3.11^{ab}	61.83±3.25^{ab}	8.19±0.07^{abc}	139.33±7.23^{ab}	639.25±98.66^{ab}	38.47±1.07^{bc}	46.33±0.4

Values with differen superscript down the column are significantly different (P<0.05)

WBC: White blood cell count, RBC: red blood cell count, Hb: haemoglobin, HCT: heamatocrict, PLT: platelets, MCV: mean corpuscular volume, MCHC mean corpuscular haemoglobin concentration, MCH: mean corpuscular haemoglobin

Table 4.11: Effect of dietary supplementation with *Ocimum gratissimum* leaf supplementation on haematological parameters

up	WBC($\times10^9$/L	Lymph(%)	RBC($\times10^{12}$/L	HGB(×g/L	PLT($\times10^9$/L	HCT(%)	MCV(fl)
trol feed	11.66±1.53^{ab}	76.775±12.23^{b}	7.99±0.19^{abc}	117.75±22.11^{a}	680.5±64.3^{ab}	36.77±1.37^{a}	46.1±1.39 ^{a}
U Control	6.32±1.57^{c}	58.76±10.67^{a}	9.34±0.073^{d}	147.6±17.73^{b}	745±80.73^{abc}	43.76±0.49^{c}	47.53±0.15 ^{a}
% + D. Control	10.18±1.11^{b}	64.27±3.86^{ab}	7.36±0.29^{ab}	125.25±1.26^{ab}	751.5±61.1^{abcd}	36.3±0.45^{a}	47.73±0.60 ^{a}
U + 2.5% supplementation	10.6333±0.51^{ab}	54.68±10.39^{a}	7.64±0.32^{abc}	131.75±6.29^{ab}	904±53.11^{cd}	39.53±1.95^{ab}	51.5±5.47^{b}
U + 5% supplementation	11.52±2.6^{b}	60.66±10.66^{a}	8.00±1.38^{abc}	137.5±24.34^{ab}	796.±131.6^{abcd}	35.87±7.20^{a}	47.63±1.58 ^{a}
U + 10% supplementation	10.025±1.71^{b}	66.78±7.11^{ab}	6.78±2.00^{a}	116.6±33.5^{a}	602±303.66^{a}	36.33±3.48^{a}	47.425±0.8 ^{a}

Values with differen superscript down the column are significantly different ($P<0.05$)

WBC: White blood cell count, RBC: red blood cell count, Hb: haemoglobin, HCT: heamatocrict, PLT: platelets, MCV: mean corpuscular volume, MCHC mean corpuscular haemoglobin concentration, MCH: mean corpuscular haemoglobin

CHAPTER FIVE

5.0 DISCUSION

Carcinoembryonic Antigen (CEA) of Colorectal Cancer is the most frequently used tumor Biomarker to monitor patients with colorectal cancer for early reoccurance Duffy *et al.,* (2003). CEA is usually present only at very low levels but raised in colorectal cancer, Boehm *et al* (2010). The result obtained from the level of Carcinoembryonic Antigen (CEA) in rats treated with N-methyl-N-nitrosourea (MNU) and *Corchorus olitorius* and *Ocimum gratissimum* diet supplementation from (Table 4.4) shows there was a significant increases ($P<0.05$) in the level of CEA with MNU control when compared with the normal control whereas, the different levels of supplementation show decrease in the level of CEA with increase supplementation, thus, this result suggests that the leaves of *C. olitorius* and *Ocimum gratissimum* may contain anti-carcinogenic properties.

Histopathogical finding confirms the carcinoembryonic antigen analysis carried out with the MNU control group and the lowest supplemented diet group showing distorted colonic architecture with extensive necrosis of the lamina propria. Also, an observable cell proliferation showing hyperchromacity and cellullar pleomorphism with anisocytosis and Poikilocytosis were observed alongside an atrophy of glands which are biomarkers for colon carcinogenesis whereas, group treated with the highest supplementation and diet control group shows no clear histopathology different when compared with that of the normal control group which can be attributed to the preventive potential of the plant at a high supplemented diet.

Elevated rates of reactive oxygen species (ROS) have been identified in almost all cancers, where they promote many aspects of tumor development progression, Geou-Yarh liou and Peter

storz, (2010). This is in agreement with the result of the TBARS carried out in (**Table 4.7**) with an observable elevated lipid peroxidation in the liver and kidney of the MNU treated groups which was more evident in that of the MNU control when compared with the normal control group, this may be as a result of cellular membrane degeneration and DNA damage due to singlet oxygen production Skrzydlewska *et al*., (2005). Also in the battle of the free radicals (ROS), the body possesses some defense machineries which includes endogenous antioxidant such as superioxide dismutase and catalase and also immune system which comprises the white blood cell, lymph etc. superoxide dismutase (SOD) is an essential enzyme that eliminates superioxideradicals (O_2^-) and thus protects cells from damage induced by radicals. Low SOD activity in cancer cells may render the malignant cells highly dependent on SOD for survival and sensitive to inhibition of SOD Huang *et al.,* (2000). This is in accordance with the result obtained in (*Table 4.6*) with a lowered SOD when compared to the normal which may be as a result of the suppressing potential of the carcinogen induced so as to enable the carcinogen exert it maximum effect whereas in the group treated with the different diet supplement we observed an increase in SOD activity with increased level of supplementation which may be due to the exogenous antioxidant in the supplemented diet to help appreciate that of the endogenous antioxidant also, a preventive potential of the plant toward colon carcinogenesis. Inhibition of endogenous antioxidant seen in (Table 4.6) causes accumulation of cellular O_2^- and leads to free radicals seen in (Table 4.7) mediated damage to mitochondria membrane and other cellular damage, Huang *et al*., (2000). Catalase (CAT), common endogenous enzymes found in nearly all living organisms exposed to oxygen such as vegetable, fruit or animals. CAT plays it major role by protecting the cells from the accumulation of hydrogen peroxide via dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase Lenzi *et al.,* (1996). The result

obtained, a decrease level of Catalase was observed in the MNU control group with its corresponding induced supplemented diet groups when compared with the Normal control group. This decrease is in agreement with the report of Skrzydlewska and Luczaj, (2005) whereas upon treatment with the supplemented diet an increase in the level of catalase with increase level of supplementation was observed.

Several medicinal plants have been used in traditional medicine worldwide and is now recognized by World Health Organization (WHO) as an essential building block for primary healthcare Onayade *et al*., (1990). According to the WHO, more than 80% of the world population still relies on naturally occurring medicinal plants as their primary source of healthcare Adeyemi *et al*., (2009). Thus, dietary plant plays an important role in healthcare management system. Dietary medicinal plant, thus are plants that posses some substances either in their leaves, stem, root etc that can therefore be used in the treatment of ailments. Phamacognostical evaluation of *Ocimum gratissimum* used as traditional medicine s in various diseases was drawn up according to WHO guidelines Matasyoh, (2012). Additionally, recent study on the phytochemical of this plant by Barku *et al.,* (2013) shows the presence of polyphenols and flavonoid and other phytochemical constituent similarly to that of *Corchorus olitorius* Lin *et al.,* (2002). The presence of these materials in these plants accounts for their usefulness as Medicinal plants. Thus, the antioxidant capacity possessed by phenolic compounds is mainly due to their redox properties which enable them act as a reducing agent via quenching of singlet oxygen or Hydrogen donation. Besides their other roles such as: anti-inflammatory, antibacterial and antimicrobial activity Balasundram *et al.,* (2006). It is expedient we determine the total polyphenolic and total flavonoid of the plant in relation to it medicinal potentials. The result shows that the total polyphenolic content of the plant of both *Corchorus olitorius* and

Ocimum gratissimum was the highest from (Table 4.8**)** whereas the total polyphenolic content in other supplemented diet group increases with increasing level of supplementation when compared to the normal feed which shows the lowest total polyphenolic content, similar result was also obtained for flavonoid (Table 4.9). It was proposed that the food containing multiple antioxidant phytochemical which plays a role in attenuating the cancer are far more effective for cancer prevention than a single chemical in the food, Narisawa *et al*., (2000).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

I. The carcinoembryonic antigen assay (CEA) shows that there was a significant increase ($P<0.05$) with MNU control group (3.47 ± 0.29) when compared with the normal control (1.17 ± 0.3) and the varying supplemented diet both for *Corchorus olitorius* and *Ocimum gratissimum*, also the level of CEA decreased with increased supplementation.

II. The histopathology of the colon of the MNU control group shows a distorted colonic architecture with distorted blood vessels, hyperchromaticity, cellular pleomorphism and atrophy in the gland, while normalcy increases in the architecture of the colon with increasing level of dietary supplementation.

III. The thiobarbituric acid reactive substance (TBARS) was significantly increased ($P<0.05$) in both kidney and liver of MNU control group when compared to the normal control group whereas there was significant decrease ($p<0.05$) in superoxide dismutase (SOD) and catalase (CAT) in MNU control and MNU induced group when compared to the normal control group.

IV. Haematological parameters shows that there was a significant difference ($P<0.05$) in the WBC, LYMPH, HGB,RBC, HCT,MCHC,MCH of the MNU control group when compared with that of the normal control group whereas there was no significant difference ($P>0.05$) between the PLT and MCV of the normal control group and the MNU control group.

6.2 CONCLUSION

The result of this study showed that dietary supplementation with the leaves of *Corchorus olitorius* and *Ocimum gratissimum* possess anticarcinogenic activity with the highest activity in 10% leaves supplementation of *Corchorus olitorius* and *Ocimum gratissimum*. This is obvious from the result of the Carcinoembryonic antigen (CEA) levels and the improvement in the architecture of the colon. Leaves of *Corchorus olitorius* and *Ocimum gratissimum* are rich in antioxidant activity thus, can serve as therapy to preventing oxidative damage.

6.3 RECOMMENDATIONS

i. The dietary consumption of the leaves of *Corchorus olitorius* and *Ocimum gratissimum* is recommended.

ii. The active components of the supplemented leaves should be isolated for further pharmacological test.

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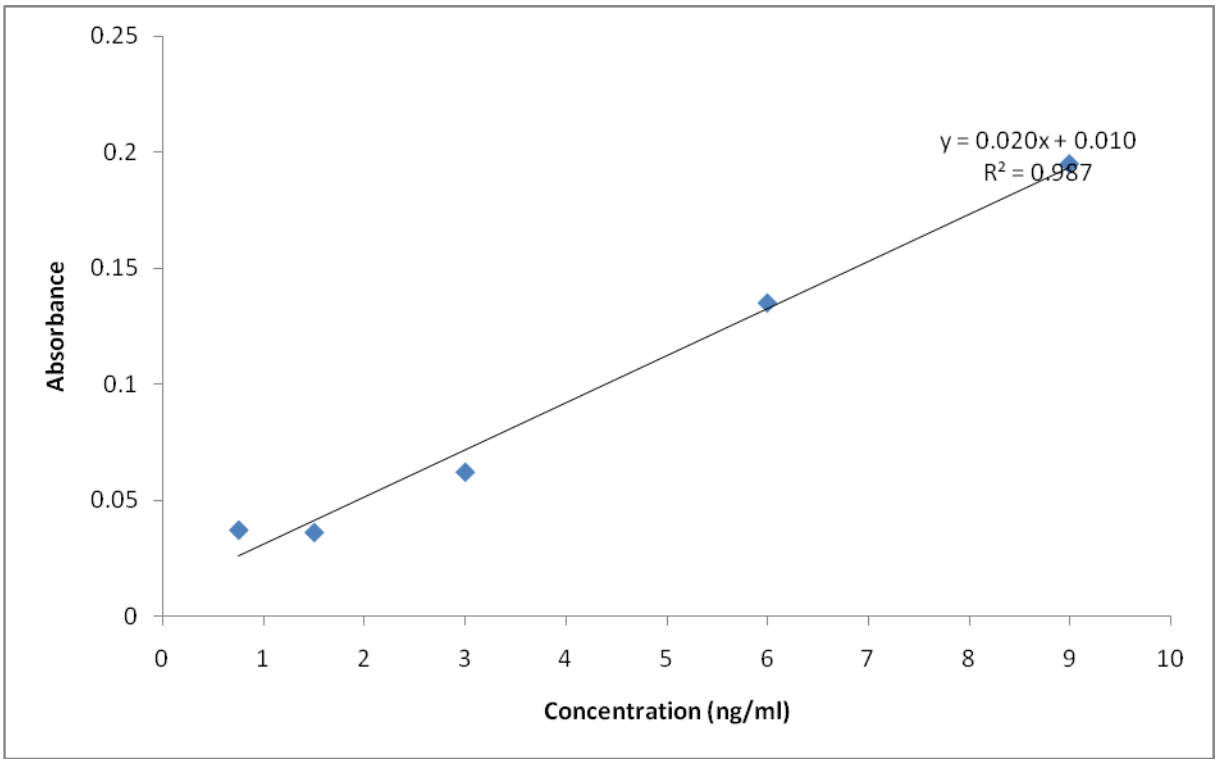
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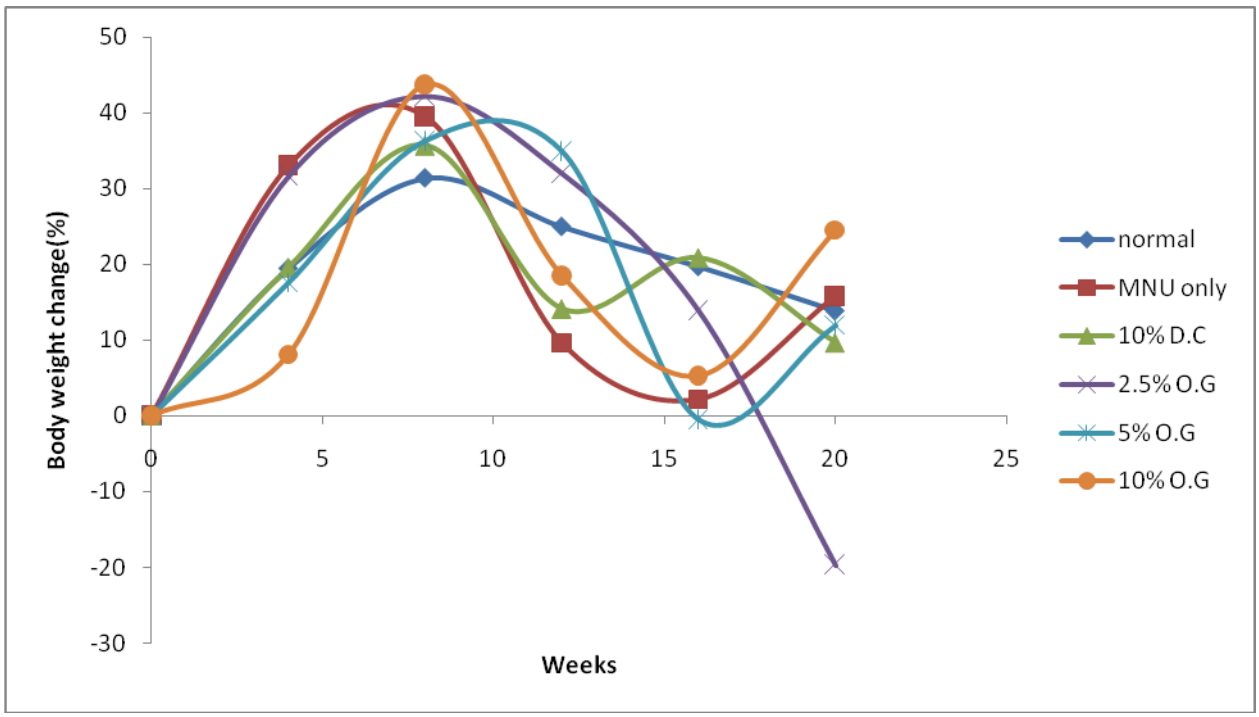
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APPENDICES

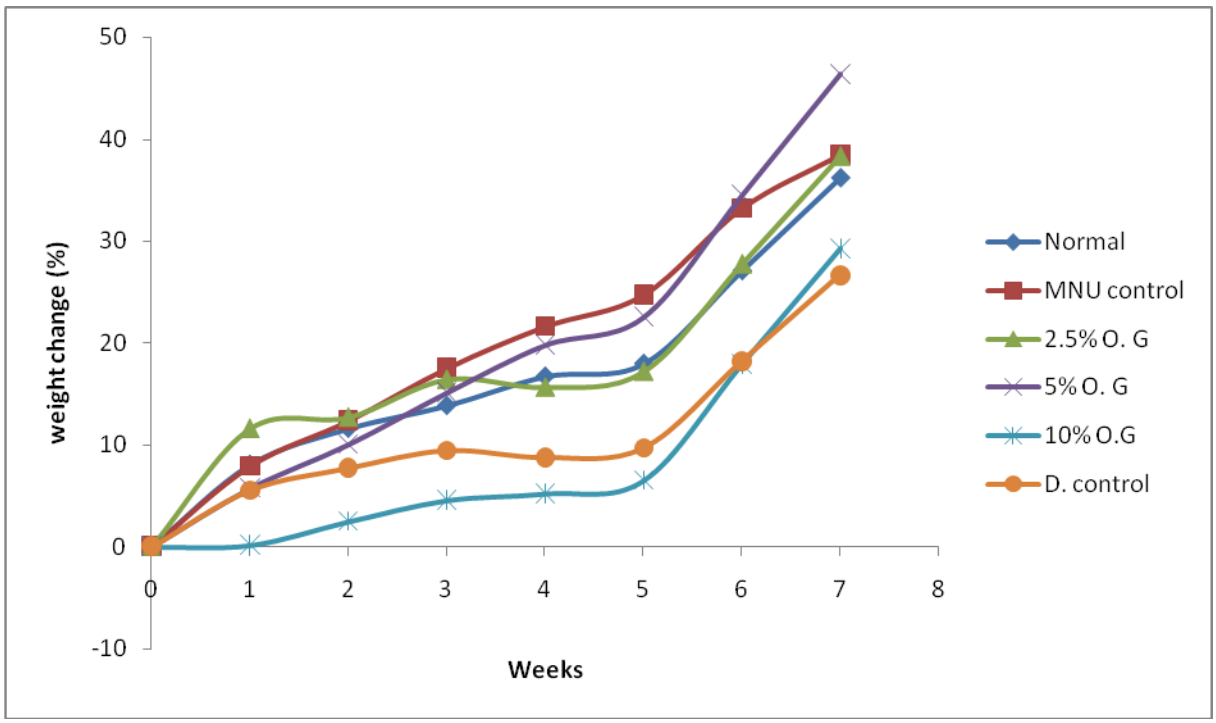


APPENDIX 1.0: Standard Curve for Carcinoembryonic Antigen by ELISA at 450nm

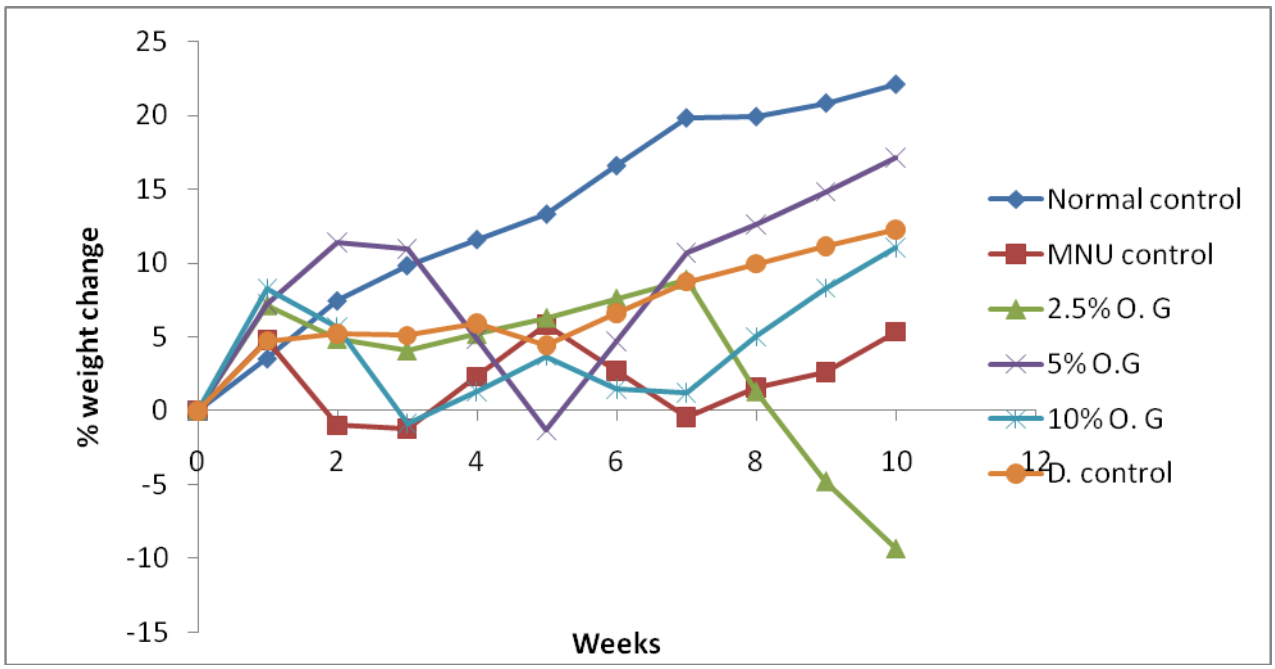


Appendix 2.0: The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *O. gratissimum* on MNU Induced Colon Cancer Wistar Rats

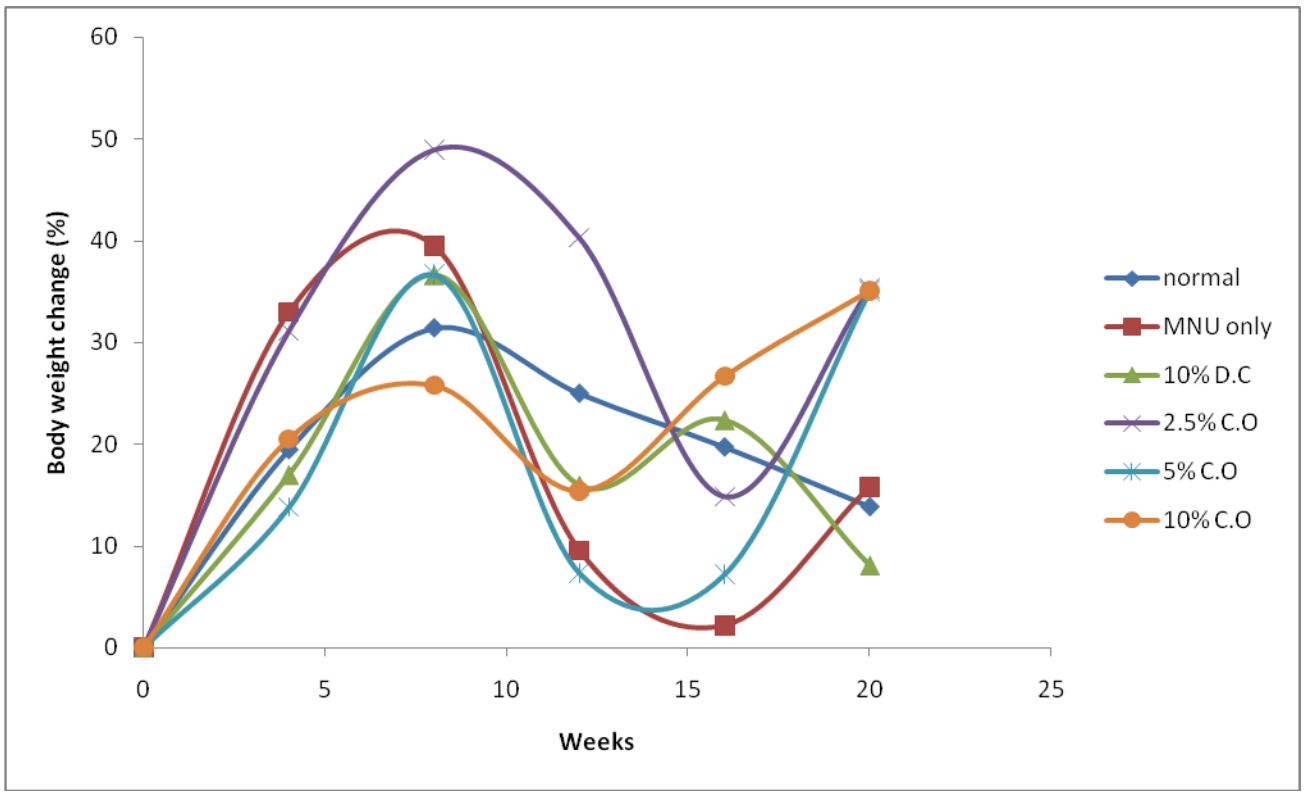
D.C: diet Control, O.G: *Ocimum gratissimum*



Appendix 3.0: The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *Ocimum gratissimmum* on Pre-MNU (first 2 quarters) Induced Colon Cancer Wistar Rats on weekly basis

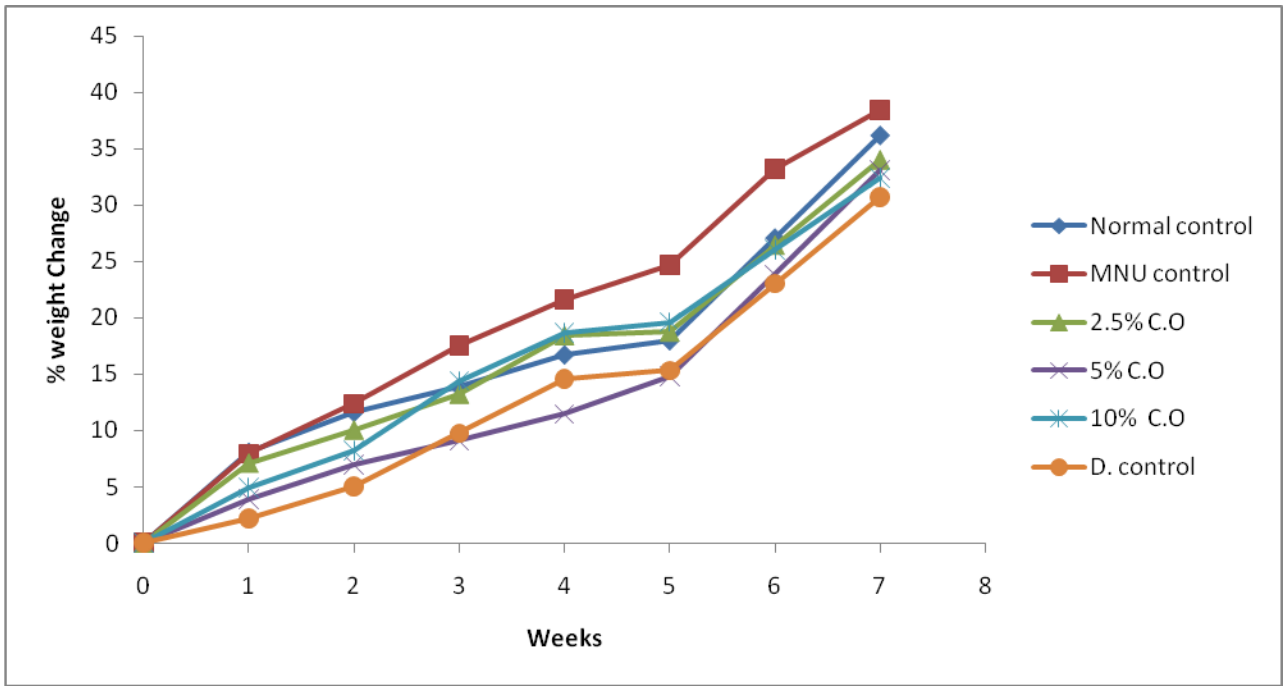


Appendix 4.0: The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *O. gratissimum* on Post-MNU Induced Colon Cancer Wistar rats on weekly basis

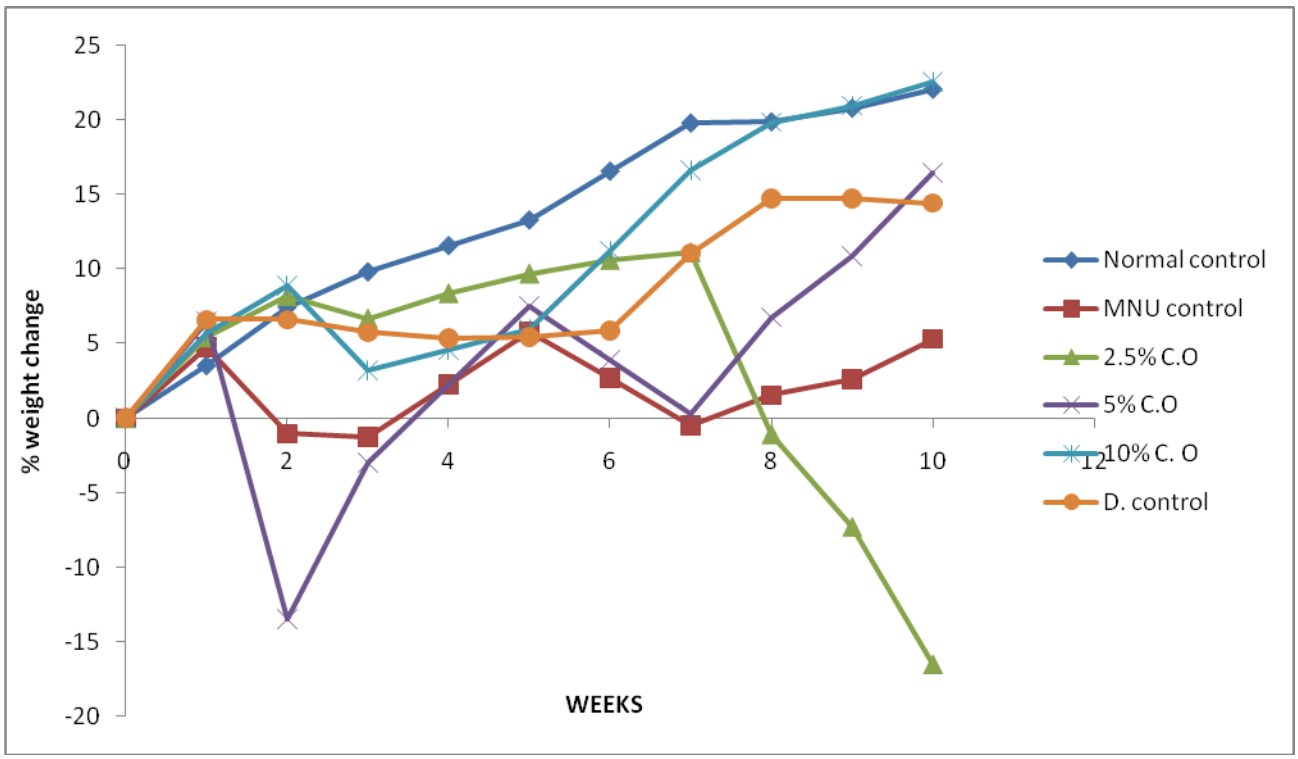


Appendix 5.0: The 18 weeks (BEFORE AND AFTER) Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *C. olitorius* on MNU Induced Colon Cancer Wistar Rats

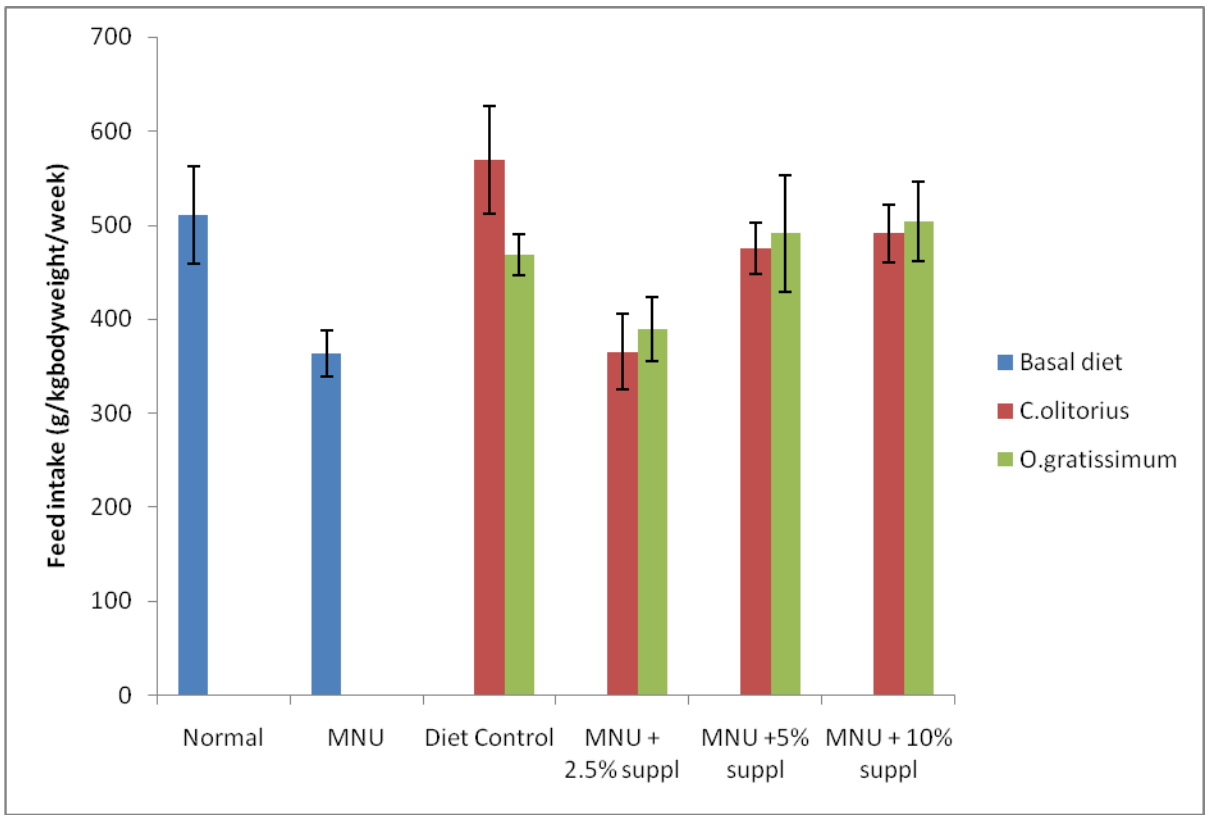
D.C: Diet Control, C.O: *Corchorus olitorius*



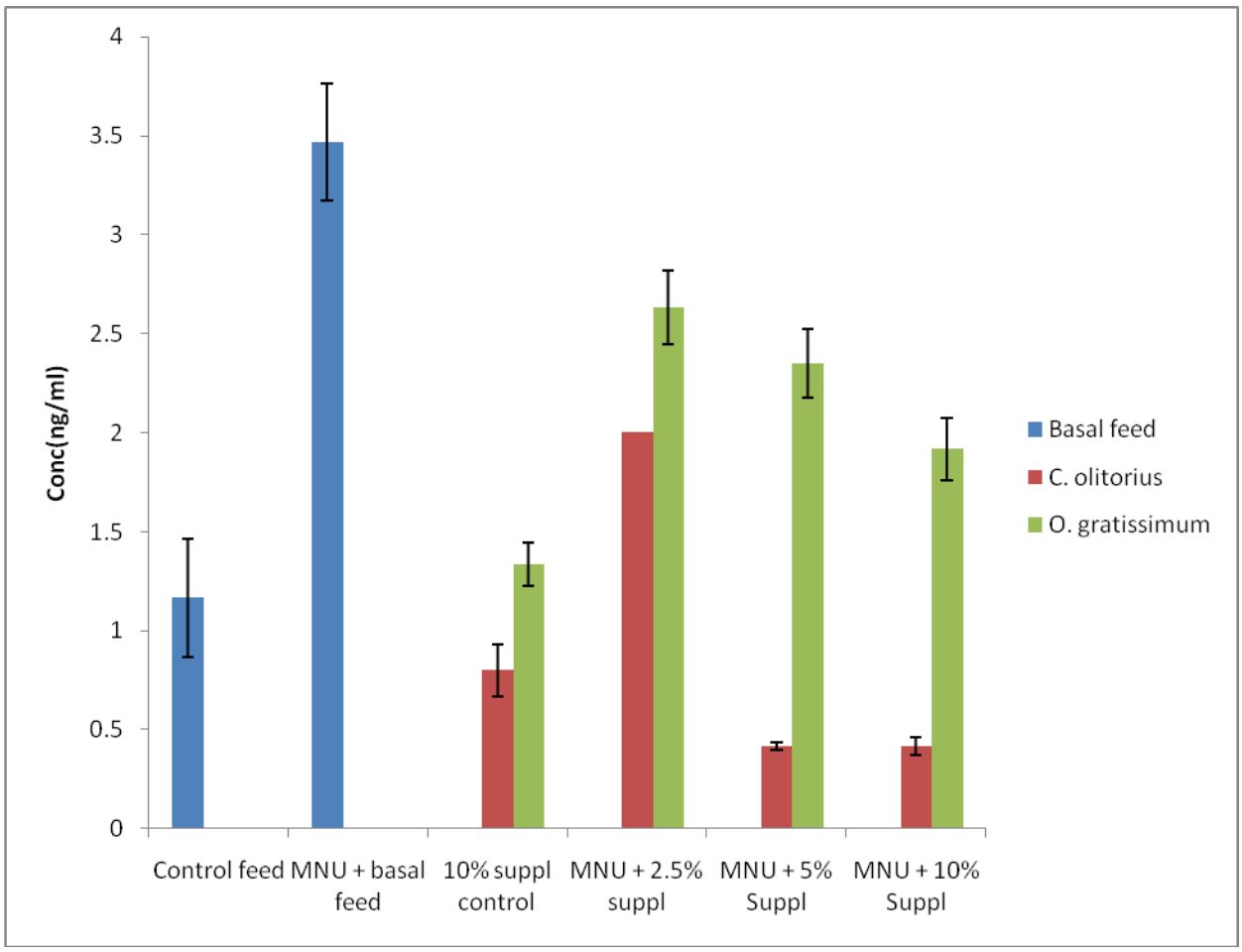
Appendix 6.0: The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *C. olitorius* on Pre-MNU (first two quarters) Induced Colon Cancer Wistar Rats on weekly basis.



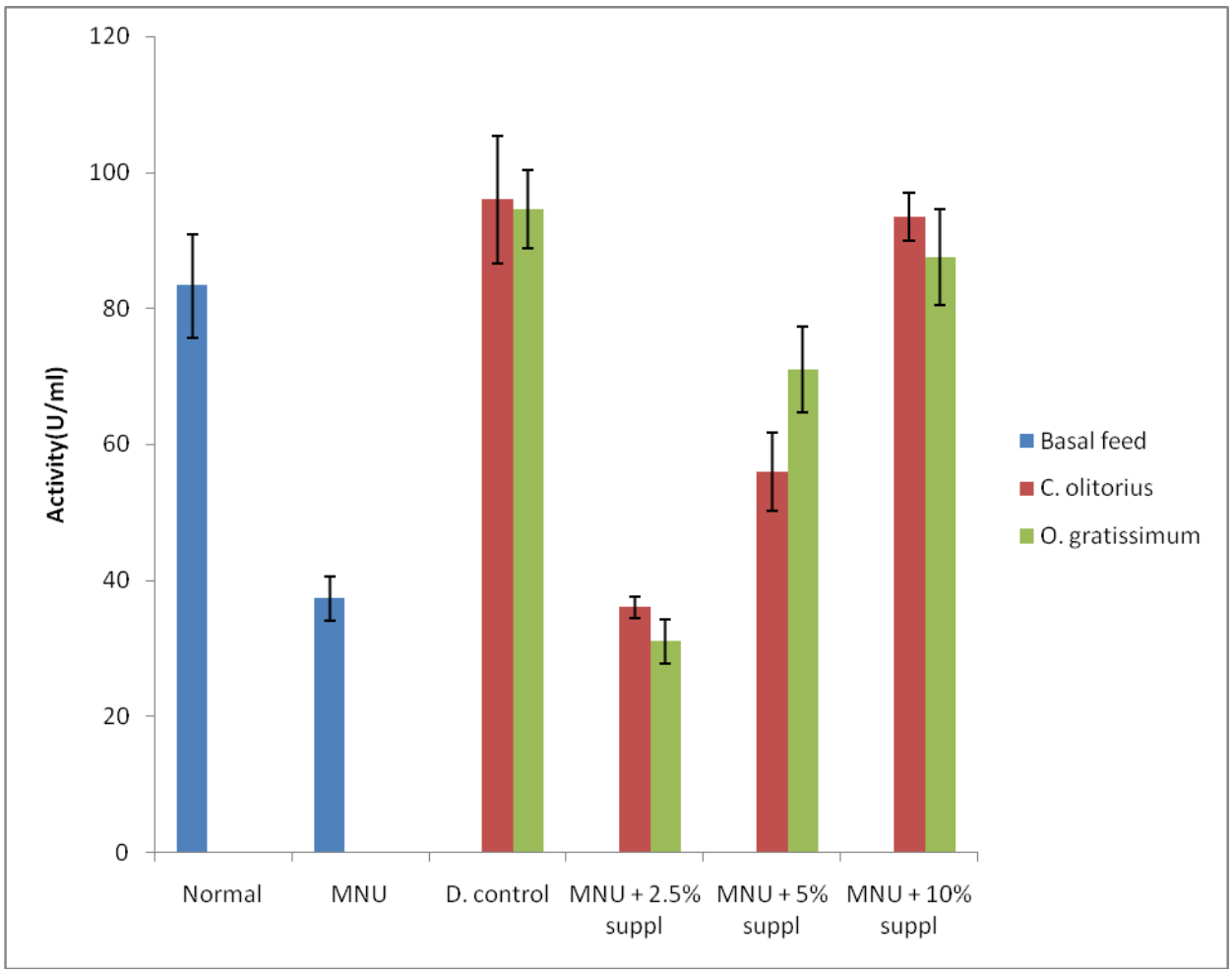
Appendix 7.0: The Body Weight Change in Percentage (%) of Diet Supplementation with Leaves of *C. olitorius* on Post-MNU (last 3 quarters) Induced Colon Cancer Wistar Rats on Weekly basis



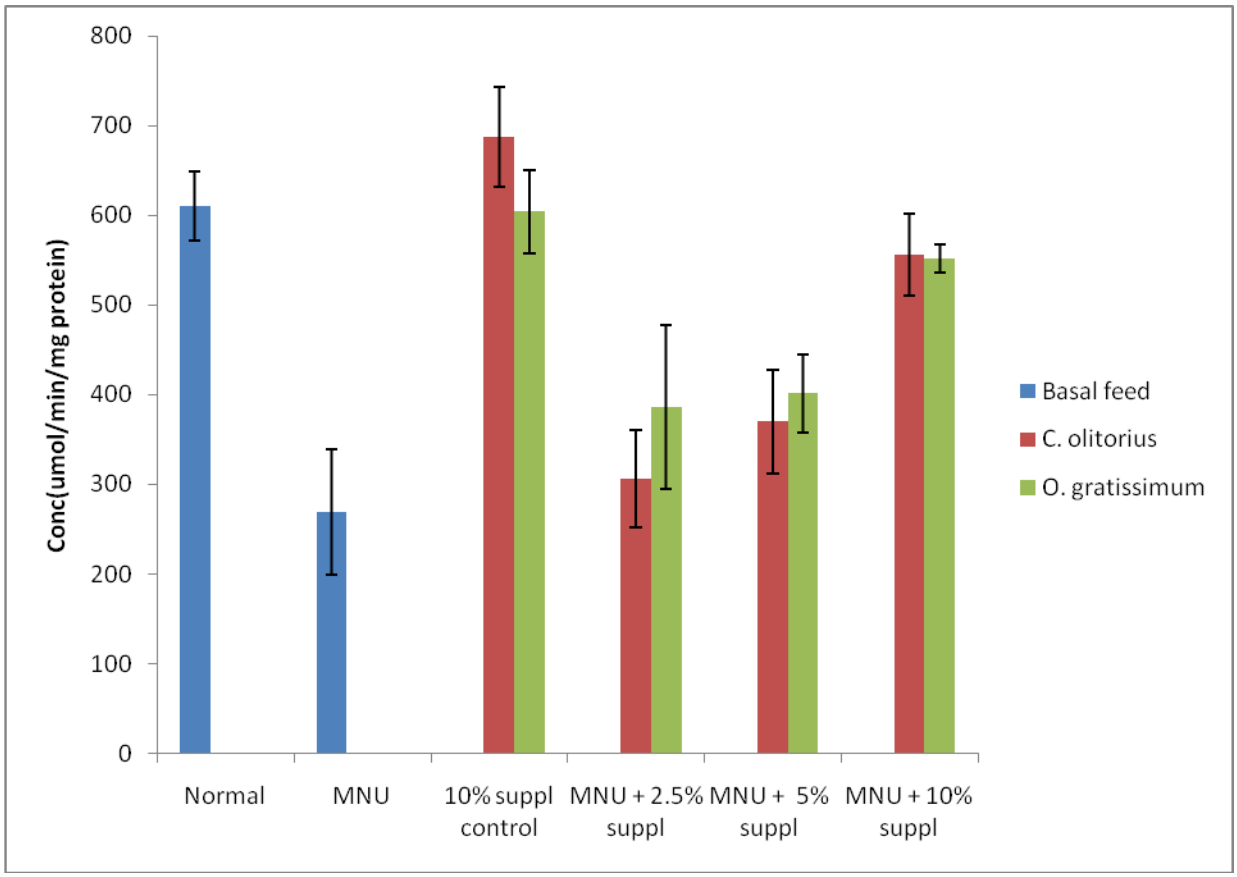
Appendix 8.0: The Effect of Leave Supplementation of *Corchorus olitorius* and *Ocimum gratissimum* on Feed Intake (g/kgbodyweight/week) for a Period of 18 weeks (before and after induction) on MNU Induced Colon Cancer Rats.



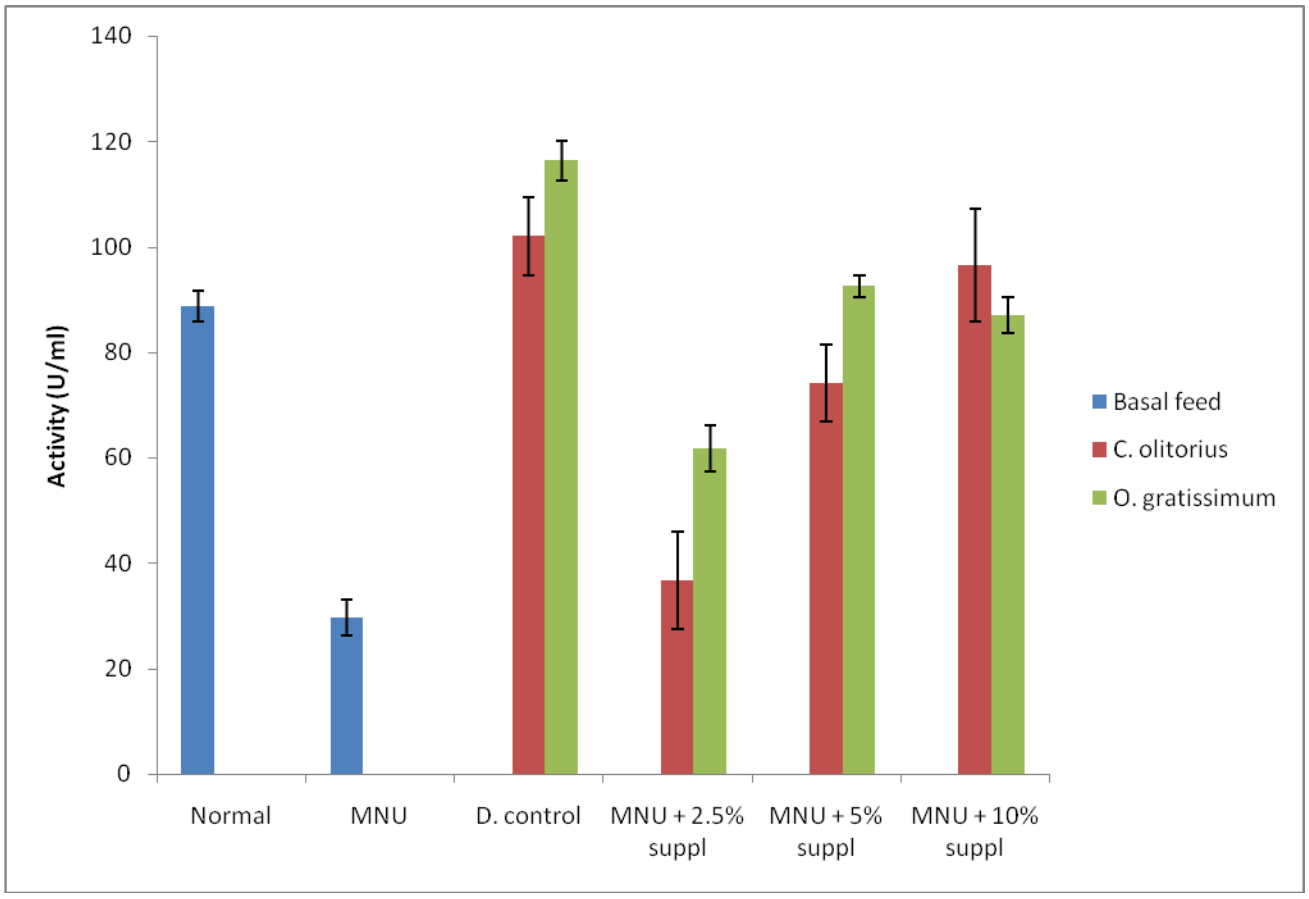
Apendix 9.0: The Level of Carcinoembryonic Antigen (CEA) in Rat Treated with N-Methyl-N-nitrourea (MNU) alongside with Leaf Supplemented Diet of *Corchorus olitorius and Ocimum gratissimum.*



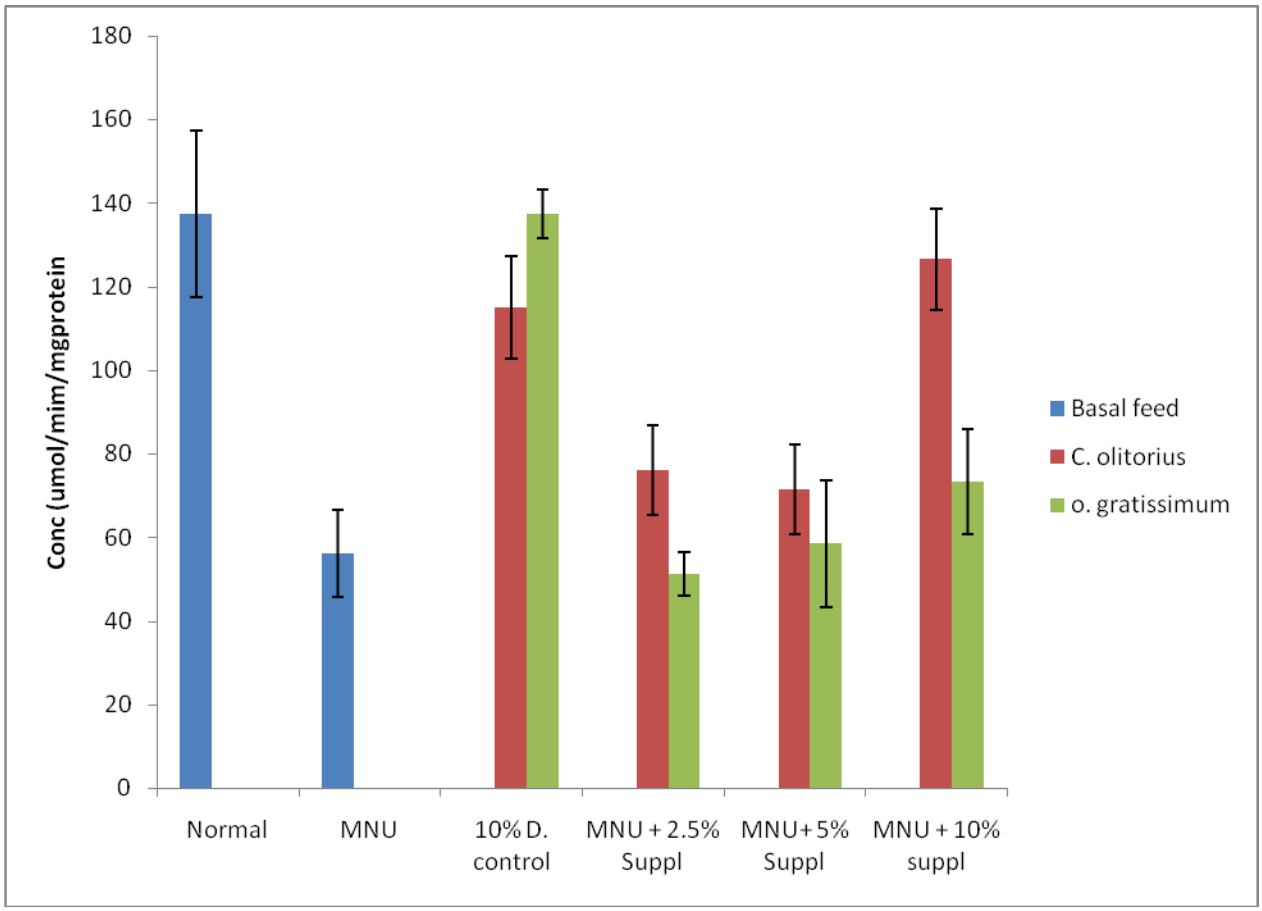
Apendix 10.0: The Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* and *Ocimum gratissimum* on Superioxide Dismutase Activity of Kidney.



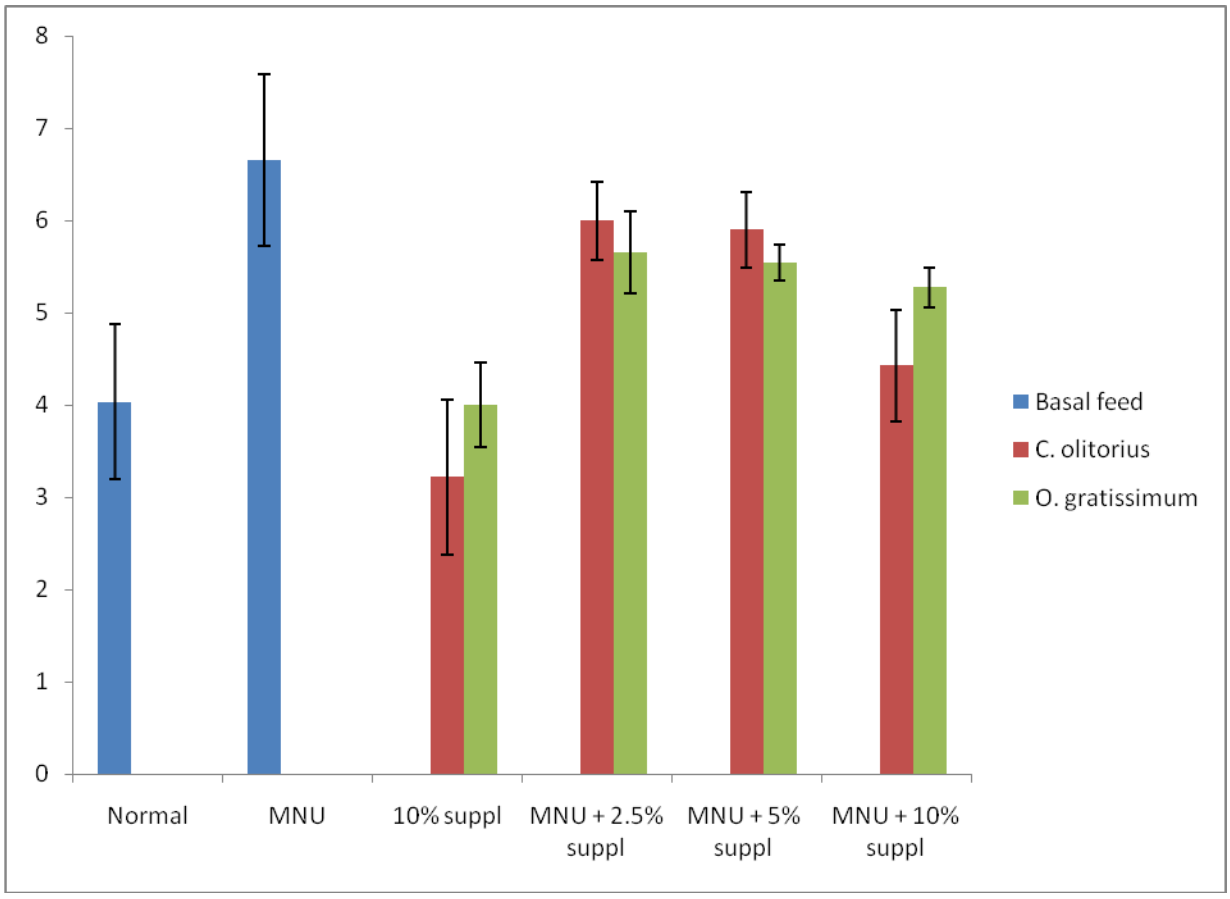
Appendix 11.0: The Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* and *Ocimum gratissimum* on Catalase Activity of Kidney.



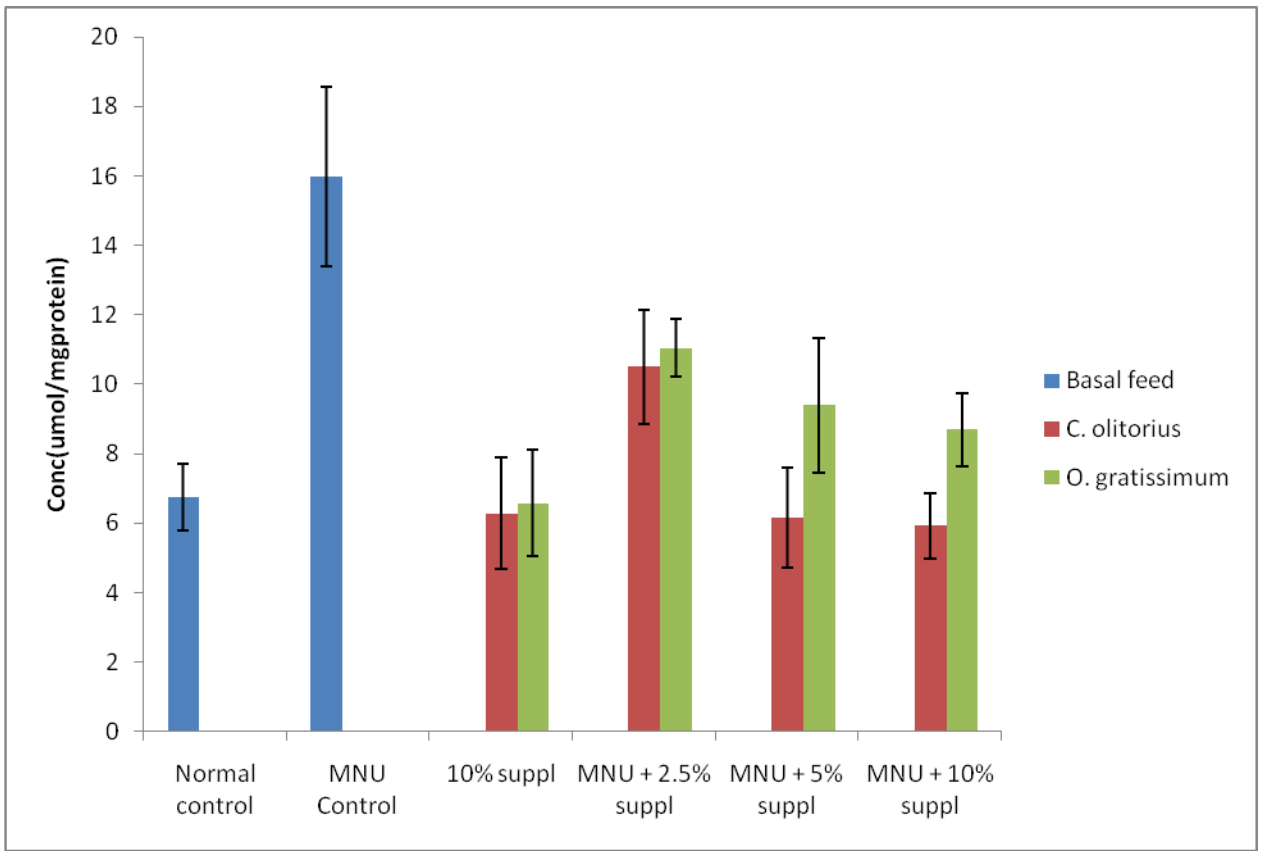
Appendix 12.0: The Effect of Dietary Supplementation with Leaves of Corchorus olitorius and Ocimum gratissimum on Superoxide Dismutase Activity of Liver.



Appendix 13.0: The Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* and *Ocimum gratissimum* on Catalase Activity of Liver.



Appendix14.0: The Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* and *Ocimum gratissimum* on Lipid Peroxidation of Kidney.



Appendix 15.0: The Effect of Dietary Supplementation with Leaves of *Corchorus olitorius* and *Ocimum gratissimum* on Lipid Peroxidation of Liver.