

**SUPPRESSIVE AND THERAPEUTIC EFFECT OF *ARACHIS HYPOGAEA* SEEDS  
ON 1,2- DIMETHYL HYDRAZINE INDUCED COLON CARCINOGENESIS IN  
MICE**

**BY**

**IHAZA SHALOM OSAKPOLOR**

**LSC1705133**

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OF BENIN, BENIN CITY**

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## CERTIFICATION

This is to certify that this work was written by **IHAZA SHALOM OSAKPOLOR** with matriculation number **LSC1705133** of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City Edo State under the supervision of **DR. (MRS) R.O USIFO (Ph.D)**, in partial fulfillment of the requirement for the Bachelor of Science (B.Sc) degree in Biochemistry.

.....

**DR. (MRS) R.O USIFO**  
**(Project Supervisor)**

.....

**Date**

.....

**DR. OJEABURU**  
**(Project Co-ordinator)**

.....

**Date**

.....

**PROF. (MRS) K.E IMAFIDON**  
**(Head of Department)**

.....

**Date**

.....

**EXTERNAL EXAMINER**

.....

**Date**

## **DEDICATION**

I hereby dedicate this research paper to my most esteemed lecturers, university staff and my beloved parents for their highly appreciated contribution in the achievement of my first Bachelor Degree.

## **ACKNOWLEDGEMENT**

I give thanks to God almighty for His love, care and blessings. I also want to thank those who in one way or the other, contributed to the success of this work. I appreciate the efforts made by my supervisor, who saw me through the writing of this research project.

## ABSTRACT

Colon cancer is the second most common cancer for women and the third most common for men. Its morbidity and mortality are increasing as it is the second largest cause of death related to cancer in the developing countries. In this study, the effects of *Arachis hypogaea* seeds on 1, 2 dimethylhydrazine (DMH)-induced colon carcinogenesis was examined. Four (4) groups of wistar albino mice were used. While the other groups were induced with DMH, group one (1) acted as the positive control group and was fed a normal diet. Group two (2) was induced with DMH only, across a 12 week period. Over the course of a 12-week period, group three (3) was induced with DMH and fed *Arachis hypogaea* diet. Group four (4) underwent a six-weeks DMH induction procedure before being administered *Arachis hypogaea* diet following the induction. The outcome revealed that twelve weeks of subcutaneous induction of DMH in group 2 significantly increased the total protein and malondialdehyde (MDA) concentration levels in colon, liver and kidney when compared to the control (Group 1). However, the activity of antioxidant enzymes such as super oxide dismutase (SOD) and catalase (CAT) in the colon, liver and kidney was significantly reduced following the induction of DMH for twelve weeks when compared to the control. Conversely, the antioxidant parameters showed a significant increase in group 3 and group 4 that were fed *Arachis hypogaea* diet when compared to group 2. Histological studies revealed normal colon architecture in group 1 but presence of many neoplastic crypts and glands irregular in shape in group 2. In group 3 and group 4 that received *Arachis hypogaea* diet, histology revealed pronounced inhibition of crypts and hyperchromasia. Overall, the *Arachis hypogaea* diet was able to reduce the negative effects of DMH on the colon, liver and kidney.

## TABLE OF CONTENTS

CERTIFICATION .....	ii
DEDICATION .....	iii
ACKNOWLEDGEMENT .....	iv
ABSTRACT .....	v
TABLE OF CONTENTS .....	vi
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1    Colon Carcinogenesis .....	1
1.2    Cancer Incidence (influence of age, body weight and sex) .....	2
1.2.1    Age .....	2
1.2.2    Body weight .....	2
1.2.3    Sex .....	2
1.3    Significance of Study .....	3
1.4    Aim and Objectives .....	3
1.5    Literature Review .....	3
1.5.1    Cancer pathogenesis .....	3
1.5.2    Carcinogens .....	4
1.5.3    Oxidative Stress .....	5
1.5.4    Carcinogen-Induced Models (in Rodents) .....	5
1.6    Dimethylhydrazine (DMH) .....	7
1.6.1    1,2-Dimethylhydrazine .....	7
1.6.2    History of DMH .....	9
1.6.3    DMH Metabolism .....	9
1.7    Other Colon Carcinogens .....	12
1.8    Diet and Cancer .....	12
1.8.1    Diet and the Gut Microbiota Interaction .....	12
1.8.2    Arachis Hypogaea (Groundnut) .....	13

1.8.2.1	Phytosterols .....	14
1.8.2.1	Flavonoids .....	14
CHAPTER TWO .....		15
MATERIALS AND METHODS .....		15
2.1	Materials .....	15
2.1.1	Equipment and Reagents .....	15
2.1.2	Sample Size .....	15
2.2	Methods .....	16
2.2.1	Groundnut Preparation .....	16
2.2.2	DMH Administration .....	16
2.2.3	Sample Collection .....	16
2.3	Biochemical Assays .....	16
2.3.1	Estimation of malondialdehyde (MDA) concentration .....	16
2.3.2	Determination of Catalase Activity .....	17
2.3.3	Determination of superoxide dismutase (SOD) activity .....	17
2.3.4	Determination of glutathione peroxidase activity .....	18
2.3.5	Estimation of total protein .....	18
2.4	Histopathological Examination .....	19
2.4.1	Dehydration .....	19
2.4.2	Clearing .....	19
2.4.3	Impregnation .....	19
2.4.4	Embedding .....	20
2.4.5	Sectioning .....	20
2.4.6	Slide Preparation .....	20
2.4.7	Staining Procedure .....	20
CHAPTER THREE .....		21
RESULTS .....		21
3.1	Statistical Analysis .....	21

3.2	Effect of 1,2- Dimethylhydrazine in the Colon of Mice Fed with Arachis hypogaea Compounded Diet .....	21
3.3	Effect of 1,2- Dimethylhydrazine in the Liver of Mice Fed with Arachis hypogaea Compounded Diet .....	23
3.4	Effect of 1,2- Dimethylhydrazine in the Kidney of Mice Fed with Arachis hypogaea Compounded Diet .....	25
3.5	Histological Analysis .....	27
CHAPTER FOUR .....		31
DISCUSSION AND CONCLUSION .....		31
4.1	Discussion .....	31
4.1	Conclusion .....	333
REFERENCES .....		314

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Colon Carcinogenesis

We now live at a time when average living standards around the world have increased, and access to high-quality healthcare has significantly improved disease diagnosis and treatment, thus increasing the average life expectancy of individuals in most countries of the world (Kuipers *et al.*, 2015). Despite the fact that these medical developments have reduced the number of people dying worldwide from communicable diseases, the mortality rate attributable to cancer has increased by more than 40% over the past 40 years. Another 60% increase is anticipated over the following 15 years, with 13 million people likely to lose their lives to cancer by 2030 (Kuipers, *et al.*, 2015).

Cancer is a condition in which aberrant cells multiply uncontrollably. Cancer, according to the World Health Organization (WHO), is a broad category of diseases that can begin in almost any organ or tissue of the body and develop when abnormal cells grow uncontrollably and spread beyond their normal boundaries, invading adjacent parts of the body and/or spreading to other organs. Colorectal cancer (CRC) arises when this type of tumor occurs in the colon or rectum (American Cancer Society, 2020). The large intestine, which includes the colon and rectum (colorectum), as well as the anus, is the last section of the gastrointestinal (GI) system. The large intestine functions to absorb water and electrolytes from food and expel waste. The first part of the large intestine is the colon, a muscular tube about 1.5 meters long and 5 centimeters (2 inches) in diameter. The colon is divided into 4 sections viz:

- The ascending colon which starts at the caecum (a pouch where the small intestine receives undigested food) and runs up the right side of the abdomen.
- The transverse colon runs from right to left across the body and is collectively referred to as the proximal, or right colon when combined with the ascending colon.
- The descending colon which runs down the left side.
- The sigmoid colon, named for its "S" shape, is the last section of the colon and is referred to as the distal, or left colon when combined with the descending colon (Lee, *et al.*, 2015).

The sigmoid colon empties into the rectum, which is ultimately ejected through the anus. Cancers of the anus and rectum are classed independently despite their anatomic proximity because they frequently originate from different cell types and so have different characteristics.

However, colorectal cancers differ in their molecular, biological, and clinical characteristics, as well as their relationship to risk factors. Physical inactivity, for example, is linked to an increased risk of cancer in the colon but not in the rectum. Furthermore, patients who are older (versus younger), black (against white), or female (versus male) are more likely to be diagnosed with proximal colon tumors (Lee, *et al*, 2015).

## **1.2 Cancer Incidence (influence of age, body weight and sex)**

Cancer morbidity has gone up over the last 100 years. The high incidence of cancer is caused by two main factors: (i) longer life expectancy; and (ii) exposure to substances that cause cancer, including radiation from X-rays, airline travel, and other causes. We live 30 years longer now than we did a century ago. Furthermore, older tissues are more frequently affected by cancer, making the elderly more vulnerable than children (Venkatachalam, *et al.*, 2020).

### **1.2.1 Age**

Age raises the likelihood of getting colon cancer. Colon cancer is most prevalent in those over the age of 50, and the risk of developing it increases with each passing decade. However, younger people are now becoming more susceptible to the condition. (Venkatachalam, *et al.*, 2020).

### **1.2.2 Body weight**

One of the risk factors for colon cancer is obesity. In general, overweight men (BMI > 30 kg/m<sup>2</sup>) have a higher risk of 53% when compared to healthy males. The incidence of colon cancer may be 13–14% higher in people who lead sedentary (somewhat inactive) lifestyles. It carries a bigger attributable risk than family history. (Venkatachalam, *et al.*, 2020).

### **1.2.3 Sex**

Males have a 1.5-fold greater risk of getting colon cancer than females across all ages and countries. Women are more likely than men to develop right-sided colon cancer, which, when

compared to left-sided colon cancer, is linked to a more aggressive form of neoplasia. (Rawla, *et al.*, 2019).

### **1.3 Significance of Study**

This study was aimed at assessing the carcinogenic potential of 1,2 – Dimethylhydrazine and the anti-carcinogenic potential of *Arachis hypogaea* seeds on 1,2-Dimethylhydrazine induced carcinogenesis in mice colon.

### **1.4 Aim and Objectives**

- To evaluate the effect of intraperitoneal administration of 1,2-Dimethylhydrazine in colon
- To evaluate the effect of intraperitoneal administration of 1,2-Dimethylhydrazine on antioxidant potential in colon
- To evaluate the effect of *Arachis hypogaea* seeds on 1,2-Dimethylhydrazine induced carcinogenesis in mice colon

The objectives of this study were to determine the general mechanisms of tumor initiation elicited by the carcinogen 1,2- Dimethylhydrazine (DMH) and to estimate the effects of feeding Groundnut meal at 20% level on 1,2 – Dimethylhydrazine induced tumor in mice.

### **1.5 Literature Review**

#### **1.5.1 Cancer pathogenesis**

Studies undertaken first in animal models and then inspired by pathological research of human tumors, verified that carcinogenesis is a multi-stage process in which a summation of events is required to produce a malignant tumor (Stuart, 2000). The term "initiation" was used to characterize an irreversible result of a tissue's contact with carcinogens, a change that was often undetectable as a diseased entity but resulted in cells that were progenitors to future tumors (Stuart, 2000).

Malignant development is governed by six fundamental alterations in cell physiology: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of programmed cell death (apoptosis), infinite ability for replication, prolonged angiogenesis, tissue invasion and metastasis (Paolo, *et al.*, 2010). The key time-dependent step of cancer pathogenesis was

identified as the conversion from a pre-malignant phenotype to a malignant cell type (Stuart, 2000).

### 1.5.2 Carcinogens

Both endogenous and external mechanisms can degrade DNA. Regardless of where they come from, carcinogens can cause DNA damage through a number of different mechanisms. This includes, for instance, the covalent attachment of a carcinogen to DNA or DNA double-strand breaks (DSBs) brought on by the production of free radicals by ionizing radiation (IR) (Smith, *et al.*, 2016). Chemical or physical substances that cause DNA damage due to their physico-chemical characteristics, such as DNA molecule deformation or DNA cross-linking, are categorized as carcinogens (Desouky, *et al.*, 2015).

There are two types of carcinogens: those that depend on activation and those that don't. Direct-acting carcinogens are those that can cause DNA damage without metabolic activation or any kind of chemical alteration. Examples include nitrosamines, ultraviolet (UV), infrared (IR), and alkylating agents (Ravanat *et al.*, 2016). These substances' electrophilic groups provide them the capacity to interact directly with DNA and other biological components. Because of their innate reactivity, these electrophilic groups can interact with nitrogen and oxygen atoms in negatively charged biological macromolecules to cause molecular alterations and distortion. Depending on the type of carcinogen, changing the DNA bases leads to a rearrangement of the genetic material and the production of DNA adducts (Lodish *et al.*, 2000). Failure of DNA repair systems causes DNA lesions to be passed on to daughter cells, gradually accumulating DNA damage and resulting in the onset of cancer (Cohen and Arnold, 2011).

The parent substances of indirect-acting carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAAs), N-nitrosamines, mycotoxins, and aristolochic acid, are often less reactive. These normally need to be bioactivated in the host cells to become reactive intermediates or carcinogenic metabolites that might cause genotoxic consequences (Smith *et al.*, 2016). Metabolic processes in phases I and/or II frequently mediate this. Phase I reactions include oxidation, reduction, and hydrolysis; these processes typically include CYPs, or cytochrome P450 (CYP) mixed function oxidase isoforms. These enzymes can either work alone or in tandem with phase II enzymes like sulfotransferases and N-acetyltransferases to activate carcinogens (Wohak *et al.*, 2016).

Although some indirect carcinogens can increase bioactivation by altering gene expression, most indirect carcinogens depend on activation (Walsh *et al.*, 2013).

### **1.5.3 Oxidative Stress**

DNA damage brought on by oxidative stress is caused by both exogenous and internal causes, including UVA, IR, and internally produced oxygen molecules. Reactive oxygen species (ROS), such as hydroxyl radicals (OH), singlet oxygen, or reactive nitrogen species, such as peroxynitrite, are the most common forms of oxidative stress that is predominantly produced (Kryston, *et al.*, 2011). It is known that these mutagenic substances interact with macromolecules, impairing DNA synthesis and repair processes as well as deactivating a number of essential proteins and repair enzymes. The primary targets of these organisms, particularly ROS, are guanine bases (Evans *et al.*, 2004).

Modified bases, apurinic/aprimidinic (AP) sites, and single-strand breaks are the results of ROS-induced damage. The oxidative product, 8-oxo-7,8-dihydro-2'-deoxyguanosine, is created when OH is added to position C8 of the guanine ring (8-oxodG). Similar to this, adding OH to deoxyadenosine at position C8 results in the oxidative product 8-oxo-7,8-dihydro-2'-deoxyadenosine (Achanta and Huang, 2004). Thymine glycol, created by the insertion of OH at position C5 of thymine rings, is another common oxidative product (Evans *et al.*, 2004). Similar to this, cytosine glycol is another oxidation byproduct of cytosine, and when it is deaminated, it produces uracil glycol. DNA glycosylase enzyme removes these nucleotides through base excision repair (BER). The accumulation of these changed nucleotides over time increases genomic instability and structural flaws (Kryston, *et al.*, 2011).

### **1.5.4 Carcinogen-Induced Models (in Rodents)**

Carcinogen-induced models (CIM) in rodents have a lengthy history, yet they continue to be relevant for specific applications today. They serve as a platform for dietary research and reveal information about the pathways of food-borne carcinogens and colitis-related carcinogenesis. Oral gavage, Ad libitum feeding, intraperitoneal or subcutaneous or intramuscular injection, or enema are all options for administering chemical substances (Burtin, *et al.*, 2020).

During the early stages of research on colon carcinogenesis, chemical substances such as azoxymethane (AOM) and its precursor molecule, 1,2-dimethylhydrazine, as well as methylazoxymethyl acetate, were widely used to induce colon carcinogenesis in mice and rats (Rosenberg, *et al.*, 2009). Methylazoxyformaldehyde, which can alkylate the DNA bases guanine and thymine, is formed when all three chemicals are metabolized. Surprisingly, the carcinogenic potential of these chemicals varies depending on the mouse strain, housing conditions, and delivery method (Suzuki, *et al.*, 2006). Although recent reports show that azoxymethane and dimethylhydrazine have some organotropism, tumor growth occurs primarily in the small intestine, with significant levels of alkylated DNA adducts seen in the liver and kidneys (Burtin, *et al.*, 2020). Furthermore, oral or rectal application of the direct alkylating target agents N-methyl-N-nitrosourea (MNU), 3,2'-dimethyl-4-aminobiphenyl, and N-Methyl-N'-nitro-N-nitrosoguanidine, (of which the latter two are traditionally employed in rats), might cause intestinal cancer (Burtin, *et al.*, 2020).

Other carcinogens have come to light as a result of the link between meat consumption and an elevated risk of colon cancer (Bouvard, *et al.*, 2015). Heterocyclic aromatic amines (HAA) are formed when free amino acids, sugars, and creatine react at high temperatures during the cooking of meat and fish, with 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) and 2-amino-9H-pyrido [2,3-b] indole being the most frequent in various foods (Jaegerstad and Skog, 2005). PhIP is converted to N2-Hydroxy-PhIP by the liver enzyme Cytochrome P450 1A2 (CYP1A2), which is subsequently sulfated or acetylated to generate active esters capable of forming DNA adducts (Cheung, *et al.*, 2005). Glutathione conjugation and glucuronidation promote the detoxification of PhIP and its metabolites. Conjugated glucuronides are eliminated in the urine and bile (Burtin *et al.*, 2020). It's worth noting that PhIP metabolism in rodents produces less reactive metabolites than in humans, suggesting that its carcinogenic potential in animal studies may be even higher in people (Frandsen, 2008). Furthermore, in a mouse model of chemical-induced colitis, PhIP caused high-grade dysplasia and adenocarcinomas. Although various heterocyclic aromatic amines included in food have been demonstrated to cause dysplasia and carcinomas in mice, they are rarely employed to simulate colon carcinogenesis (Sugimura, *et al.*, 2004).

When discussing chemical-induced colon carcinogenic animal models, dextran sodium sulfate (DSS) must also be highlighted (Burtin, *et al.*, 2020). Dextran sodium sulfate, as a sulfated polysaccharide, causes direct damage to the colonic epithelium, impairing the

mucosal barrier and allowing luminal bacteria and related antigens to enter the mucosa, causing inflammation (Eichele and Kharbanda, 2017). Mice can develop acute and chronic colitis, as well as colitis-induced dysplastic lesions, depending on the animal strain, DSS dosage, and administration pattern (Munyaka, *et al.*, 2016).

## 1.6 Dimethylhydrazine (DMH)

### 1.6.1 1,2-Dimethylhydrazine

Dimethylhydrazine is an organic compound with the formula  $(\text{CH}_3\text{NH})_2$  and occurs as 1,2-dimethylhydrazine and 1,1-dimethylhydrazine isomers. Both substances are colorless, transparent liquids. 1,1-Dimethylhydrazine is utilized as a fuel in rockets and jets, as well as a plant growth regulator and a feedstock in chemical syntheses (Venkatachalam *et al.*, 2020).

DMH and its metabolite, azoxymethane (AOM), are procarcinogens that need to be metabolically activated in order to produce DNA-reactive compounds (Venkatachalam *et al.*, 2020). The mutagenic activity of the alkylating chemicals dimethylhydrazine and azoxymethane begins with the methylation of guanine at the N-7 position in DNA. By providing a proton, alkylated guanine is coupled with thymidine instead of cytosine, resulting in base modification. Mispairing of guanine to thymine and cytosine to adenine happens during subsequent replication, resulting in DNA mutations. Various metabolic enzymes, particularly xenobiotic-metabolizing enzymes, are involved in the metabolism of these procarcinogenic substances. These enzymes process many N oxidation hydroxylation stages, including the creation of the final carcinogen methylazoxymethanol (MAM) (Venkatachalam *et al.*, 2020).

Due to methylazoxymethanol being a substrate of the nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase found in the colon and liver, it's possible that the active metabolite of methylazoxymethanol is the corresponding aldehyde. These cytochrome P450 2E1 (CYP2E1) metabolites are transported to the colon via the bile or bloodstream (Sohn *et al.*, 2001). The main pathway involves the conversion of DMH to azoxymethane and azoxymethanol in the liver, followed by glucuronic acid conjugation and biliary excretion. However, azoxymethanol toxicity affects the liver, cell membranes, and other organelles, as evidenced by the release of aspartate and alanine amino transferases as well as alkaline

phosphatase. When the glucuronides enter the colon, they are hydrolyzed by bacterial enzymes, resulting in an active carcinogen in the colonic lumen (Venkatachalam *et al.*, 2020).

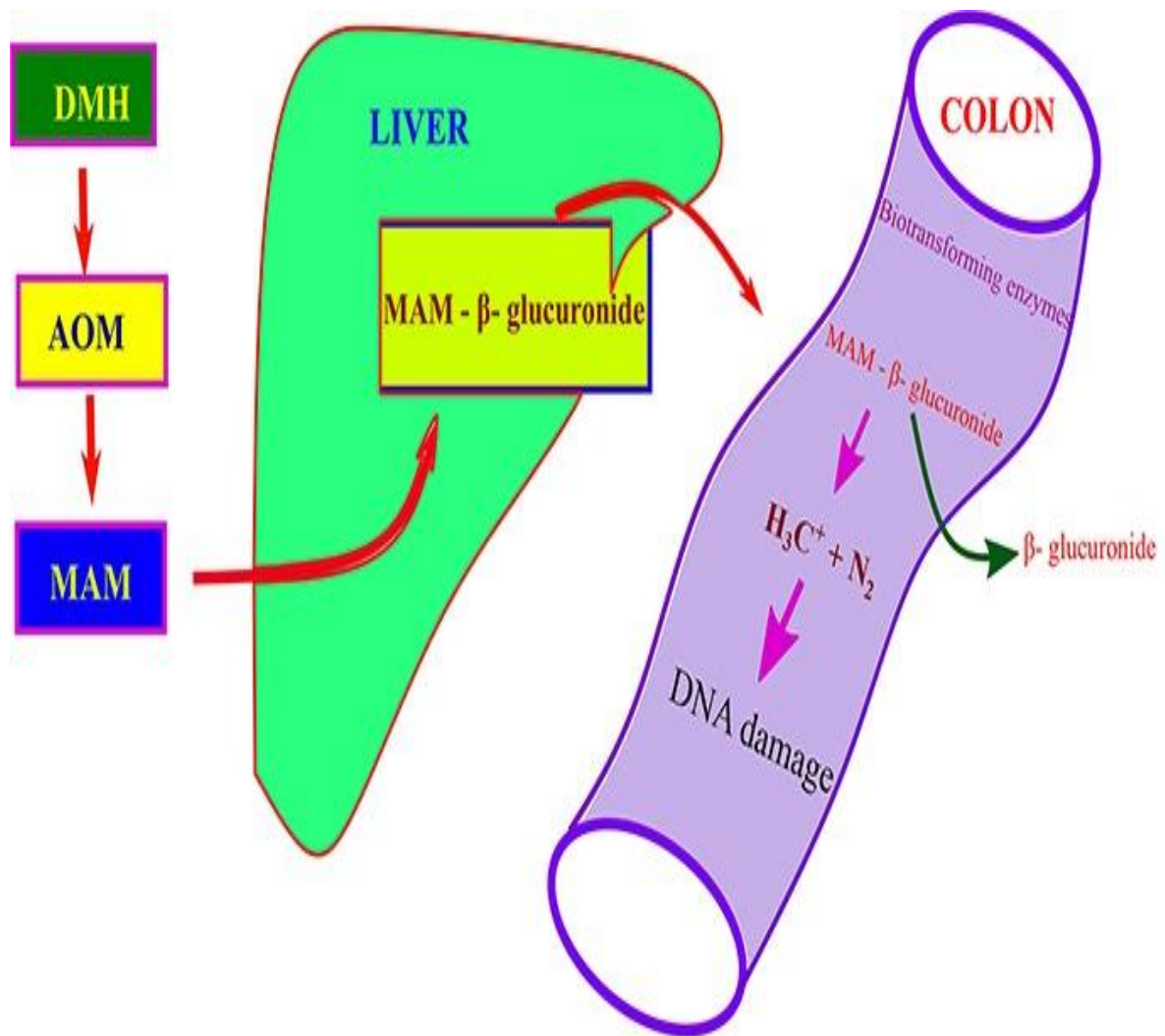


Figure 1.1: Transport of DMH from subcutaneous site to colon through glucuronidation (Venkatachalam *et al.*, 2020)

### 1.6.2 History of DMH

The history of dimethylhydrazine dates back to 1965, when Laquer studied the neurotoxicity of *Cycas circinalis*. Rats fed a crude extract of cycad meal developed tumors in different organs, including the gut, liver, and kidney (Venkatachalam *et al.*, 2020). To establish processes for diagnosis and, eventually, prevention of the disease, it is vital to have a thorough understanding of the chemical carcinogen DMH and its mechanism of action.

Hydrazine derivatives can be found in a variety of places, including the environment, industry, agriculture, and medicine. DMH, an organotropic colon carcinogen, is a frequently utilized alkylating chemical for inducing benign and malignant tumors in rodents' colons (Kuipers *et al.*, 2015).

Repeated exposure to DMH causes colon tumors in animals, which have pathological characteristics that are similar to sporadic forms of human colon cancer. In the majority of the investigations, DMH was administered subcutaneously. The enzymes that can metabolize or react with DMH are not present in the subcutaneous injection site. As a result, subcutaneously injected DMH slowly enters the circulation before reaching the liver and being converted into numerous intermediates (Venkatachalam *et al.*, 2020).

### 1.6.3 DMH Metabolism

DMH is first oxidized to azomethane, which is then transformed to azoxymethane (AOM), which is finally hydroxylated to methylazoxymethanol (MAM). Hydroxylation takes place mostly in the liver, most likely via the cytochrome P450 pathway, and to a lesser extent in the intestinal mucosa. MAM may enter the colon via bile, the intestinal lumen, and circulation as glucuronides and glucosides, and to a lesser extent as sulfates. The enzymes glucuronidase, glucosidase, and sulfatase, which are found in both enterocytes and the gut microbiota, cleave them. At body temperature, methylazoxymethanol is chemically unstable and decomposes spontaneously into formaldehyde, water, and nitrogen (Venkatachalam *et al.*, 2020). The likelihood of DNA mispairing, which has been considered to be crucial in mutagenesis and carcinogenesis, is turned off by alkylation of the oxygen atoms found in nitrogenous bases. Methylazoxymethanol has also been discovered to be a substrate for NAD<sup>+</sup> dependent dehydrogenase in the colon and liver, implying that methylazoxymethanol's active metabolite is the equivalent aldehyde (Martinello, *et al.*, 2017).

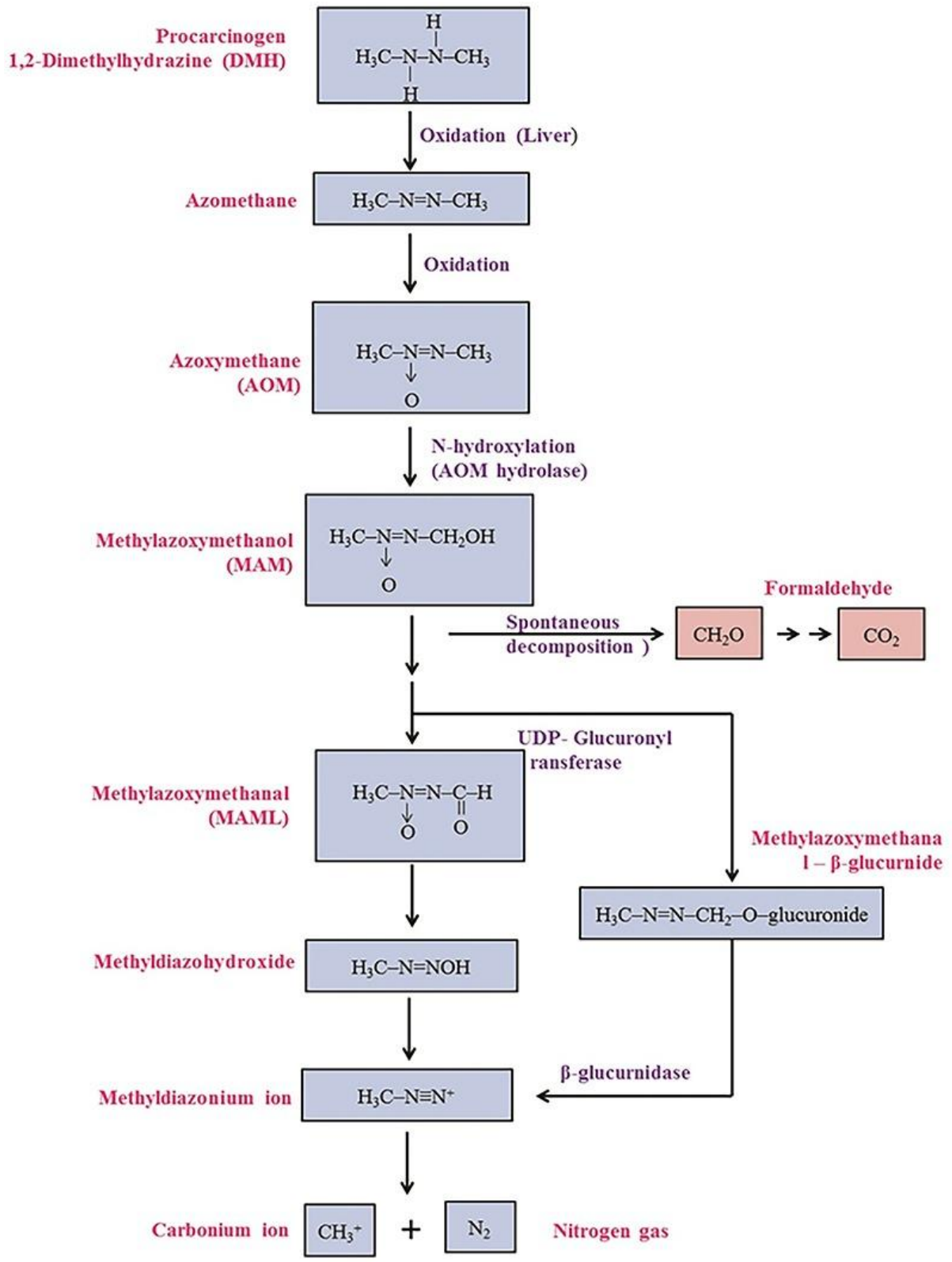


Figure 1.2: Metabolism of DMH (Venkatachalam *et al.*, 2020)

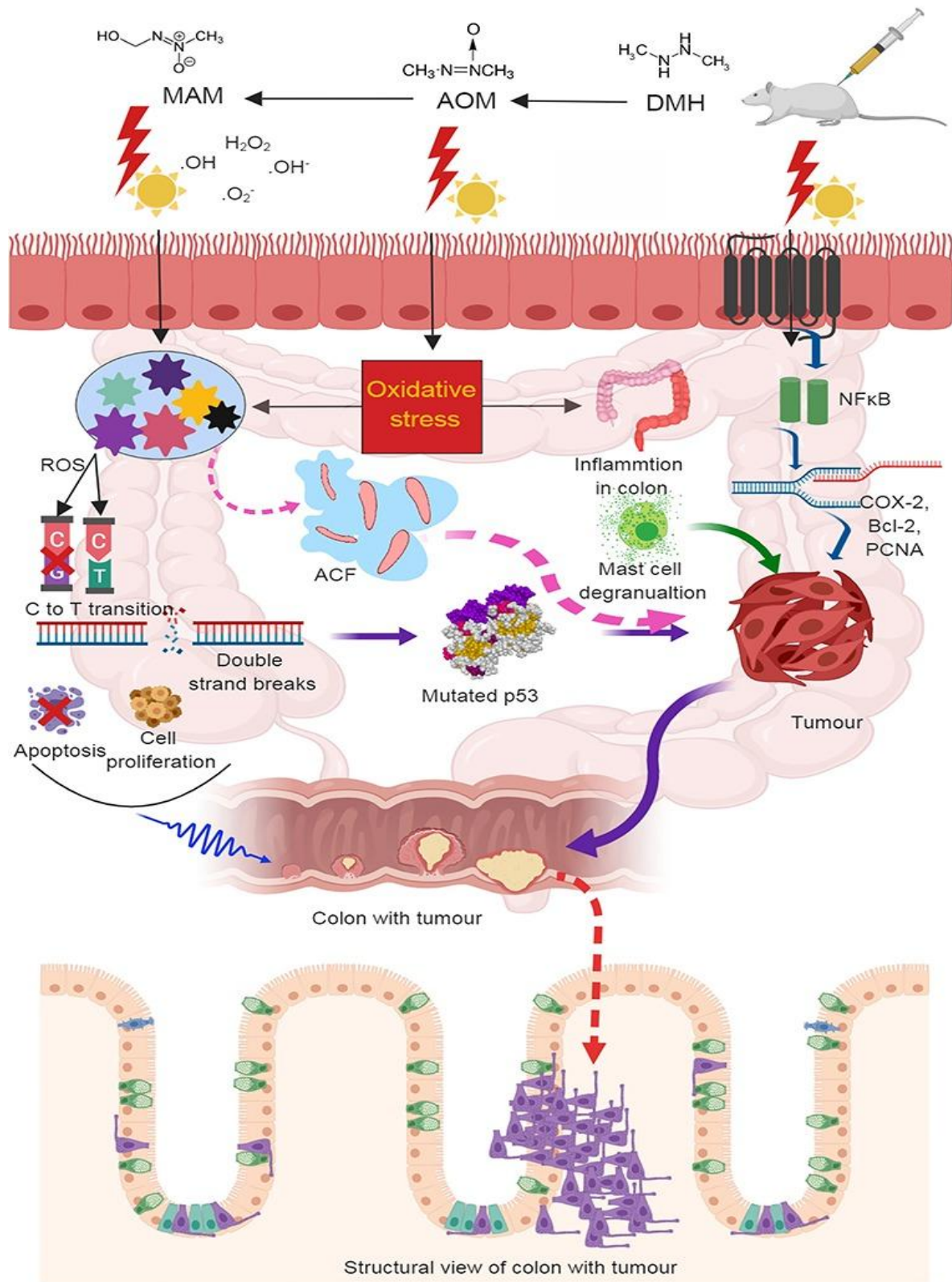


Figure 1.3: A Schematic expression of the overall mechanism of DMH-induced carcinogenesis. (Venkatachalam, *et al.*, 2020)

## 1.7 Other Colon Carcinogens

Other carcinogens that cause colon cancers to differing degrees, in addition to MAM acetate, AOM, and DMH, include:

- i. Heterocyclic amines (HCAs), e.g. 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) and 2-amino-3,3-dimethylimidazo[4,5-*f*] quinoline (IQ).
- ii. Aromatic amines, e.g. 3,2'-dimethyl-1,4-aminobiphenyl (DMAB).
- iii. Alkyl nitrosamide compounds, e.g. methylnitrosourea (MNU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

These carcinogens cause a range of epithelial lesions, including malignancies in the colon that are identical to neoplastic lesions seen in humans (Gurley *et al.*, 2015).

## 1.8 Diet and Cancer

### 1.8.1 Diet and the Gut Microbiota Interaction

The known risk factors of colon carcinogenesis includes low physical activity, obesity, and dietary habits such as excessive consumption of red, processed meats, and alcohol, as well as a lack of dietary fibre (Loke, *et al.*, 2020).

Firmicutes (30–50%), Bacteroidetes (20–40%), and Actinobacteria (1–10%) are the three major phyla that dominate the human gut microbiota (Gagnière, *et al.*, 2016). The major groups of bacteria in the human gut are strict anaerobes such as Bifidobacterium, Fusobacterium, Bacteroides, Eubacterium, Peptostreptococcus, and Atopobium, whereas facultative anaerobes such as Lactobacilli, Enterococci, Streptococci, and Enterobacteriaceae are present in 1,000-fold lower numbers (Loke *et al.*, 2020). During the first year of life, the gut microbiota evolves rapidly in terms of variety and composition before remaining relatively consistent as an adult. Nonetheless, external variables, particularly the influence of nutrition, can affect the composition of the resident microbiota. The balance between beneficial and detrimental gut microbiota can be upset when dietary components modify the gut microbial composition and diversity, and the resulting compromised gut homeostasis can pave the scene for cancer development. A continuous relationship between colon cancer and gut microbiota has been discovered in numerous research. Colorectal cancer-associated

bacteria such as *Fusobacterium nucleatum*, *Streptococcus gallolyticus*, *Clostridium difficile*, *Clostridium septicum*, *Enterococcus faecalis*, *Escherichia coli*, *Peptostreptococcus stomatis*, and *Bacteroides fragilis* are differentially enriched in fecal or colonic mucosa samples of colorectal cancer patients compared to healthy individuals (Loke *et al.*, 2020).

### 1.8.2 *Arachis Hypogaea* (Groundnut)

*Arachis hypogaea*, also referred to as the groundnut or peanut, is a tropical and temperate region of the world annual herbaceous legume. It is a member of the genus *Arachis* and family *Leguminosae*. Groundnuts are a composite food that contains a wide range of nutrients, including fiber, carbs, proteins, fats, vitamins, and minerals. Bioactive substances such as stilbenes, amino acids, phytosterols, and flavonoids, which possess anti-oxidant, anti-cancer and anti-tumor properties, have been extracted from Groundnuts. Allergies and aflatoxins contamination are two potential health issues with *Arachis hypogaea* seeds. In the food business, peanuts are frequently used to make flour, protein concentrates and isolates, oils, and other products (Syed, *et al.*, 2021).



Figure 1.6: *Arachis hypogaea* Seeds

### **1.8.2.1 Phytosterols**

Phytosterols are organic substances found in plants that mimic cholesterol in structure. Intestinal cholesterol absorption is known to be decreased by phytosterols, which lowers blood LDL-cholesterol and prevents cardiovascular issues. It has been suggested that diets high in phytosterols may cut the risk of cancer by 20%. Additionally, phytosterols may have an impact on the host's systems, enhancing the immune system's ability to recognize cancer, influencing the formation of hormone-dependent endocrine tumors, and modulating sterol biosynthesis. Additionally, phytosterols have demonstrated characteristics that directly impede tumor growth, such as slowed cell cycle progression, induction of apoptosis, and suppression of tumor spread (Shahzad, *et al.*, 2017).

### **1.8.2.1 Flavonoids**

Flowers' color and scent are produced by secondary plant metabolites called flavonoids, which also have antibacterial, antiviral, antioxidant, anti-allergic, and anti-inflammatory properties. Flavonoids interfere with a number of signal transduction pathways throughout the development of cancer, limiting growth, angiogenesis, and metastasis or boosting apoptosis., peanuts are frequently used to make flour, protein concentrates and isolates, oils, and other products (Abotaleb, *et al.*, 2018).

Flavonoids can be divided into various families, including anthocyanidins, chalcones, flavonols, flavanones, flavan-3-ols, flavanonols, flavones, and isoflavonoids, depending on their chemical makeup, degree of oxidation, and unsaturation of the connecting chain. Additionally, plants contain flavonoids in both free and glycoside-bound aglycone forms. The most prevalent flavone and flavonol type ingested in food is the one that is coupled to a glycoside (Rodríguez-García, *et al.*, 2019).

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.1 Materials

##### 2.1.1 Equipment and Reagents

Sodium chloride (NaCl), Distilled water, Sodium hydroxide (NaOH), Hydrogen chloride (HCL), EDTA disodium, Formalin, Normal saline, 1,2-Dimethyl hydrazine, 5-fluorouracil, 6M sulphuric acid, Carcinogenic antigen (CEA) kits, Visible Spectrophotometer, Ultra-Violet spectrophotometer (PG Instruments Ltd, Model-T80+, China), Water bath, Centrifuge, Weighing balance, Digital pH meter, Cuvette (glass and quartz), Micropipette (microlux).

##### 2.1.2 Sample Size

A total of 32 male albino mice were used for this study (provided by the Faculty of Pharmacy, University of Benin, Benin city). The mice were divided into 4 groups. Each group contained 8 mice. They were housed in standard plastic cages and allowed to acclimatize for 14 days under normal laboratory conditions with free access to feed (growers mesh) and drinking water.

After acclimatization, the mice in the various groups were treated once a week for twelve weeks based on the experimental design below.

<b>GROUP DESIGNATION</b>	<b>TREATMENT</b>
Group 1	Control
Group 2	DMH only for twelve weeks
Group 3	DMH and compounded diet for twelve weeks
Group 4	DMH for six weeks, After which Compounded diet for six weeks

## **2.2 Methods**

### **2.2.1 Groundnut Preparation**

The groundnut seeds were air-dried in the laboratory at the Department of Biochemistry, University of Benin, Benin city. The seeds were then grinded to powdered form and mixed the feed using the ratio 20g of groundnut to 80g of feed for a 100g diet.

### **2.2.2 DMH Administration**

1,2-dimethyl hydrazine was dissolved in normal saline and administered subcutaneously once a week at 20mg/kg body weight of mice.

### **2.2.3 Sample Collection**

After the treatment weeks, the mice were fasted overnight and sacrificed the following morning. Vital organs such as the liver and kidney were collected and a portion of the colon was kept in formalin for histological analysis. The colons harvested were kept in appropriate EDTA containers stored in ice. The tissues were homogenized in 10ml of normal saline using homogenizer fitted with Teflon pestle, mechanically and centrifuged at 300g for 10mins to completely separate the supernatant which was collected using a micropipette and stored in ice for subsequent analysis. Blood was collected, allowed to clot and the serum was separated from the blood by centrifuging it at 4000 rpm for 10 minutes. The serum samples were stored at -200C until required for the biochemical assays.

## **2.3 Biochemical Assays**

### **2.3.1 Estimation of malondialdehyde (MDA) concentration**

Principle:

This method depends on the formation of MDA as the end product of lipid peroxidation which reacts with 2-thio-barbituric-acid. A pink coloured complex which absorbs spectrophotometrically at 535 nm is formed.

Procedure:

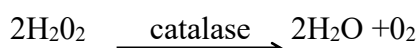
Aliquots (1ml) of the liver, colon and kidney homogenates and plasma were respectively added to 2ml of TCA-TBA-HCl reagent, and mixed thoroughly by swirling. The mixture was heated for 15 minutes in boiling water. After cooling, the flocculent precipitates were

removed by centrifugation at 3000rpm for 10 minutes. The absorbance of the clear supernatant was measured against a blank at 535nm. The MDA concentration of each sample was calculated using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### **2.3.2 Determination of Catalase Activity**

Principle:

Catalase is present in nearly all animal cells, plants and bacteria and also to prevent accumulation of noxious hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) which is converted to oxygen and water.



Procedure:

Aliquot of the plasma or homogenate supernatant (0.5ml) was put into ice-cold test tubes, while the blank contained 0.5ml distilled water. The reaction was initiated by adding 5ml of 30mM  $\text{H}_2\text{O}_2$ . This was mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 1.5ml of 6M  $\text{H}_2\text{SO}_4$ . 7ml of 0.01M potassium permanganate was added and mixed by inversion. Absorbance was read at 480nm within 30-60 seconds. The spectrophotometric standard was prepared by adding 7ml of 0.01M potassium permanganate to a mixture of 0.05M phosphate buffer, pH 7.0 and 1ml of 6M sulphuric acid solution.

### **2.3.3 Determination of superoxide dismutase (SOD) activity**

Principle:

Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined at 420nm using a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions. The degree of inhibition is a reflection of the activity of SOD and is determined at one unit of the enzyme activity.

Procedure:

The spectrophotometer was set and zeroed with a blank (distilled water). Distilled water was added to the reference tube, while 0.2ml of the appropriate homogenate supernatant or plasma was added to appropriately labeled test tubes. To each of these was added 2.5ml of carbonate buffer. 0.3ml of 3mM adrenaline solution was then added to the reference test tube and other test tubes with homo

genate supernatant and plasma. These were mixed and absorbance read at 420nm.

#### **2.3.4 Determination of glutathione peroxidase activity**

Principle:

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a brown colour disposition which absorbs spectrophotometrically at 430nm.

Procedure:

To an aliquot of 0.2ml of plasma, 2.5ml of phosphate buffer, 2.5ml of potassium peroxide, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A brown colour was formed and read spectrophotometrically at 430nm.

#### **2.3.5 Estimation of total protein**

Principle:

Cupric ions in an alkaline solution react with the peptide bonds of proteins and polypeptides containing at least two peptide bonds to produce a violet colored complex. The absorbance of the complex at 546 nm is directly proportional to the concentration of protein in the sample.

Procedure:

This assay was done using the Randox total protein reagent kit which contained the Biuret reagent (Sodium hydroxide, Na-K tartrate, potassium iodide and cupric sulphate) referred to as R1, Blank reagent, R2 which contained sodium hydroxide and Na-K tartrate, and a standard protein of known concentration. Distilled water (0.02ml) and 1.0mL of R1 were added to a test tube and used as the reagent blank. In another test tube, 0.02mL of the standard protein and 1.0mL of R1 was added and used as the standard. Aliquot of each sample (0.02ml) and 1.0mL of R1 were pipetted into separate test tubes. The solutions were mixed and left at room temperature for 30 minutes. The absorbance values of the sample and the standard were then measured against the reagent blank.

### **2.3.6 Estimation of Lactate Dehydrogenase Activity**

Principle:

Lactate dehydrogenase is an oxidoreductase that catalyses the interconversion of pyruvate and lactate as well as the simultaneous interconversion of NADH and NAD<sup>+</sup>.

Procedure:

The assay for lactate dehydrogenase was done using LDH Randox test kit which contained reagent R1a (buffer/substrate) and reagent R1b (NADH). The vials of freeze dried NADH (R1b) were each reconstituted with 3 mL of R1a (buffer/substrate) and mixed gently. The sample (0.1 mL) and 3 mL of the rehydrated NADH solution were pipetted into each test tube. After carefully combining the mixture, it was transferred into a quartz cuvette and the initial absorbance was read at 340 nm. The clock was started instantaneously and the absorbance was taken again after exactly 1, 2 and 3 minutes.

## **2.4 Histopathological Examination**

Fixed tissue sections were processed for histo-pathological examination. The tissues were washed in tap water for 30 minutes, dehydrated, cleared, impregnated, embedded, sectioned and stained with eosin and haematoxylin.

### **2.4.1 Dehydration**

The graduated alcohol concentrations were applied to a piece of the sectioned tissue (3mm) at predefined intervals. Dehydration was required in order for paraffin wax to properly penetrate the portions.

### **2.4.2 Clearing**

Here, tissues were cleared of alcohol for 2.5 hours using equal measures of chloroform and xylene. For proper impregnation, the tissues were then put through two cycles of pure xylene for 2.5 hours each.

### **2.4.3 Impregnation**

This treatment cleared the tissue segment of any clearing agents. 2.5 hours were spent processing the tissues via two cycles of molten paraffin wax at 60°C in an electric oven with a thermostat.

#### **2.4.4 Embedding**

The Impregnated tissues were embedded in a mould, and when the blocks had time to set, they were removed. Thus, solid tissue blocks in paraffin wax were produced.

#### **2.4.5 Sectioning**

A wooden handled spatula was heated over a Bunsen burner and closed on the opposite sides of the paraffin and wooden blocks. After a few seconds, the spatula was carefully withdrawn and the paraffin blocks stuck firmly onto the wooden block. The wood paraffin block was fixed to the disc of the microtome and trimmed before sectioning. The microtome was set to the required thickness of 15 $\mu$ M and the knife was positioned and the embedded specimen was cut into thin paraffin ribbons.

#### **2.4.6 Slide Preparation**

On a clear slide, a drop of albumin was added and equally distributed out in the middle of the slide. With a clean finger, the surplus albumin was eliminated. Some distilled water was applied to the albumin that had been spread. After that, the slice was placed on the distilled water of the albumin-smear surface and slowly warmed until it was flat. The sample was kept to dry while the extra water was drained from it.

#### **2.4.7 Staining Procedure**

The paraffin tissue section was cleared with three changes of xylene for 2 hours each. The prepared sections were hydrated by passing the slides through two changes of absolute ethanol for 2 hours each, 90% ethanol for 2 hours and finally to distilled water for some minutes. The prepared slides were stained in haematoxylin for 30 minutes and then washed in tap water. This was followed by differentiation with 1% acid alcohol until correct differentiation was clear under the microscope. The specimens were counterstained in 1% eosin for 1 minute and washed in running water to remove excess eosin.

The specimens were hydrated again by passage through a series of graded concentrations (80%, 70%, 90%) of ethanol for 1 minute each. The specimens were cleared in xylene and mounted using a drop of Canada balsam on the specimens and covered with the glass cover slips for study under the microscope.

## CHAPTER THREE

### RESULTS

#### 3.1 Statistical Analysis

The experimental results obtained were expressed as mean  $\pm$  standard error of mean (S.E.M) and were analysed for statistical significance by one way analysis of variance (ANOVA). The mean values that were significantly different from each other were determined by one-way statistical probability of social science (SPSS). A P-value  $\leq 0.05$  was considered significant and P-value ( $>0.05$ ) was considered not statistically significant.

#### 3.2 Effect of 1,2- Dimethylhydrazine in the Colon of Mice Fed with *Arachis hypogaea* Compounded Diet

The result shown in Table 3.1 revealed that twelve weeks of subcutaneous induction of DMH significantly elevated the colon total protein and MDA concentration relative to the normal control ( $p < 0.05$ ). Consequently, the activity of colon antioxidant enzymes was significantly reduced following the induction of DMH for twelve weeks relative to the normal control ( $p < 0.05$ ).

On a six weeks induction of DMH, concentrations of total protein and MDA were significantly elevated ( $p < 0.05$ ) relative to the normal control but not as much as the twelve weeks induction. However, the activity of colon antioxidant enzymes was significantly reduced in the group induced with DMH relative to the normal control ( $p < 0.05$ ). A single dose induction of DMH followed by twelve weeks of exposure to compounded diet exhibited a modulation in the elevation of colon total protein and MDA concentration relative to the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). A similar modulatory response was observed in the activities of colon antioxidant enzymes in the group exposed to *Arachis hypogaea* compounded diet for twelve weeks after a single dose subcutaneous induction of DMH when compared with the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). The characteristic response of mice colon exposed to the compounded diet for both twelve (12) and six (6) weeks were in similar order.

Table 3.1: Effect of 1,2- Dimethylhydrazine in the Colon of Mice Exposed to *Arachis hypogaea* Compounded Diet

GROUPS	MDA(nmol/mg)	GPx(U/mg)	SOD(U/mg)	CAT(U/mg)	Total Protein (g/dL)
G1 (CONTROL)	0.34 ± 0.26 <sup>a</sup>	1.05 ± 0.04 <sup>a</sup>	1.30 ± 0.10 <sup>a</sup>	1.34 ± 0.07 <sup>a</sup>	1.65 ± 0.16 <sup>a</sup>
G2 (DMH 12wks)	1.38 ± 0.54 <sup>b</sup>	0.05 ± 0.03 <sup>b</sup>	0.11 ± 0.05 <sup>b</sup>	0.27 ± 0.04 <sup>b</sup>	3.41 ± 0.15 <sup>b</sup>
G3 (DMH + Diet 12WKS)	0.38 ± 0.20 <sup>a</sup>	0.78 ± 0.04 <sup>a</sup>	1.93 ± 0.02 <sup>a</sup>	1.21 ± 0.02 <sup>a</sup>	1.80 ± 0.08 <sup>a</sup>
G4 (DMH 6wks + Diet 6wks)	0.30 ± 0.45 <sup>a</sup>	0.81 ± 0.11 <sup>a</sup>	1.77 ± 0.15 <sup>a</sup>	1.25 ± 0.02 <sup>a</sup>	1.42 ± 0.12 <sup>a</sup>

Key: DMH = 1,2- Dimethylhydrazine; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; MDA = malondialdehyde; G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4; Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ( $p < 0.05$ ).

### **3.3 Effect of 1,2- Dimethylhydrazine in the Liver of Mice Fed with *Arachis hypogaea* Compounded Diet**

The result shown in Table 3.2 revealed that twelve weeks of subcutaneous induction of DMH significantly elevated the hepatic total protein and MDA concentration relative to the normal control ( $p < 0.05$ ). Consequently, the activity of hepatic antioxidant enzymes was significantly reduced following the induction of DMH for twelve weeks relative to the normal control ( $p < 0.05$ ).

On a six weeks induction of DMH, concentrations of total protein and MDA in the liver of mice were significantly ( $p < 0.05$ ) elevated relative to the normal control but not as much as the twelve weeks induction. However, the activity of liver antioxidant enzymes was significantly reduced in the group induced with DMH relative to the normal control ( $p < 0.05$ ). A single dose induction of DMH followed by twelve weeks of exposure to compounded diet exhibited a modulation in the elevation of liver total protein and MDA concentration relative to the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). A similar modulatory response was observed in the activities of liver antioxidant enzymes in groups exposed to compounded diet for twelve weeks after a single dose subcutaneous induction of DMH when compared with the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). The characteristic response of mice liver exposed to the compounded diet for both twelve (12) and six (6) weeks was in similar order.

Table 3.2: Effect of 1,2- Dimethylhydrazine in the Liver of Mice Exposed to *Arachis hypogaea* Compounded Diet

GROUPS	MDA(nmol/mg)	GPx(U/mg)	SOD(U/mg)	CAT(U/mg)	Total Protein (g/dL)
G1 (CONTROL)	0.23 ± 0.68 <sup>a</sup>	1.06 ± 0.11 <sup>a</sup>	1.70 ± 0.56 <sup>a</sup>	1.15 ± 0.00 <sup>a</sup>	2.56 ± 0.08 <sup>a</sup>
G2 (DMH 12wks)	1.30 ± 0.95 <sup>b</sup>	0.08 ± 0.09 <sup>b</sup>	0.36 ± 0.18 <sup>b</sup>	0.20 ± 0.01 <sup>b</sup>	3.04 ± 0.08 <sup>b</sup>
G3 (DMH + Diet 12WKS)	0.35 ± 0.43 <sup>a</sup>	0.88 ± 0.57 <sup>a</sup>	1.67 ± 0.23 <sup>a</sup>	1.18 ± 0.02 <sup>a</sup>	2.33 ± 0.13 <sup>a</sup>
G4 (DMH 6wks + Diet 6wks)	0.35 ± 0.70 <sup>a</sup>	0.87 ± 0.41 <sup>a</sup>	1.80 ± 0.29 <sup>a</sup>	0.95 ± 0.00 <sup>a</sup>	2.65 ± 0.08 <sup>a</sup>

Key: DMH = 1,2- Dimethylhydrazine; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; MDA = malondialdehyde; G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ( $p < 0.05$ ).

### **3.4 Effect of 1,2- Dimethylhydrazine in the Kidney of Mice Fed with *Arachis hypogaea* Compounded Diet**

The result shown in Table 3 revealed that twelve weeks of subcutaneous induction of DMH significantly elevated the renal total protein and MDA concentration relative to the normal control ( $p < 0.05$ ). Consequently, the activity of renal antioxidant enzymes was significantly reduced following the induction of DMH for twelve weeks relative to the normal control ( $p < 0.05$ ).

On a six weeks induction of DMH, concentrations of total protein and MDA in the kidney of mice were significantly ( $p < 0.05$ ) elevated relative to the normal control but not as much as the twelve weeks induction. However, the activity of renal antioxidant enzymes was significantly reduced in the group induced with DMH relative to the normal control ( $p < 0.05$ ). A single dose induction of DMH followed by twelve weeks of exposure to compounded diet exhibited a modulation in the elevation of renal total protein and MDA concentration relative to the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). A similar modulatory response was observed in the activities of renal antioxidant enzymes in groups exposed to compounded diet for twelve weeks after a single dose subcutaneous induction of DMH when compared with the normal control ( $p > 0.05$ ) and DMH (12 weeks) group ( $p < 0.05$ ). The characteristic response of mice renal exposed to the compounded diet for both twelve (12) and six (6) weeks was in similar order.

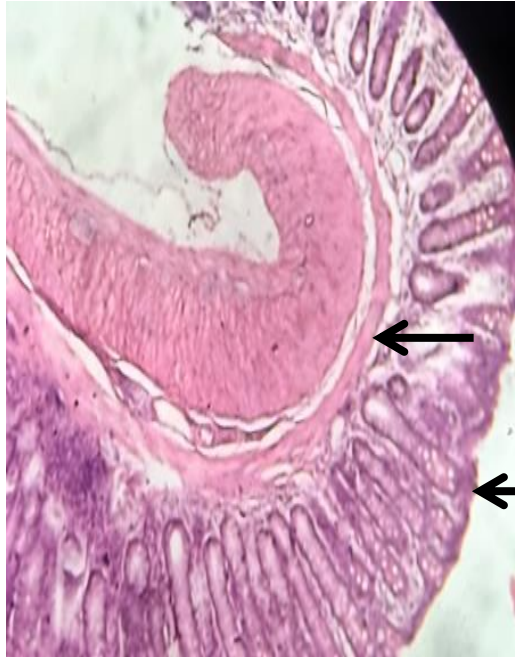
Table 3.3: Effect of 1,2- Dimethylhydrazine in the Kidney of Mice Exposed to *Arachis hypogaea* Compounded Diet

GROUPS	MDA(nmol/mg)	GPx(U/mg)	SOD(U/mg)	CAT(U/mg)	Total Protein (g/dL)
G1 (CONTROL)	0.40 ± 0.26 <sup>a</sup>	1.08 ± 0.04 <sup>a</sup>	1.78 ± 0.56 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	2.82 ± 0.12 <sup>a</sup>
G2 (DMH 12wks)	1.47 ± 0.54 <sup>b</sup>	0.08 ± 0.03 <sup>b</sup>	0.75 ± 0.18 <sup>b</sup>	0.17 ± 0.01 <sup>b</sup>	3.63 ± 0.27 <sup>b</sup>
G3 (DMH + Diet 12WKS)	0.42 ± 0.20 <sup>a</sup>	0.77 ± 0.04 <sup>a</sup>	1.75 ± 0.23 <sup>a</sup>	1.17 ± 0.02 <sup>a</sup>	2.50 ± 0.10 <sup>a</sup>
G4 (DMH 6wks + Diet 6wks)	0.49 ± 0.45 <sup>a</sup>	0.82 ± 0.11 <sup>a</sup>	1.77 ± 0.29 <sup>a</sup>	1.18 ± 0.00 <sup>a</sup>	2.65 ± 0.13 <sup>a</sup>

Key: DMH = 1,2- Dimethylhydrazine; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; MDA = malondialdehyde; G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ( $p < 0.05$ ).

### 3.5 Histological Analysis

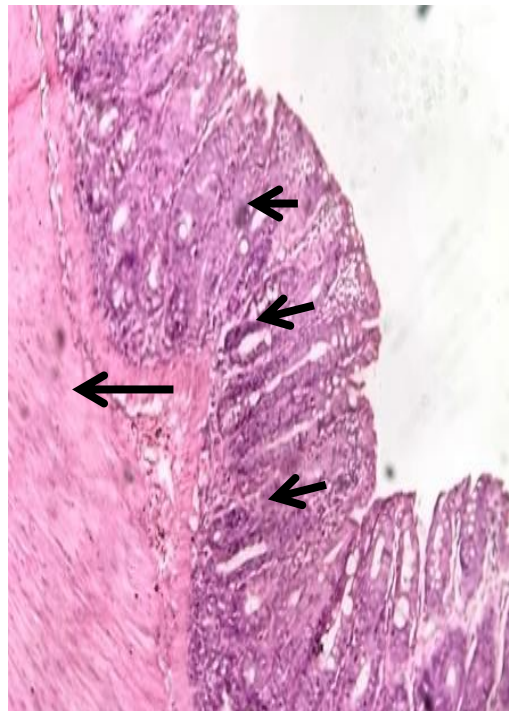
#### Group 1: CONTROL RH 12/5 COLON



**Plate 3.5.1 :** Photomicrograph of colon histology that served as control

**Features:** Revealed prominent histology with normal mucosal details bound by muscularis externa (long arrow) and distinct columnar epithelium and villi in the mucosal (short arrow)

**Group 2: RHLL 12/5 COLON**



**Plate 3.5.2 :** Photomicrograph of colon section from mice induced with DMH for 12 weeks

**Features:** Revealed many neoplastic crypts and glands irregular in shape and size with a tendency to an exophytic growth. Crypts are lined by a tall, hyperchromatic, somewhat disordered epithelium without differentiation into mature goblet cells (short arrow). There is marked fibrosis in muscularis externa (long arrow).

**Group 3: RL 12/5 COLON**



**Plate 3.5.3 :** Photomicrograph of colon section from mice treated with DMH + *Arachis hypogaea* seeds for 12 weeks

**Features:** Revealed visible histology with prominent mucosal details bound by muscularis externa (long arrow) and columnar epithelium and villi in the mucosal revealing mild visible proliferating changes(short arrow).

**Group 4: RL 12/5 COLON**



**Plate 3.5.4:** Photomicrograph of colon section from mice treated with DMH for 6 weeks and then fed *Arachis hypogaea* seeds for 6 weeks

**Features:** Revealed visible histology with prominent mucosal details bound by thick muscularis externa (long arrow) and columnar epithelium and villi in the mucosal revealing mild visible proliferating changes (short arrow).

## CHAPTER FOUR

### DISCUSSION AND CONCLUSION

#### 4.1 Discussion

This study was carried out to investigate the effect of *Arachis hypogaea* seeds on 1,2 dimethylhydrazine (DMH) induced colon carcinogenesis in mice.

In the result tables, super oxide dismutase (SOD) and catalase (CAT) activity showed a reduction in groups induced with DMH when compared to control. Under normal conditions, the intracellular levels of reactive oxygen species (ROS) are steadily maintained to prevent cells from damage (Ferraro D, *et al*, 2006) as seen in control. As a result of increased metabolic activity, elevated levels of reactive oxygen species have been seen in almost all cancer forms, where they promote many aspects of tumor formation and cancer progression. The most prevalent types of oxidative stress that are primarily formed are reactive oxygen species, such as hydroxyl radicals (OH), singlet oxygen, or reactive nitrogen species, such as peroxynitrite (Kryston, *et al.*, 2011).

At week 12, constant enhanced lipid peroxidation was observed in the liver, colon and kidney of mice treated with only DMH. The organ SOD, CAT and GSH levels were significantly lowered while malondialdehyde (MDA) and total protein levels were higher in DMH induced mice compared to the control and thus may have caused increase in lipid peroxidation. DMH is a procarcinogen that is metabolized to a methyl free radical and produces a hydroxyl radical or hydrogen peroxide in the presence of metal ions that may contribute to the development of cancer and lipid peroxidation (Hamiza, *et al.*, 2012). One of the significant and pertinent indicators of oxidative damage is the development of lipid peroxide, or MDA, and treatment with DMH has been associated with higher levels of this lipid peroxidation product (MDA) (Hamiza, *et al.*, 2012). Free radical removal from biological systems is accomplished through enzymatic and non-enzymatic antioxidants, which serve as important free radical defense mechanisms (Nandhakumar *et al.*, 2012). GSH and its oxidized counterpart make up a significant portion of the cell's redox buffer system. By directly interacting with ROS through the -SH group, GSH can function as a non-enzymatic antioxidant. Alternatively, it can be involved as a cofactor or coenzyme in the enzymatic detoxification reaction for ROS (Nandhakumar *et al.*, 2012).

Free radicals are produced by DMH treatment in colonic tissue, although GSH and other enzymatic antioxidants such (SOD, Catalase, GPx etc.) limit their level by scavenging the free radicals. In this investigation, mice treated with DMH without *Arachis hypogaea* diet supplementation had significantly reduced GSH, SOD and Catalase levels. As a result, DMH reduced antioxidant enzyme levels (Hamiza, *et al.*, 2012).

However *Arachis hypogaea* diet supplementation significantly decreased hepatic and colonic MDA, accompanied by an increase in total protein (TP), and enzymes including super oxide dismutase and catalase. This is due to the presence of bioactive substances such as phytosterols and flavonoids in *Arachis hypogaea* which have antioxidant and anti-inflammatory properties. Flavonoids interfere with a number of signal transduction pathways throughout the development of cancer, limiting growth, angiogenesis, and metastasis or boosting apoptosis (Abotaleb, *et al.*, 2018). *Arachis hypogaea* supplementation, proved important in inhibiting carcinogenesis induced by DMH.

In group 2 mice, histology revealed numerous neoplastic crypts and glands irregular in shape and size with a tendency for exophytic development. Chronic inflammation promotes hyperchromasia, which in turn may predispose to the development of cancer. In groups 3 and 4, histology revealed a modest hyperchromatic disorganized epithelium. Colon tumorigenesis modifies normal intestinal epithelial cells by spontaneous mutation, environmental mutagens or inflammation-induced genetic and epigenetic alterations which may lead to disordered epithelial (Venkatachalam, *et al.*, 2020). However in groups 3 and 4, *Arachis hypogaea* diet supplementation had a significant inverse influence on crypt formation in the distal colon, showing a higher antioxidant property with fewer distorts in the colon epithelium.

#### **4.1 Conclusion**

The findings of this study demonstrated that DMH-induced oxidative stress had negative effects on the experimental animals, leading to hepatocellular and hepatic injury as well as imbalances in enzyme distributions. In contrast, the *Arachis hypogaea* diet demonstrated remarkable hepatoprotective potential, highlighting its capacity to suppress and reverse the injuries associated with the adverse effect of DMH in mice.

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