INFLUENCE OF *ACALYPHA WILKISENIA* ON MALONDIALDEHYDE AND GLUTATHIONE PEROXIDASE ACTIVITY IN 1,2-DIMETHYLHYDRAZINE-INDUCED COLON TUMORS IN WISTAR RATS

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc (HONS) MEDICAL BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY

JUNE, 2024

CERTIFICATION

This is to certify that this project work was carried out by MAYAKI OZOZA DEBORAH with matriculation number BMS1902152, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry

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DEDICATION

I dedicate this Thesis first and foremost to God Almighty who has been there for me right from birth and kept me in good health for the duration of my studies. I also dedicate this to my closest friends I found in school as well as my classmates, the Department of Medical Biochemistry and the University of Benin

ACKNOWLEDGEMENTS

My utmost gratitude is to God Almighty who was with me all through this journey, who made Himself manifest even in my weakest points.

Deepest gratitude and a million accolades to my research (project) supervisor, Dr. Mrs. S.O. Olubodun, PhD, Medical Biochemistry, University of Benin, Benin City, Nigeria, for giving me the opportunity to do this research under her supervision and providing invaluable guidance throughout the research.

I would not fail to mention Mazi Chimezie Nzoputam and Mr. Ekene Asoya in the success of this project, for their consistent and unwavering support and contributions. God bless you

Special thanks to the staff of Department of Medical Biochemistry for making this a part of my study. I am thankful and blessed enough to get constant encouragement, support and guidance from all teaching staff of the Department of Medical Biochemistry, for their assistance in helping us complete this work successfully.

This work wouldn't have been possible without the financial, mental and spiritual support from my mother , Mrs. Maryann Mayaki and my big mummy, Mrs. Dupe Mayaki, I also want to say a huge thank you to my grandmother Mrs/ Funmilayo Mayaki as well as my God mother Pastor Mrs. Joan Gamra without them none of this would have been possible. I also want to acknowledge my younger sister, Hadassah Myrtle Mayaki , who has been inspirational, supportive and a source of motivation to strive for excellence and good success.

.Big shout out to my friends .Aize, Tessie, Rosemary, Sidikatu, Kelvin and Victor for their unwavering support also to my colleagues/entire MBC190 class especially the amazing twelve who were also on this project.

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ABSTRACT

This research examines the effect of ethanol leaf extract of *Acalypha wilkisenia* on malondialdehyde (MDA) and glutathione peroxidase (GPx) levels in wistar rats with 1,2 dimethylhydrazine(DMH) induced colon tumors. Colon cancer is a leading cause of cancer related mortality ,is often characterized by elevated oxidative stress markers such as MDA and altered antioxidant enzyme activities, including GPx. *Alcalypha wilkisenia*, a plant commonly known for its antioxidant properties, was evaluated for its potential therapeutic effects. Wistar rats were divided into six groups: Control, DMH treated only, Xeloda, DMH plus ethanol leaf extract of *Alcalypha wilkisenia* . Over the experimental period, MDA and GPx levels were measured using standard biochemical assays. Results indicated that DMH significantly increased MDA levels and decreased GPx activity, indicative of oxidative stress and impaired antioxidant defense. Conversely administration of *Acalypha wilkisenia* extract to DMH treated rats resulted in a significant reduction in MDA levels and restoration of GPx activity , suggesting a protective effect against colon carcinogenesis through the modulation of oxidative stress markers, highlighting its potential as a complementary therapeutic agent in colon cancer management .Further studies are needed to elucidate the underlying mechanisms and confirm these effects in clinical settings.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Colon cancer, also known as colorectal cancer (CRC), is a type of cancer that originates in the colon or rectum, which are parts of the large intestine, it arises from epithelial cells lining the colon or rectum and it is characterized by uncontrolled cell growth. It is a significant health concern worldwide, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths globally (Siegel *et al*., 2019). Colon cancer often begins as benign clumps of cells known as polyps on the inner lining of the colon or rectum. These polyps can become cancerous over time through a complex multistep process involving both genetic mutations and epigenetic changes. This progression is well explained by the adenoma-carcinoma sequence, which involves the accumulation of mutations in key oncogenes and tumor suppressor genes. For instance, mutations in the APC gene are commonly seen in early adenomas, while further mutations in genes such as KRAS, and later in TP53, lead to the development of carcinoma (Sameer, 2013; Forsmark *et al*., 2023). This transformation underscores the importance of early detection and removal of polyps to prevent the development of colon cancer.

There are a number of risk factors for colorectal cancer; aging, dietary habits, lifestyle choices, family history and inflammatory bowel disease. Some common symptoms include:

- Alterations in bowel movements
- Presence of blood in the stool
- Abdominal pain
- Significant weight loss.

Diagnosis is usually achieved through colonoscopy, imaging techniques and biopsy. There are different treatment strategies for colorectal cancer including, surgery,(this is usually the best route for Early-stage cancers) while advanced levels require surgery, chemotherapy and radiation therapy that are aimed at specific targets within

Growing evidence points to oxidative stress as a major contributor to colon cancer development. The imbalance that leads to this illness is the body's generation of reactive oxygen species (ROS) and its utilization of antioxidants to counteract these damaging chemicals. According to (Boccellino *et al*., 2019), high ROS can cause cellular signaling pathways to be disrupted, DNA damage to occur, and mutations to be promoted. All of these factors increase the risk of cancer. The level of lipid peroxidation and cellular damage within tissues is indicated by malondialdehyde (MDA), a noteworthy biomarker for oxidative stress. Ozone damage plays a significant role in the advancement of cancer, as evidenced by the correlation between elevated MDA levels and increased oxidative stress in malignant tissues (Lee *et al*., 2014). Furthermore, by lowering hydrogen peroxide and organic hydroperoxides, the vital antioxidant enzyme glutathione peroxidase (GPx) lessens oxidative stress. Numerous malignancies, including colon cancer, have been shown to have decreased GPx activity, highlighting the need for therapeutic approaches that strengthen antioxidant defenses (Li et al., 2018).

A growing number of people are developing colon cancer, and while treatment strategies have improved over time, new therapeutic approaches are still needed. Traditional medicinal plants have drawn interest because of their bioactive compounds, which have antioxidative, anti-inflammatory, and anticancer properties. Of these, Acalypha wilkesiana has shown promise because of its rich phytochemical composition and documented pharmacological activities (Md

Arshad et al., 2020). The plant's antioxidant properties could help mitigate oxidative stress, thereby playing a role in cancer prevention and therapy.

Acalypha wilkisenia, often referred to as Irish Petticoat. Belongs to the Euphoribiacce family and originates from the south pacific islands (Kingsley and Marshall, 2014). It has been employed traditionally in medicine for many years to treat various health conditions. The plant's leaves have been found to possess significant antioxidant properties, which could be beneficial in mitigating oxidative stress associated with colon cancer. Previous research has demonstrated the plant's potential to enhance antioxidant defenses and protect against oxidative damage, suggesting its applicability in cancer therapy. Scientific evidence has been gathered to suggest that the plant might possess anti-cancer benefit, research from this plan has shown that extracts from this plant suppresses the growth of colon cancer tumors n rats, making it a liable candidate for further investigation in its potential role in preventing colon tumors and cancer. Ethanol extracts of Acalypha wilkisenia leaves contain flavonoids, tannins, and saponins known for their ability to scavenge free radicals and inhibit lipid peroxidation (Adedapo *et al*., 2009; Ashafa *et al*., 2010).

1.1 JUSTIFICATION OF THE STUDY

Plants are essential to human survival and the foundation of his continuous existence since they provide all of his basic requirements, including food, clothing, and shelter, as well as indirectly by influencing the climate and maintaining both his immediate and remote environments. The effectiveness with which man, with all of the resources and technology at his disposal, harnesses, develops, and uses plants and plant products, in turn, determines our survival and continued existence.

Oxidative stress plays a pivotal role in cancer development, and assessing its modulation by therapeutic agents is crucial. *Acalypha wilkisenia* , known for its traditional medicinal uses and rich antioxidant content, presents a promising avenue for exploration. By evaluating the ethanol leaf extract of *Acalypha wilkisenia* in a 1,2-dimethylhydrazine-induced colon tumor model, this study seeks to elucidate its effects on malondialdehyde (MDA) and glutathione peroxidase (GPx) levels, addressing a notable gap in current research.

1.2 AIM OF THE STUDY

The aim of this study is to evaluate the influence of ethanol leaf extract of *Acalypha wilkisenia* on oxidative stress markers, specifically malondialdehyde (MDA) and glutathione peroxidase (GPx), in Wistar rats with colon tumors induced by 1,2-dimethylhydrazine (DMH).

1.3 OBJECTIVE OF THE STUDY

The objectives of the study are to:

1. To determine the effect of *Acalypha wilkisenia* ethanol leaf extract on malondialdehyde (MDA) levels in the blood of DMH-induced colon tumor-bearing Wistar rats.
2. To assess the impact of *Acalypha wilkisenia* ethanol leaf extract on glutathione peroxidase (GPx) activity in the blood of DMH-induced colon tumor-bearing Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.0 ACALYPHA WILKISENIA

Acalypha wilkisenia commonly referred to as Copperleaf or Jacob's Coat, belongs to the Euphorbiaceae family (Ahmad *et al*., 2019). This tropical and subtropical ornamental plant is celebrated for its vibrant and variegated foliage, displaying an array of colors including red, bronze, green, and pink. Its aesthetically pleasing leaves make it a sought-after choice for garden landscapes and indoor settings alike. The plant typically grows as a shrub, attaining heights of 1 to 3 meters, influenced by environmental factors and cultivation practices (Gupta *et al*., 2017). The leaves are broad, ovate to elliptical, and serrated, with sizes ranging from 10 to 20 cm in length. The variability in leaf colors across different varieties adds to its visual appeal. Additionally, the plant produces small, inconspicuous flowers arranged in terminal spikes (Ahmad *et al*., 2019).

Originating from the South Pacific islands, particularly Fiji, *Acalypha wilkisenia* thrives in warm, humid climates and is extensively cultivated in tropical and subtropical regions globally (Nguyen *et al*., 2020). It prefers well-drained soils enriched with organic matter and favors areas with partial to full sunlight exposure. While adaptable to various soil types and conditions, it performs optimally in slightly acidic to neutral pH soils.

Propagation of *Acalypha wilkisenia* primarily occurs through stem cuttings, a method valued for its simplicity and high success rate (Khan *et al*., 2020). Semi-hardwood cuttings, sourced from healthy, mature plants, are commonly used. These cuttings, typically measuring 10 to 15 cm, are taken just below a node and stripped of lower leaves. Rooting hormone application may enhance

root development before planting in a well-draining potting mix. Maintaining a warm, humid environment with indirect sunlight facilitates root formation, typically achieved within a few weeks (Ahmad *et al*., 2019).

In Nigeria, *Acalypha wilkisenia* is recognized by different names in local languages. Among the Hausa people, it is referred to as "Kafinta," while the Yoruba people call it "Agbala" (Ojekale *et al*., 2016). These indigenous names underscore the plant's significance in traditional medicine and horticulture within these communities..

In addition to these scientific names, it is also known by the common names "copperleaf," "Joseph's coat," "fire dragon," and "beef steak plant." Both indoors and out, they do best in lots of sunlight, with direct sunlight showcasing their colorful foliage. Aphids, spider mites, mealy bugs, and other pests are among the many pests that can affect *Acalypha Wilkisenia*. It also acts as a little animal's microhabitat.

2.1.TAXONOMY

KINGDOM: Plantae

PHYLUM: Magnoliophyta

CLASS: Magnoliopsida(Dicotyledoneae)

ORDER: Malpighiales

FAMILY: Euphorbiaceae

GENUS: Acalypha

SEPCIES: WILKESIANA

Life cycle: ANNUAL

Origin: Fiji and the Pacific islands

Distribution: Africa and Asia and America

2.2 NUTRITIONAL VALUE

Acalypha Wilkisenia leaves are nutritionally rich, containing essential proteins, vitamins such as (Vitamin A and C), minerals which include (calcium,iron and magnesium),dietary fibres and a few bioactive compounds like flavonoids,tannins, sapponins and phelonics these contribute to its anti-oxidant, anti-inflammatory and its anti-caancer properties (Adedapo *et al*., 2009)

2.3 MEDICINAL BENEFITS

The medicinal herb *Acalypha wilkisenia* is said to have been utilized to cure a wide variety of illnesses. Therefore, research into the molecular causes of its therapeutic effectiveness is essential. Numerous antioxidants and phytochemicals with antibacterial and antifungal activities are found in *A. wilkisenia*.

- Antimicrobial properties; Several studies have demonstrated the potent antimicrobial properties of Acalypha wilkisenia. The plant's extracts have been tested against several bacterial pathogens, demonstrating noteworthy antibacterial activity. The methanolic extract of Acalypha wilkesiana, for example, has been found to inhibit the growth of Staphylococcus aureus and Escherichia coli, among other bacteria. This antimicrobial efficacy is attributed to the presence of bioactive compounds like flavonoids, tannins, and saponins, which disrupt microbial cell membranes and inhibit enzyme activities essential for bacterial survival (Okaiyeto and Oguntibeju, 2021).

- Antioxidant Activity; Acalypha wilkesiana has been shown to possess substantial antioxidant properties. The plant's extracts have been observed to scavenge free radicals, thereby reducing oxidative stress. This antioxidative potential is largely due to its rich phytochemical composition, including phenolic compounds and flavonoids, which are known for their ability to donate electrons and neutralize free radicals. Studies have highlighted the strong correlation between these compounds and the plant's antioxidant capacity, suggesting its potential use in managing oxidative stress-related conditions (Okaiyeto and Oguntibeju, 2021).

- Anti-inflammatory and Analgesic Effects; The anti-inflammatory and analgesic effects of *Acalypha wilkisenia* have been validated through various studies. The plant has been observed to reduce inflammation and alleviate pain in animal models, corroborating its traditional use for managing conditions like arthritis (Iwalewa *et al*., 2014).

- Antidiabetic and Antihypertensive Benefits;. It has been shown from different researches that the plant's extracts significantly reduce blood glucose levels in diabetic rats, indicating its potential in diabetes management. Furthermore it also shows that the plants extract helps to reduce high blood pressure, supporting its use in treating hypertension (Akinmoladun *et al*., 2016).

2.4 PHYTOCHEMISTRY

The phytochemical analysis of the aqueous leaf extract of *Acalypha wilkisenia* showed a significant presence of analysis has identified the presence of flavonoids, tannins, saponins, alkaloids, glycosides, and phenolic acids in the plant (Idu *et al*., 2016) .

2.4.1 ANTI-CARCIONOGENIC EFFECTS OF ALCALYPHA WILKISENIA

Acalypha wilkisenia has been identified as a plant with notable medicinal benefits, particularly in its potential to combat cancer. A research revealed that extracts from *A. wilkisenia* exhibit considerable cytotoxic effects against various types of cancer cells, indicating the plant's capability to prevent the growth of these cells. The study attributes the anticarcinogenic effects to bioactive compounds such as flavonoids, alkaloids, and saponins present in the plant (Nworu *et al*., 2018).

Subsequent research showed additional insights into the mechanisms behind *A. wilkisenia's* anticancer properties. Their findings showed that the plant extracts trigger apoptosis in cancer cells through the mitochondrial pathway, indicating a specific method of action. Moreover, the study highlighted the antioxidant properties of the extracts, which help mitigate oxidative stress—a factor known to contribute to cancer development. These results suggest that *A. wilkisenia* could be an important natural resource for new anticancer drug development (Adeyemi *et al*., 2020) .

2.4.2 FLAVONOIDS(2-phenyl-2-3-dihydrochromen-4-one):

Flavonoids are a class of polyphenolic compounds, are known for their diverse biological activities. In *Acalypha wilkisenia*, notable flavonoids include quercetin and kaempferol.

- Quercetin: This has been well-documented for its antioxidant capabilities, which involves neutralizing free radicals and protecting cells from oxidative damage. Additionally, quercetin also exhibits anti-inflammatory effects by suppressing the production of pro-inflammatory cytokines.

- Kaempferol: Known as another flavonoid found in *A. wilkisenia*, it has demonstrated potential in preventing cancer cell proliferation and inducing cell death For example, research indicates that kaempferol can cause cell cycle arrest and initiate apoptosis in various cancer cell lines (Okwu and Omodamiro, 2014).

2.4.3 ALKALOIDS:

Alkaloids are nitrogen-containing compounds that exert significant physiological effects on humans and animals. They are known for their analgesic, anti-inflammatory, and antimalarial properties. In *Acalypha wilkisenia*, alkaloids such as indole alkaloids and pyrrolizidine alkaloids have been identified:

- Indole Alkaloids; They are known for their antitumor and antimicrobial activities. They interfere with DNA replication in cancer cells, leading to cell death. Indole alkaloids also possess antimicrobial properties, making them effective against a variety of pathogens. Their presence in *Acalypha wilkisenia* contributes to its potential in cancer treatment and infection control (Cushnie *et al*., 2014).

- Pyrrolizidine Alkaloids; These are another class of alkaloids found in some species of the Acalypha genus. These alkaloids can have both therapeutic and toxic effects, depending

on the dosage and mode of administration. They are known for their hepatotoxicity, have also shown potential in anticancer research (Fu *et al*., 2015).

The presence of alkaloids in *Acalypha wilkisenia* enhances its pharmacological potential. These compounds provide a range of therapeutic benefits, from antitumor and antimicrobial effects to analgesic and anti-inflammatory properties, supporting the plant's traditional use in treating pain, infections, and inflammation.

2.4.4 SAPONINS

Saponins are glycosides with soap-like properties that play a crucial role in enhancing immune responses. In *Acalypha wilkisenia*, saponins exhibit antitumor activities by modulating the immune system and inducing apoptosis in cancer cells. Saponins in *A. wilkisenia* enhance the cytotoxicity of immune cells against cancer cells, aiding in tumor suppression. Furthermore, saponins can increase cancer cell membrane permeability, facilitating the entry of chemotherapeutic agents and enhancing their efficacy (Adeoye *et al*., 2020). In *A. wilkisenia* , sapponins such as tritipenoid sapponins and steroidal sapponins have been identified .

- Triterpenoid saponins: uch as those found in this plant, are known for their immune-enhancing and anti-inflammatory effects. These compounds can induce apoptosis in cancer cells, thereby exhibiting antitumor activities (Adeoye *et al*., 2020).

- Steroidal saponins: These are also present in *A. wilkisenia*, are recognized for their ability to lower cholesterol levels and boost immune responses (Siddiqui *et al*., 2017).

Saponins in *Acalypha wilkisenia* enhance its medicinal value by providing a range of health benefits, including anticancer and immune-boosting effects, as well as cholesterol-lowering properties. These benefits support the plant's traditional use in various therapeutic applications,

2.4.5 TANNINS

Tannins are polyphenolic compounds with strong astringent properties. In *Acalypha wilkisenia*, tannins contribute to its antimicrobial activities. These compounds can bind to proteins and other organic substances, forming stable complexes that inhibit the growth of pathogens. Research highlighted the effectiveness of tannins from *A. wilkisenia* in combating bacterial and fungal infections. For example, tannins can disrupt bacterial cell walls, leading to bacterial death and preventing infection spread (Parekh and Chanda, 2017).

2.5 CANCER

Cancer is a complex group of diseases characterized by uncontrolled cell division and the ability of these cells to invade other tissues. This disease begins when genetic mutations occur within a cell, leading to rapid and uncontrolled proliferation. Unlike normal cells, cancer cells do not respond to the regulatory mechanisms that maintain cell division and growth, allowing them to grow and divide uncontrollably (Hanahan and Weinberg, 2014).

One of the hallmarks of cancer is its ability to form tumors, which are masses of abnormal cells. These tumors can be benign (non-cancerous) or malignant (cancerous). Malignant tumors have the ability to invade surrounding tissues and spread to other parts of the body through a process known as metastasis. During metastasis, cancer cells break away from the original tumor, travel through the bloodstream or lymphatic system, and form new tumors in other organs or tissues (Siegel *et al*., 2020).

Cancer development involves multiple stages, including initiation, promotion, and progression. In the initiation stage, genetic mutations occur, often as a result of errors during DNA replication or exposure to carcinogens. During promotion, these mutated cells begin to proliferate abnormally, often influenced by factors such as inflammation or hormonal changes. Progression is characterized by further genetic alterations and changes in cell behavior, leading to increased malignancy and the ability to metastasize (Vogelstein *et al*., 2013).

It was emphasized that the significant association between diets heavy in red meat and processed foods and a higher risk of colorectal cancer. Therefore, comprehending cancer necessitates a thorough analysis of both hereditary susceptibilities and environmental factors, highlighting the intricate and heterogeneous character of the illness (Keum and Giovannucci, 2019).

2.5.1 CAUSES

Cancer arises from a confluence of genetic, environmental, and lifestyle factors. Genetic mutations, which can be inherited or acquired, are at the heart of cancer development. These mutations can occur due to errors during cell division or in response to environmental carcinogens. Researchers pointed out that such mutations disrupt normal cellular functions, providing cancer cells with the ability to grow uncontrollably, evade death signals, and invade other tissues. Environmental factors, including exposure to carcinogens such as tobacco smoke, ultraviolet (UV) radiation, and certain chemicals, significantly increase the risk of cancer (Henderson *et al*., 2014). Tobacco smoke, for instance, is responsible for about 22% of cancer deaths globally, illustrating the profound impact of environmental carcinogens on cancer incidence (Siegel *et al*., 2020).

2.5.2 TYPES

Cancer is classified into several major types based on the origin of the abnormal cells. Carcinomas, the most common type, arise from epithelial cells that line organs and tissues and include cancers such as breast, lung, and colorectal cancer (Siegel *et al*., 2020). Sarcomas originate in connective tissues like bones, muscles, and fat, and tend to be more aggressive (Fletcher, 2014). Leukemia's are cancers of the blood and bone marrow, characterized by the excessive production of abnormal white blood cells. Lymphomas, including Hodgkin and non-Hodgkin lymphoma, originate in the lymphatic system, affecting the lymphocytes, a type of white blood cell (Swerdlow *et al*., 2016). Other types include melanomas, arising from melanocytes in the skin, and central nervous system cancers, developing in the brain and spinal cord (Louis *et al*., 2016).

2.5.3 SYMPTOMS AND SIGNS

The symptoms and signs of specific cancer types in young adults closely resemble those observed in both younger and older patients (Bleyer, 2009). These manifestations can vary based on the cancer type and tumor location, with some shared symptoms.

1. Change in bowel habits, such as diarrhea, constipation, or narrowing of the stool

2. Rectal bleeding

3. Abdominal pain, cramps or bloating

4. Unexplained weight loss

5. Constant fatigue

6. Weakness

7. Lump in Brest and other part of the body

8. Difficulty swallowing

8. Difficulty swallowing

9. Persistent caugh

10. Headaches

11. Vision cha10. Headaches

11. Vision changes

12. Change in the appearance of a mole or other skin lesion.

Research indicates that while individuals with cancer commonly exhibit these symptoms, it's important to note that not everyone experiencing these symptoms necessarily has cancer. The prevalence and distribution of cancer types differ significantly among young adults compared to both younger and older individuals, resulting in distinct patterns of symptoms and signs (Bleyer, 2009). Early detection plays a crucial role in the effective treatment of cancer.

2.5.4 CANCER PREVENTION

Preventing cancer involves a combination of lifestyle modifications, early detection, and preventive medical treatments. Adopting a healthy diet rich in fruits, vegetables, whole grains, and lean proteins while avoiding processed foods and red meat can significantly reduce cancer risk. Regular physical activity and maintaining a healthy weight are also crucial, as obesity is

linked to an increased risk of various cancers (Kushi *et al*., 2019). Avoiding known carcinogens, such as tobacco and excessive alcohol, and protecting the skin from excessive sun exposure are critical preventive measures. Furthermore, regular screenings and vaccinations, like those for human papillomavirus (HPV) and hepatitis B, can prevent cancers associated with these infections (Jemal *et al*., 2018).

2.6 COLORECTAL CANCER

Colorectal cancer (CRC) remains a significant public health concern, being one of the most prevalent forms of cancer globally. This malignancy arises from the inner lining of the colon or rectum, often beginning as benign adenomatous polyps which can progressively transform into invasive cancer over time (Siegel *et al*., 2020).

The imncidence of colorectal cancer varies worldwide, with higher rates observed in developed countries. This geographic variation is largely attributed to differences in diet, lifestyle, and screening practices. CRC is the third most common cancer diagnosed in both men and women in the United States, with significant mortality rates despite advancements in detection and treatment (Arnold *et al*., 2017). Key risk factors include age, with most cases occurring in individuals over 50, a family history of CRC, inherited conditions such as Lynch syndrome and familial adenomatous polyposis, and lifestyle factors such as a diet high in red and processed meats, physical inactivity, obesity, smoking, and heavy alcohol use (World Cancer Research Fund, 2018).

The development of colorectal cancer is a multistep process involving genetic and epigenetic alterations. The adenoma-carcinoma sequence describes the progression from normal epithelium

to adenoma to carcinoma, which involves the accumulation of mutations in oncogenes, tumor suppressor genes, and genes involved in DNA repair.

Early detection of colorectal cancer significantly improves prognosis. Screening methods include fecal occult blood testing (FOBT), fecal immunochemical testing (FIT), and colonoscopy. Colonoscopy remains the gold standard for CRC screening, allowing for direct visualization of the colon and rectum and the ability to remove polyps during the procedure (Levin et al., 2018). Other diagnostic tools include sigmoidoscopy, double-contrast barium enema, and CT colonography. Once a diagnosis of CRC is suspected, biopsy and histopathological examination confirm the presence of cancer. Imaging studies such as CT and MRI scans are employed to determine the extent of disease spread (stage).

Staging of colorectal cancer is performed using the TNM system, which assesses tumor size and extent (T), involvement of regional lymph nodes (N), and the presence of distant metastasis (M). The stages range from I (early-stage) to IV (advanced-stage), with prognosis worsening with higher stages. Stage I CRC is localized, stage II involves the wall of the colon or rectum, stage III involves regional lymph nodes, and stage IV indicates distant metastasis, often to the liver, lungs, or peritoneum (Edge et al., 2010). The five-year survival rate for localized CRC is approximately 90%, but it drops to about 14% for metastatic disease (Siegel et al., 2020).

Lifestyle factors such as diet, physical inactivity, and obesity, alongside genetic predispositions, significantly influence CRC risk (Chan and Giovannucci, 2019). These findings underscore the importance of preventive strategies targeting modifiable risk factors to mitigate the rising incidence of CRC globally.

Colorectal cancer is a frequently preventable malignancy, and with the widespread implementation of colon cancer screening in developed countries, there has been a decline in the incidence and mortality of colon cancer in targeted populations. Unfortunately, in the last 25 years, there has been a rising trend in the incidence and mortality of colorectal cancer (CRC) among young adults under the age of 50 (Ahmed, 2020).

2.6.1 RISK FACTORS

Colorectal cancer (CRC) is influenced by a variety of risk factors, including both genetic and environmental elements. Understanding these risk factors is crucial for prevention and early detection strategies.

- Age: Age is a significant risk factor for CRC, with the incidence increasing sharply after the age of 50 (Chan and Giovannucci, 2019). This age-related risk underscores the importance of regular screening, particularly for individuals aged 50 and older.

- Family History and Genetic Predisposition:Individuals with a family history of CRC, especially in first-degree relatives, have an elevated risk of developing the disease (Chan and Giovannucci, 2019). Moreover, specific genetic syndromes, such as familial adenomatous polyposis (FAP) and Lynch syndrome, significantly increase the risk of CRC (Dienstmann *et al*., 2019).

- Inflammatory Bowel Disease (IBD): Individuals with inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, have an elevated risk of developing CRC, particularly with long-standing and extensive disease involvement (Chan and Giovannucci, 2019).

- Smoking: Smoking is a well-established risk factor for CRC, with current smokers having a higher risk compared to former or non-smokers (Chan and Giovannucci, 2019). The carcinogens present in tobacco smoke can promote the development of CRC through various mechanisms.

- Obesity and Physical Inactivity: Obesity and physical inactivity are associated with an increased risk of CRC (Chan and Giovannucci, 2019). The mechanisms underlying this association may involve insulin resistance, chronic inflammation, and alterations in hormone levels.

2.6.2 SYMPTOMS

Colorectal cancer (CRC) manifests with various symptoms, although in early stages, it may be asymptomatic. As the disease progresses, symptoms can include changes in bowel habits such as diarrhea, constipation, or narrowing of stools, which may persist over time (Chan and Giovannucci, 2019).

The symptoms may include the following;

- Rectal bleeding: Also known as blood in the stool , is a common symptom and may present as bright red blood or as dark, tarry stools indicative of bleeding higher up in the digestive tract.
- Abdominal discomfort, pain, or cramping: This is often localized in the lower abdomen, may occur and may be accompanied by bloating or a feeling of fullness.
- Unexplained weight loss
- Fatigue,
- Weakness

These are non-specific symptoms that may signal advanced disease and warrant further evaluation.

In addition to these symptoms, CRC can present with more alarming signs such as:

- the presence of a palpable mass in the abdomen or rectum: This may indicate advanced disease with tumor growth
- Bowel obstruction: This characterized by severe abdominal pain, distension, and an inability to pass stool or gas, can occur as a late complication of CRC.

Though some of these symptoms may overlap with benign conditions, their persistence or worsening over time should prompt medical evaluation, particularly in individuals with known risk factors for CRC. Early detection and diagnosis are crucial for initiating timely treatment and improving outcomes for CRC patients.

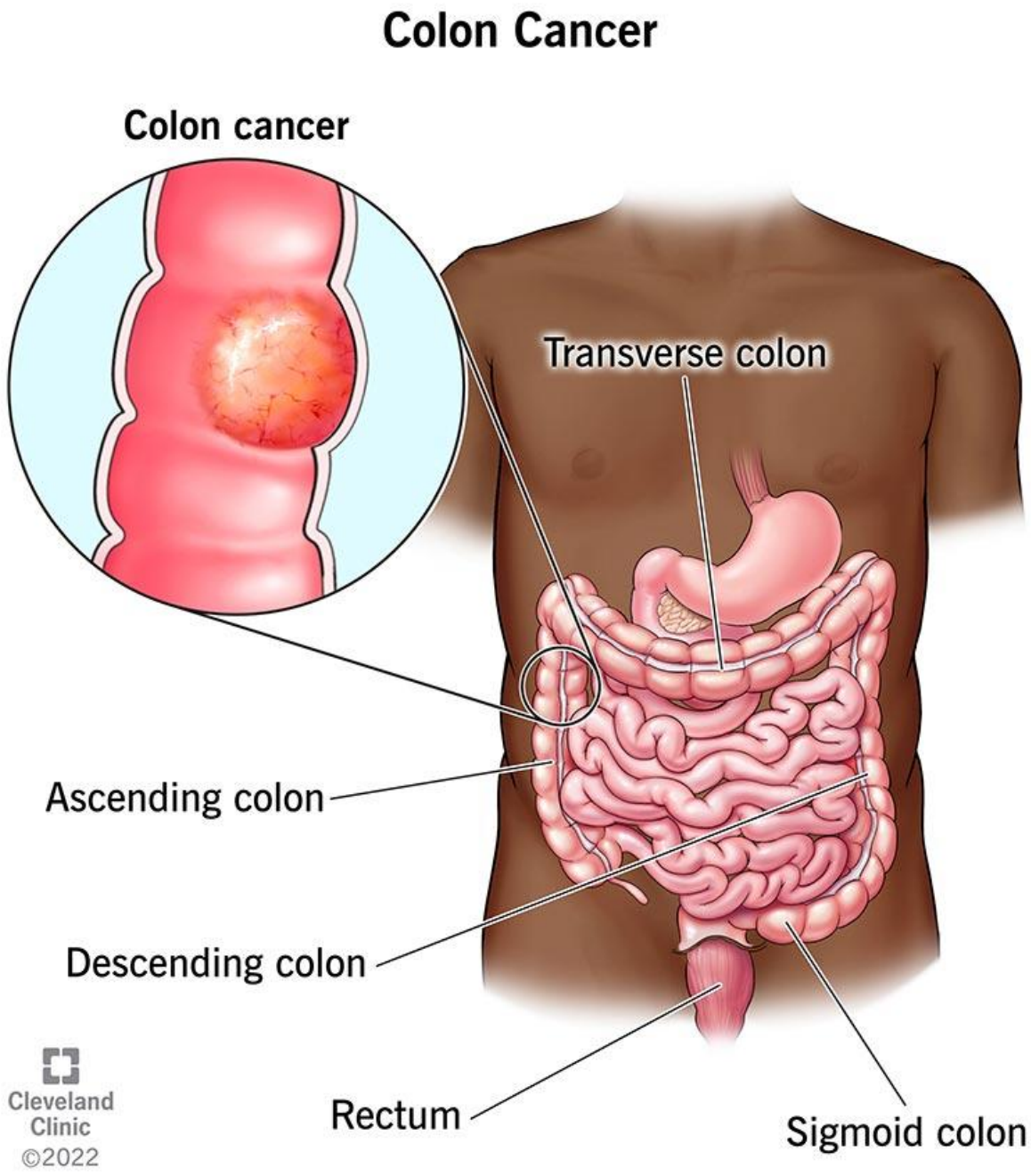


Fig 2.1. An image of the colon (Chan and Giovannucci, 2019).

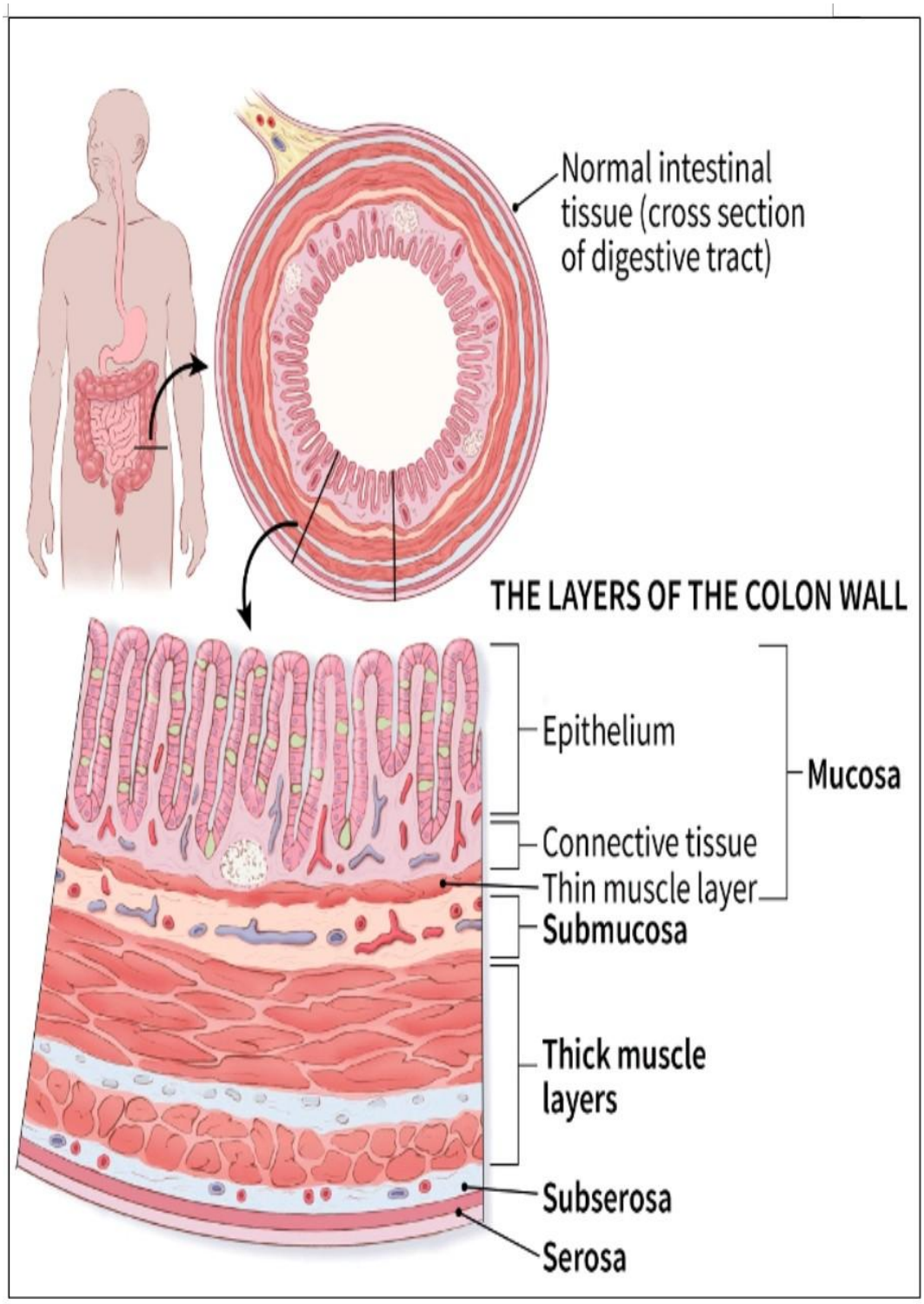


Fig.2.2: Layers of the Colon wall (American Cancer Society, 2020).

2.6.3. CURRENT TREATMENT

Treatment options for colorectal cancer depend on the stage of the disease, the patient's overall health, and specific molecular characteristics of the tumor. Surgery is often the primary treatment for localized CRC. In early-stage disease, minimally invasive techniques such as laparoscopic surgery may be used. For more advanced stages, a colectomy or proctectomy, involving the removal of part of the colon or rectum along with nearby lymph nodes, is typically performed (Van Cutsem *et al*., 2016). Adjuvant chemotherapy is standard for stage III and high-risk stage II CRC to eliminate residual microscopic disease. Common chemotherapeutic agents include fluoropyrimidines (e.g., 5-fluorouracil), oxaliplatin, and irinotecan. For metastatic CRC, combination chemotherapy regimens such as FOLFOX (folinic acid, fluorouracil, and oxaliplatin) or FOLFIRI (folinic acid, fluorouracil, and irinotecan) are commonly used (Van Cutsem *et al*., 2016).

Advances in the understanding of molecular pathways in CRC have led to the development of targeted therapies. Drugs like bevacizumab, an anti-VEGF antibody, and cetuximab and panitumumab, anti-EGFR antibodies, are used in combination with chemotherapy for metastatic CRC. These agents inhibit specific pathways involved in tumor growth and angiogenesis (Heinemann *et al*., 2018). Immunotherapy has emerged as a promising treatment for certain subsets of colorectal cancer. Pembrolizumab and nivolumab, immune checkpoint inhibitors targeting PD-1, have shown efficacy in MSI-high or mismatch repair-deficient CRC by enhancing the body's immune response against cancer cells (Overman *et al*., 2017). Radiation therapy is commonly used in rectal cancer, either preoperatively to shrink tumors or

postoperatively to eliminate residual cancer cells. It is often combined with chemotherapy for a synergistic effect (National Comprehensive Cancer Network, 2020).

Prevention strategies for colorectal cancer focus on lifestyle modifications and regular screening. A diet high in fruits, vegetables, and whole grains and low in red and processed meats can reduce the risk. Regular physical activity, maintaining a healthy weight, avoiding smoking, and limiting alcohol consumption are also recommended preventive measures (Kushi *et al*., 2019). Regular screening starting at age 50 (or earlier for those at high risk) is crucial for early detection and removal of precancerous polyps, significantly reducing the incidence and mortality of CRC (Levin *et al*., 2018).

In summary, treatment options for colon cancer encompass a multidisciplinary approach, incorporating surgery, chemotherapy, targeted therapy, and immunotherapy, tailored to the individual patient's disease characteristics and stage. Advances in treatment modalities have significantly improved outcomes for patients with colon cancer, highlighting the importance of ongoing research and innovation in the field.

2.7 OXIDATIVE STRESS

Oxidative stress occurs due to an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to counteract their harmful effects through antioxidant defenses (Halliwell , 2006). ROS, including superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), are natural byproducts of cellular metabolism, but their overproduction or insufficient neutralization can lead to oxidative damage to cellular components such as lipids, proteins, and DNA.

Lipid peroxidation, is one of the consequences of Oxidative stress, and it occurs when ROS attacks lipid molecules in cell membranes, resulting in the production of reactive lipid species

and products such as Malondialdehyde (MDA) (Ayala *et al* ., 2014). These lipid peroxidation products may lead to disruption of membrane integrity and function, thereby contributing to cellular dysfunction and disease progression. Protein oxidation is, another hallmark of oxidative stress which involves the modification of amino acid residues in proteins by ROS, leading to structural alterations and impaired protein function (Dalle-Donne *et al*., 2006).

Furthermore, Oxidative stress-induced DNA damage poses a significant threat to genomic stability and cellular homeostasis. ROS can directly attack DNA molecules, causing DNA strand breaks, base modifications, and DNA-protein crosslinks (Cooke *et al*., 2003). These DNA lesions can then lead to mutations, chromosomal aberrations, and genomic instability, which then contributes to the development of cancer and other age-related diseases. Additionally, oxidative stress-induced DNA damage can interfere with critical cellular processes such as DNA replication, transcription, and repair, further exacerbating cellular dysfunction and disease progression (Jackson and Bartek , 2009).

Moreover, oxidative stress influences cellular signaling pathways involved in the regulation of key cellular processes, including proliferation, differentiation, and apoptosis. ROS serve as signaling molecules in redox-sensitive signaling pathways, modulating the activity of transcription factors, protein kinases, and phosphatases (Sena and Chandel , 2012). Dysregulation of redox signaling pathways by oxidative stress can disrupt cellular homeostasis and contribute to the development of inflammation, metabolic disorders, and cancer. Glutathione peroxidase (GPx), an important antioxidant enzyme, plays a crucial role in neutralizing hydrogen peroxide and lipid peroxides, thereby protecting cells from oxidative damage (Brigelius-Flohe and Maiorino, 2013).

Oxidative stress exerts a wide range of biological effects on cells and tissues, including lipid peroxidation, protein oxidation, DNA damage, and modulation of cellular signaling pathways. Understanding the mechanisms underlying oxidative stress-mediated damage is crucial for elucidating its role in disease pathogenesis and developing strategies for therapeutic intervention.

2.7.1 OXIDATIVE STRESS MARKERS

Oxidative stress markers encompass a diverse array of molecules, including reactive species themselves , products of oxidative damage to lipids, proteins, and nucleic acids, as well as antioxidant enzymes and non-enzymatic antioxidants. These markers are measured using various analytical techniques, such as spectrophotometry, chromatography, and immunoassays, allowing researchers to assess oxidative stress levels in biological samples with high precision and sensitivity.

Among the most widely studied oxidative stress markers are :

- Malondialdehyde (MDA): A byproduct of lipid peroxidation.
- Glutathione peroxidase (GPx): A key antioxidant enzyme.
- Superoxide dismutase (SOD): This catalyzes the dismutation of superoxide radicals.
- Glutathione (GSH): A major intracellular antioxidant.

These markers provide valuable information about the extent of oxidative damage, antioxidant defense mechanisms, and redox signaling pathways implicated in various physiological and pathological processes.

2.7.2 MALONDIALDEHYDE (MDA)

Malondialdehyde (MDA), is derived from the peroxidation of polyunsaturated fatty acids, which serves as a biomarker of Oxidative stress-induced lipid damage. In the context of colon cancer, where oxidative stress plays a pivotal role in tumor initiation and progression, MDA emerges as a key player in elucidating the mechanistic underpinnings of carcinogenesis (Ayala *et al*., 2014).

The accumulation of MDA in colon cancer tissues reflects the heightened oxidative burden within the tumor microenvironment. ROS-mediated lipid peroxidation generates MDA, this results in the formation of DNA adducts and protein modifications that contribute to genomic instability, oncogene activation, and tumor suppressor gene inactivation (Reuter *et al*., 2020).

The dysregulation of MDA levels not only fuels tumor growth and metastasis but also influences therapeutic responses and clinical outcomes in colon cancer patients. Elevated MDA levels correlate with tumor aggressiveness, metastatic potential, and resistance to chemotherapy and radiation therapy, underscoring its prognostic significance (Bian *et al*., 2019).

However, despite its detrimental effects, MDA also represents a potential therapeutic target in colon cancer management. Strategies aimed at reducing MDA levels or inhibiting its downstream signaling pathways hold promise for overcoming therapeutic resistance and improving patient outcomes (Chen *et al*., 2022).

MDA emerges as a multifaceted biomarker in colon cancer research, serving not only as an indicator of oxidative stress-induced damage but also as a mediator of tumorigenesis, inflammation, and therapeutic resistance.

2.7.3 Biochemistry of MDA

Malondialdehyde (MDA) is a low-molecular-weight aldehyde, a byproduct of polyunsaturated fatty acid (PUFA) peroxidation, which occurs during lipid peroxidation. Lipid peroxidation is a chain reaction mechanism leading to the oxidative degradation of lipids, prominently involving cellular membranes. This process is initiated by free radicals, particularly reactive oxygen species (ROS), which abstract hydrogen atoms from the methylene groups in PUFAs, creating lipid radicals. These lipid radicals react with molecular oxygen to form peroxyl radicals, which propagate the chain reaction, ultimately producing various aldehydes, including MDA (Niki, 2014).

MDA is considered a secondary product of lipid peroxidation and is relatively stable compared to other lipid peroxides. Its formation signifies the extent of lipid peroxidation and subsequent cell membrane damage, playing a crucial role in the pathophysiology of numerous diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Del Rio *et al*., 2005).

2.7.4 Relevance as a Marker for Oxidative Stress

Oxidative stress occurs when there is an imbalance between the production of ROS and the capability of the body to detoxify these reactive intermediates or repair the resulting damage. As an end product of lipid peroxidation, MDA is widely used as a biomarker for oxidative stress. Elevated levels of MDA are indicative of increased lipid peroxidation, reflecting oxidative damage to cell membranes and other lipid-containing structures within the cells (Ayala *et al*., 2014).

In the context of cancer, oxidative stress is implicated in all stages of carcinogenesis, including initiation, promotion, and progression. The oxidative damage to DNA, proteins, and lipids contributes to mutagenesis, genome instability, and alterations in cell signaling pathways that favor tumor growth and metastasis. Thus, monitoring MDA levels provides insights into the oxidative state of the organism and the extent of oxidative damage occurring during cancer development and progression (Reuter *et al*., 2010).

2.7.5 Studies Correlating MDA Levels with Tumor Progression

Numerous studies have demonstrated a correlation between elevated MDA levels and tumor progression. For instance, studies have shown that patients with various types of cancer, including breast, lung, and colorectal cancers, exhibit significantly higher levels of MDA compared to healthy controls. This elevation is linked to enhanced lipid peroxidation and oxidative stress in cancerous tissues (Gago-Dominguez *et al*., 2017).

- A study on colorectal cancer patients reported that serum MDA levels were significantly higher in patients with advanced-stage cancer compared to those with early-stage disease. The increased MDA levels were associated with more aggressive tumor behavior and poorer prognosis, suggesting that MDA could serve as a prognostic marker in colorectal cancer (Kharb *et al*., 2016).

- Another study investigating the role of oxidative stress in breast cancer found that patients with breast cancer had elevated MDA levels in their plasma, which correlated with tumor size and stage. The findings indicated that oxidative stress markers, including

MDA, could potentially be used to assess disease progression and therapeutic response (Fang *et al*., 2017).

2.7.6 Methods for Quantifying MDA in Biological Samples

Quantifying MDA in biological samples is essential for assessing oxidative stress levels. Several analytical methods are employed for this purpose, each with its advantages and limitations:

1. **Thiobarbituric Acid Reactive Substances (TBARS) Assay**:

Principle: The TBARS assay is one of the most commonly used methods for measuring MDA. It is based on the reaction of MDA with thiobarbituric acid (TBA) to form a pink chromogen, which can be quantified spectrophotometrically.

Procedure: Samples are mixed with TBA reagent and heated, typically at 90-100°C for 15-60 minutes. The absorbance of the resulting chromogen is measured at 532 nm.

Advantages: Simple and relatively inexpensive.

Limitations: Lacks specificity as other aldehydes can also react with TBA, potentially leading to overestimation of MDA levels (Del Rio *et al*., 2005).

2. **High-Performance Liquid Chromatography (HPLC):**

Principle: HPLC offers a more specific and accurate measurement of MDA by separating it from other interfering substances before detection.

Procedure: Biological samples are derivatized with 2,4-dinitrophenylhydrazine (DNPH) or TBA, and the MDA derivative is separated using an HPLC system equipped with a suitable detector (UV, fluorescence).

Advantages: Higher specificity and sensitivity compared to the TBARS assay.

Limitations: Requires expensive equipment and skilled personnel (Agrawal *et al*., 2015).

3. **Gas Chromatography-Mass Spectrometry (GC-MS):**

Principle: GC-MS allows for the precise and accurate quantification of MDA by separating volatile derivatives of MDA and detecting them using mass spectrometry.

Procedure: Samples are derivatized (e.g., with pentafluorobenzyl bromide) and analyzed using a GC-MS system.

Advantages: Highly specific and sensitive.

Limitations: High cost and complexity of the method (Minami and Yoshikawa, 1979).

4. **Enzyme-Linked Immunosorbent Assay (ELISA):**

Principle: ELISA kits specifically designed to measure MDA levels use antibodies that bind to MDA-adducts.

Procedure: Samples are added to microplate wells coated with MDA-specific antibodies, followed by the addition of enzyme-linked secondary antibodies and substrate for color development.

Advantages: High throughput, specificity, and ease of use.

Limitations: Potential for variability between different assay kits and manufacturers (Jentzsch *et al*., 1996).

MDA serves as a crucial biomarker for oxidative stress due to its stable presence as an end product of lipid peroxidation. Elevated levels of MDA are strongly correlated with the progression of various cancers, making it a valuable indicator of disease state and oxidative damage. Accurate quantification of MDA in biological samples is essential for research and clinical applications, with methods such as TBARS assay, HPLC, GC-MS, and ELISA offering various levels of specificity and sensitivity. As research continues, improved methodologies and a deeper understanding of MDA's role in oxidative stress and cancer will enhance its utility in diagnostics and therapeutic monitoring.

2.8.0 GLUTATHIONE PEROXIDASE (GPx)

Glutathione peroxidase (GPx) is a crucial antioxidant enzyme that plays a central role in protecting cells from oxidative damage by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides, thus maintaining cellular redox balance (Brigelius-Flohé and Maiorino, 2013). In the context of colon cancer, where oxidative stress is implicated in tumor initiation, progression, and therapy resistance, GPx serves as a critical sentinel of oxidative stress dynamics within the tumor microenvironment.

Numerous studies conducted by researchers have consistently demonstrated alterations in GPx activity in colon cancer. Decreased GPx expression and activity have been observed in colon cancer tissues and then compared to adjacent normal tissues, reflecting compromised antioxidant defense mechanisms and heightened oxidative stress levels in tumor cells (Zhang *et al*., 2015; Zhou *et al*., 2018).

The dysregulation of GPx in colon cancer not only exacerbates oxidative damage to cellular macromolecules, including lipids, proteins, and DNA, but also fuels tumor progression and

metastasis. GPx deficiency contributes to genomic instability, oncogene activation, and tumor suppressor gene inactivation, thereby facilitating the acquisition of malignant phenotypes (Guo *et al*., 2021).

The clinical implications of GPx dysregulation in colon cancer are profound. Altered GPx levels correlate with tumor aggressiveness, metastatic potential, and resistance to conventional chemotherapy and radiation therapy, underscoring its prognostic value and therapeutic significance (Bian *et al*., 2019).

Despite its role as a marker of oxidative stress, GPx represents a promising therapeutic target in colon cancer management. Strategies aimed at restoring GPx activity or enhancing its expression offer potential avenues for mitigating oxidative stress-induced damage, overcoming therapeutic resistance, and improving patient outcomes (Zhou *et al*., 2018; Chen *et al*., 2022).

2.8.1 Biochemistry of GPx

Glutathione peroxidase (GPx) is a vital enzyme in the antioxidant defense system, primarily involved in converting hydrogen peroxide (H_2O_2) and lipid hydroperoxides into water (H_2O) and corresponding alcohols, utilizing glutathione (GSH) as a substrate. During this reaction, GSH is oxidized to glutathione disulfide (GSSG). This process is essential for protecting cells from oxidative damage by detoxifying harmful peroxides produced during cellular metabolism (Brigelius-Flohé and Maiorino, 2013).

GPx exists in multiple isoforms, each with distinct roles and tissue distributions and they include:

- GPx1: The most common isoform, present in the cytoplasm and mitochondria of almost all cell types.
- GPx2: Predominantly found in the gastrointestinal tract.
- GPx3: Located in plasma and extracellular fluids.
- GPx4: Capable of reducing phospholipid hydroperoxides, found in various tissues, including testes and brain (Lubos *et al*., 2011).

2.8.2 Role of GPx in Cancer Research

GPx is crucial in maintaining cellular redox balance, which is essential for cancer prevention and treatment.

- **Maintenance of Cellular Redox Balance**: GPx helps maintain the cellular redox state by neutralizing harmful peroxides, thus preventing oxidative damage to DNA, proteins, and lipids, which can lead to mutations and cancer development (Brigelius-Flohé and Maiorino, 2013).

By regulating redox homeostasis, GPx influences various cellular signaling pathways involved in cell proliferation, apoptosis, and differentiation, which are often disrupted in cancer (Harris *et al*., 2015).

- **Tumor Suppression**:

Increased GPx activity is associated with lower tumor incidence and slower progression. GPx reduces oxidative stress, which is a critical factor in cancer initiation and progression (Klaunig *et al*., 2018).

Studies have shown that overexpression of GPx1 in transgenic mice decreases susceptibility to chemically induced tumors, highlighting its protective role against cancer development (Zhang *et al*., 2018).

2.8.3 Studies Correlating GPx Activity with Tumor Suppression

Several studies have shown a correlation between GPx activity and tumor suppression. Below are a few examples:

- **Breast Cancer:** Higher GPx1 expression in breast cancer patients is linked to better prognosis and less aggressive tumors. This protective effect is due to GPx1's role in reducing oxidative stress in the tumor microenvironment (Bera *et al*., 2016).

- **Lung Cancer:** In non-small cell lung cancer (NSCLC) patients, higher GPx3 levels correlate with longer overall survival rates. GPx3 suppresses tumor cell proliferation and enhances apoptosis, making it a potential prognostic biomarker (Li *et al*., 2017).

- **Colorectal Cancer:** Low GPx2 expression in colorectal cancer patients is associated with increased tumor progression and poor survival. GPx2 is crucial for maintaining intestinal homeostasis and protecting against inflammation-induced cancer (Zhang *et al*., 2018).

2.8.4 Measurement Techniques for GPx Activity

Accurate measurement of GPx activity in biological samples is essential for research and clinical diagnostics. Various methods are used, each with specific advantages and limitations and a few of them include :

1. **Spectrophotometric Assay:**

Principle: Measures the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP+ in the presence of glutathione reductase, which correlates with GPx activity.

Procedure: The reaction mixture includes GSH, glutathione reductase, NADPH, and H_2O_2 or a hydroperoxide substrate. The rate of NADPH oxidation is monitored spectrophotometrically.

Advantages: Simple, widely used, and applicable to various sample types.

Limitations: Interference from other thiol-containing compounds and enzymes can affect specificity (Flohé and Günzler, 1984).

2. **Enzyme-Linked Immunosorbent Assay (ELISA):**

Principle: Utilizes GPx-specific antibodies to quantify the enzyme based on antibody-antigen interactions.

Procedure: Samples are added to microplate wells coated with GPx-specific antibodies, followed by enzyme-linked secondary antibodies and a substrate for color development.

Advantages: High specificity and sensitivity, suitable for large-scale studies.

Limitations: Requires standardized kits and can be more expensive compared to other methods (Zhu *et al*., 2014).

3. **High-Performance Liquid Chromatography (HPLC):**

Principle: Separates and quantifies the products of the GPx reaction, such as GSSG.

Procedure: Samples are derivatized and injected into an HPLC system with appropriate detection, such as UV or fluorescence.

Advantages: High specificity and ability to quantify multiple components.

Limitations: Requires specialized equipment and is more time-consuming (Lee *et al*., 2011).

4. **Fluorometric Assay:**

Principle: Uses fluorescent probes that react with GPx substrates or products, allowing measurement of GPx activity via fluorescence.

Procedure: Samples are incubated with a fluorescent probe, and the fluorescence intensity is measured using a fluorometer.

Advantages: High sensitivity and suitable for small sample volumes.

Limitations: Potential interference from other fluorescent substances in the sample (Luo *et al*., 2015).

GPx is an essential enzyme in the antioxidant defense system, crucial for maintaining cellular redox balance and protecting against oxidative damage. Its role in cancer suppression is well-documented, with numerous studies linking higher GPx activity to reduced tumor progression and improved prognosis. Accurate measurement of GPx activity is vital for understanding its function and potential as a therapeutic target, with various methods available to quantify its activity in biological samples. Continued research into GPx will enhance our understanding of its role in cancer biology and its potential in clinical applications.

2.9 1,2-Dimethylhydrazine (DMH)

1,2-Dimethylhydrazine (DMH) is an organic compound widely employed in scientific research to induce colon cancer in experimental animal models. Its usefulness arises from its capacity to closely replicate the pathological and molecular features of human colorectal cancer, making it a valuable tool for investigating the disease's mechanisms and potential treatments.

DMH acts as a potent colon carcinogen primarily through its metabolites. Once administered, DMH undergoes metabolic activation in the liver, forming azoxymethane (AOM), which is further metabolized into methylazoxymethanol (MAM). MAM is then transported to the colon, where it exerts carcinogenic effects by forming DNA adducts, leading to mutations that drive carcinogenesis (Baggio et al., 2017). This metabolic pathway is essential for the carcinogenicity of DMH, as it converts the relatively inert DMH molecule into highly reactive intermediates that directly damage DNA (Manjulatha *et al*., 2018).

A specific series of histological alterations is followed when DMH induces colon cancer in animals. In the beginning, DMH causes aberrant crypt foci (ACF), which are preneoplastic lesions that are clusters of aberrant glandular crypts in the colon mucosa. As with real colorectal cancer, these ACFs can develop into adenomas and then adenocarcinomas with continued exposure, simulating the adenoma-carcinoma sequence (Arora *et al*., 2016). Because of this trend, DMH is a great model to research colorectal cancer in its early stages and find biomarkers for early identification.

Considerable alterations in the colon's inflammatory milieu are also connected to DMH-induced colon cancer. The administration of DMH is associated with increased production of pro-inflammatory cytokines, oxidative stress, and activation of inflammatory signaling pathways

such as NF-κB. Chronic inflammation is a known risk factor for colorectal cancer (Bansal *et al*., 2020). These inflammatory reactions emphasize the intricate relationship between inflammation and the development of cancer by creating a microenvironment that is favorable to tumor growth.

Besides its effects on the colon, DMH has been shown to impact other physiological systems. Studies indicate that DMH administration can alter the composition of the gut microbiota, which plays a crucial role in maintaining intestinal homeostasis and modulating immune responses (Shivappa *et al*., 2021). Dysbiosis induced by DMH may further exacerbate inflammation and promote carcinogenesis, emphasizing the significance of the gut microbiome in colorectal cancer.

DMH has been used in experimental research to shed light on possible treatment and chemopreventive measures for colorectal cancer. In DMH-induced animals, a range of natural substances, dietary treatments, and pharmaceutical medications have been evaluated for their potential to prevent or reduce tumor growth. Curcumin and resveratrol, for instance, are examples of substances having antioxidant qualities that have demonstrated promise in reducing DMH-induced oxidative damage and preventing tumor growth (Bandyopadhyay *et al*., 2017). In a similar vein, the ability of dietary fibers and probiotics to balance the gut microbiota and reduce inflammation has been investigated, potentially reducing the risk of cancer (Kim *et al*., 2019) .

There are two isomers of dimethylhydrazine (DMH): 1,2-dimethylhydrazine and 1,1-dimethylhydrazine. Both are clear, colorless liquids. 1,1-Dimethylhydrazine is used as a fuel for rockets and jets, as well as a plant growth regulator and a feedstock for chemical synthesis. DMH and its metabolite azoxymethane (AOM) are procarcinogens that require metabolic activation to produce DNA-reactive byproducts. The methylation of guanine at the N-7 position in DNA

initiates the mutagenic activity of the alkylating agents DMH and AOM. This methylation causes alkylated guanine to pair with thymine instead of cytosine, leading to base mismatches during DNA replication, resulting in guanine to thymine and cytosine to adenine substitutions. Various metabolic enzymes, particularly xenobiotic-metabolizing enzymes, facilitate the N oxidation and hydroxylation of these procarcinogens, leading to the formation of the final carcinogen, methylazoxymethanol (MAM) (Bansal *et al*., 2020).

Studies have shown that MAM, a reactive metabolite of DMH and AOM, quickly forms methyldiazonium ion, which can alkylate macromolecules in the liver and colon (Shivappa *et al*., 2021). It is proposed that the active metabolite of MAM might be the corresponding aldehyde, as MAM is a substrate for the nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase found in the colon and liver (Kim *et al*., 2019).

These CYP2E1 metabolites are transported to the colon via the bile or bloodstream. However, the toxicity of azoxymethanol doses impacts the liver, cell membranes, and other organelles, evidenced by the release of aspartate and alanine aminotransferases and alkaline phosphatase (Rao *et al*., 2017). The primary pathway involves the hepatic conversion of DMH to AOM and azoxymethanol, followed by glucuronic acid conjugation and biliary excretion (Baggio *et al*., 2017). When the glucuronides reach the colon, bacterial enzymes hydrolyze them, forming an active carcinogen in the colonic lumen (Manjulatha *et al*., 2018).

The history of DMH begins with Laquer's investigation into the neurotoxicity of Cycas circinalis L. seed. Rats fed crude cycad meal extract developed tumors in various organs, including the intestine, liver, and kidney (Arora *et al*., 2016). Laquer also identified that the first metabolite of aglycone cycasin (MAM), a glycoside called cycasin, and a compound called d-

glucosyloxyazoxymethane, were responsible for the tumors in the intestines (Shivappa *et al*., 2021).

Fiala's early research into DMH metabolism and mechanism of action involved examining its metabolites and separating them using column chromatography (Bandyopadhyay et al., 2017). Further studies confirmed that the active carcinogen is transported through the circulatory system rather than the fecal stream (Bansal *et al*., 2020). This was substantiated by research from Campbell et al. and Wittig and Ziebarth, demonstrating the systemic nature of DMH-induced carcinogenesis (Kim *et al*., 2019).

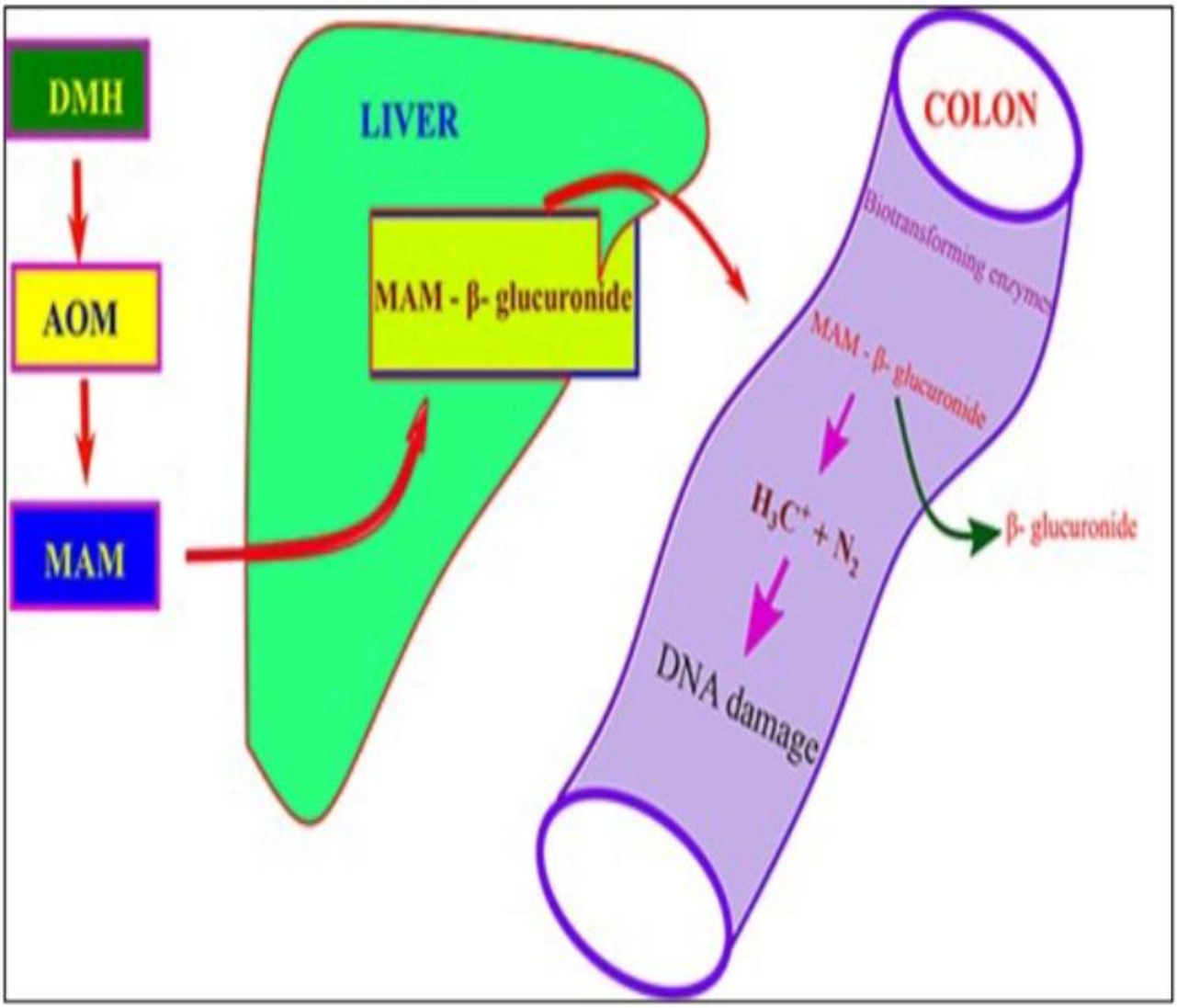


Fig 1.4. Transport of DMH from subcutaneous site to colon through glucuronidation. (Karthikkumar *et al*., 2020)

2.9.1 Metabolism of Dimethylhydrazine

The metabolism of 1,2-dimethylhydrazine (DMH) is an intricate process that converts the compound into potent carcinogens, making it an essential model in colorectal cancer research. This metabolic pathway, primarily occurring in the liver, leads to the formation of intermediates that significantly affect DNA in target tissues.

Initially, DMH undergoes metabolic activation in the liver, primarily facilitated by the cytochrome P450 enzyme system, specifically CYP2E1 (Kim *et al*., 2019). The first step in this pathway is the conversion of DMH into azoxymethane (AOM), which is crucial as it transforms DMH into a more reactive intermediate. AOM is then hydroxylated to form methylazoxymethanol (MAM), a key carcinogenic metabolite (Baggio *et al*., 2017).

MAM, a highly reactive compound, exerts its carcinogenic effects after being transported from the liver to the colon via the bloodstream. Once in the colon, MAM decomposes into formaldehyde and a methyldiazonium ion, both highly reactive species capable of alkylating DNA (Rao *et al*., 2017). The methyldiazonium ion is particularly significant in this process as it directly interacts with DNA, leading to the formation of O6-methylguanine adducts.

The formation of O6-methylguanine is a critical step in DMH-induced carcinogenesis. This DNA adduct causes mispairing during DNA replication, where O6-methylguanine pairs with thymine instead of cytosine. This mispairing results in G to A transition mutations, a common mutation type observed in many cancers, including colorectal cancer. These mutations can inactivate tumor suppressor genes or activate oncogenes, driving the transformation of normal cells into cancerous ones (Arora *et al*., 2016).

Moreover, the presence of O6-methylguanine in DNA triggers a cellular repair response. The primary repair mechanism for this lesion is the action of O6-methylguanine-DNA methyltransferase (MGMT), which removes the methyl group from the O6 position of guanine, thereby reversing the damage. However, the capacity of this repair mechanism can be overwhelmed by excessive DNA damage, leading to the accumulation of mutations (Manjulatha *et al*., 2018). In DMH-induced carcinogenesis, it is the balance between the rate of DNA damage and the efficiency of repair mechanisms that determines the extent of mutagenesis and subsequent tumorigenesis.

In addition to DNA alkylation, DMH metabolism is associated with the generation of reactive oxygen species (ROS). The metabolic activation of DMH and its intermediates can lead to oxidative stress, which further contributes to DNA, protein, and lipid damage. ROS can cause additional mutations and promote inflammatory responses, creating a tumor-promoting microenvironment in the colon (Bansal *et al*., 2020).

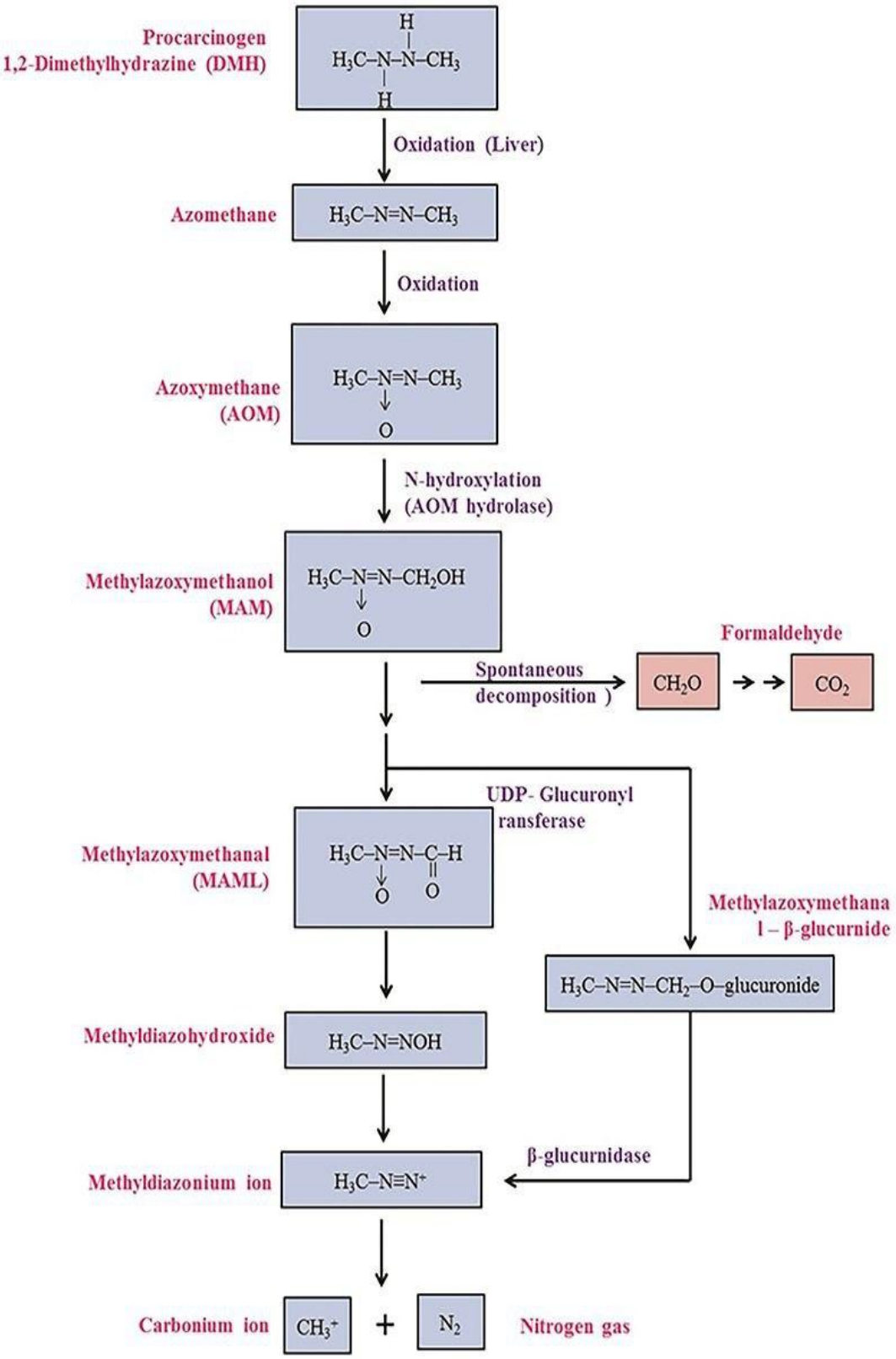
Inflammation plays a crucial role in the context of DMH-induced carcinogenesis. The metabolic byproducts of DMH can activate inflammatory pathways such as NF-κB, which in turn upregulate the production of pro-inflammatory cytokines. Chronic inflammation induced by these pathways can exacerbate DNA damage and promote the survival and proliferation of mutated cells. This inflammatory environment is a key component in the progression from preneoplastic lesions, such as aberrant crypt foci (ACF), to adenomas and eventually adenocarcinomas (Shivappa *et al*., 2021).

The impact of DMH metabolism on the gut microbiota has also been documented. The gut microbiota plays a significant role in maintaining intestinal homeostasis and modulating immune

responses. DMH-induced dysbiosis, characterized by an imbalance in the microbial community, can contribute to increased inflammation and a higher propensity for tumor formation. This interaction between the metabolic products of DMH and the gut microbiota highlights the multifaceted nature of DMH-induced carcinogenesis (Bandyopadhyay *et al*., 2017).

Research on DMH metabolism has also explored potential interventions to mitigate its carcinogenic effects. Compounds with antioxidant properties, such as N-acetylcysteine, have been shown to reduce oxidative stress and inflammation in DMH-treated animals, thereby lowering cancer risk. Additionally, dietary modifications, including the inclusion of fibers and probiotics, have been investigated for their potential to restore gut microbiota balance and reduce DMH-induced tumorigenesis (Kim *et al*., 2019).

The metabolism of 1,2-dimethylhydrazine is a complex process involving multiple biochemical transformations that lead to the formation of highly reactive and carcinogenic intermediates. These intermediates cause significant DNA damage, induce oxidative stress, and promote inflammation, all of which contribute to the development and progression of colorectal cancer. Understanding these metabolic pathways is crucial for developing strategies to prevent and treat DMH-induced carcinogenesis.



. **Fig 1.5.** Metabolism of DMH (Venkatachalam *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus

The following materials were used during the research study;

1. Petri dish
2. Glass slide and cover slip
3. Lab dissection kit
4. Digital weight balance
5. Syringe (5ml)
6. Insulin syringe (1ml)
7. Scissors
8. Pipette
9. Plain bottles
10. EDTA containers
11. Hand gloves
12. Cotton wool
13. Beakers (100ml and 50ml)
14. Multichannel micropipette
15. Universal containers
16. Dimethyl hydrazine
17. Test tube racks

18. Microplates
19. Test tubes
20. Hand gloves
21. Nose masks
22. Iceblocks
23. Wistar Rats
24. Acalypha Wilkisenia
25. Mortar and Pestle
26. Measuring cylinder
27. Cold chainCages
28. Rodent feed
29. Cheese cloth
30. Aluminium foil
31. Filter paper
32. Oral gavge
33. Funnel
34. Spatula
35. Aluminium foil
36. Ceramic plates
37. Cardboard paper

3.1.2 Equipments

1. Incubator (Techmel-Techmel USA)
2. Microplate washer (0atek China)

3. Freezer (Kiron, China)
4. Centrifuge (Oatek China)
5. Spectrophotometer (Oatek China)
6. Water bath (Techmel-Techmel USA)

3.1.3 Chemicals and Reagents

1.	1,2-dimethylhydrazine	Sigma, Germany
2.	α-tocopherol (vitamin E)	Merck, Germany
3.	Absolute ethanol	BDH, England
4.	Chloroform, hydrochloric acid (HCl)	May and Bayer, England
5.	Diphenylcarbazone indicator	Merck, Germany
6.	Distilled water	Trigas, UNIBEN
7.	DPX mountant	BDH, England
8.	Epinephrine (adrenaline)	BDH, England
9.	Ethanol	
10.	Ethylene diamine tetraacetic acid (EDTA)	BDH, England
11.	Formalin	BDH, USA
12.	Gentian Violet	
13.	Hydrogen peroxide (H2O2)	BDH, England

14.	Methylated spirit	Ivee Pharmaceutical Limited
15.	Methyline blue	BDH, England
16.	Normal Saline	Ivee Pharmaceutical Limited
17.	Petroleum ether	BDH, England
18.	Phenylhydrazine hydrochloride	[Fisher Scientific Company, New Jersey, USA]
19.	Phosphoric acid (H3PO4)	May and Bayer, England
20.	Potassium chloride (KCl)	May and Bayer, England
21.	Potassium hydroxide (KOH)	May and Bayer, England
22.	Potassium permanganate (KMnO4)	May and Bayer, England
23.	Pyrogallol	May and Bayer, England
24.	Reduced glutathione (GSH) standard	Merck, Germany
25.	Sodium carbonate (Na2CO3)	May and Bayer, England
26.	Sodium chloride (NaCl)	May and Bayer, England
27.	Sodium citrate	May and Bayer, England
28.	Sodium dihydrogen phosphate (NaH2PO4)	May and Bayer, England
29.	Sulphuric acid (H2SO4)	BDH, England
30.	Thiobarbituric acid (TBA)	Merck, Germany

31. Trichloroaceticacid (TCA) Merck, Germany

32. Vitamin E [Nature Made, USA] was purchased from a Pharmacy shop within University of Benin premises.

3.2. PLANT COLLECTION AND IDENTIFICATION

During the timeframe of January-April 2024, fresh *Alcalypha Wilkisenia* leaves were gathered from the surroundings of the University of Benin's campus in Benin City, South-Western Nigeria. The plant was then identified and / with the help of Prof. H. A Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, The herbarium specimen for archives was deposited at the University of Benin's Departmental Herbarium under the herbarium code UBH- A549. The leaves were then washed to remove dirt, dried in a room temperature no higher than 400 degrees Celsius, and pulverized with an electric grinder.

3.3. PREPARATION OF ETHANOL EXTRACT

The leaves of *Acalypha wilkisenia* was air-dried for two weeks until they became brittle, then they were ground into a fine powder using a mechanical blender. The powdered material was then extracted with ethanol using a Soxhlet apparatus, and the extract was then concentrated under reduced pressure with a rotary evaporator. The weight and percentage yield of the extract was also taking into consideration before storing it at -4°C for further studies. Additionally, fresh leaves were macerated in 250 ml of distilled w ater for 48 hours, filtered, concentrated to dryness under vacuum, and stored at -4°C until needed.

3.4 The percentage Yield of the Plant Sample

The percentage yield of the plant was calculated by dividing the weight of the dried extracted sample with the weight of the pulverized plant sample, and multiplying the value by 100.

That is: % yield = weight of dried extracted sample/weight of the pulverized plant sample × 100.

The methanol leaf extract recorded 450g of dried *Acalypha wilkisenia* sample. The percentage yield was then calculated thus:

% yield = 450g/4300g × 100

= 45000/4300

= 450/43

= 10.5

3.5. PREPARATION OF ANIMALS USED

Fourty six male Wistar rats, aged three weeks and weighing approximately 90 grams each, were acquired from the Department of Animal and Environmental Biology at the University of Benin, Edo State. They were housed in clean, serene cages and allowed to acclimatize for two weeks, during which they were fed "grower mash" rodent feed. The rats were then divided into six groups, each housed in separate cages. Their body weights were measured using a digital balance,

and each rat was marked with gentian violet on different body parts (e.g., head, tail, back, limbs) for easy identification.

3.6. EXPERIMENTAL DESIGN

The male Wistar rats were divided into six groups, with groups 1 and 2 each containing seven animals, while the other four groups had eight animals each. The experimental groups were organized as follows:

- Group 1 (Normal Control): No DMH was administered, and no treatments were given.
- Group 2 (Tumorogenic . Control): the standard drug Xeloda was adnubu for another six weeks (500mg).
- Group 3(Negative Control): DMH was administered subcutaneously three times a week for six weeks, with no subsequent treatments administered.
- Group 4: DMH was administered subcutaneously three times a week for six weeks, followed by treatment with a low dose (200 mg/kg) of the ethanol extract of Acalypha wilkesiana.
- Group 5: DMH was administered subcutaneously three times a week for six weeks, followed by treatment with a medium dose (400 mg/kg) of the ethanol extract of Acalypha wilkesiana.
- Group 6: DMH was administered subcutaneously three times a week for six weeks, followed by treatment with a high dose (800 mg/kg) of the ethanol extract of Acalypha wilkesiana.

3.6. BLOOD COLLECTION AND BIOCHEMICAL ANALYSIS

3.6.1 BLOOD COLLECTION

Blood samples of 1 ml and 4 ml were collected from each rat using syringe needles and placed into bijou bottles, either with EDTA or without EDTA (for serum). The 1 ml samples, mixed with EDTA to prevent coagulation, were used for hematological tests. The 4 ml samples were allowed to clot at room temperature, and the serum was separated by centrifuging at 1000 rpm for 10 minutes, then stored at -20°C for biochemical assays. The 1 ml blood samples were taken before and after anemia induction, while the 4 ml samples were collected post-treatment.

3.7.BIOCHEMICAL ASSAYS

3.7.1 Estimation of Malondialdehyde (MDA) concentration

Malondialdehyde (MDA), one of the end products of lipid peroxidation was estimated by the method of Buege and Aust (1978).

Principle

Malondialdehyde, a product of lipid peroxidation when heated with 2- thiobarbituric acid under alkaline condition, forms a pink colored product, which has an absorption maximum at 535 nm. The intensity of the color is directly proportional to the concentration of MDA in the sample.

Procedure

To 0.1ml of serum in the test tube, 2.0ml of TCA- TBA- HCl (25% TCA, 1% TBA and 0.4ml HCl) was added. The blank tube contained the same volume of reagents but 0.1ml of distilled water instead of serum. The solution was heated in a boiling water bath at

95°C for 15min. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10min. The absorbance was read at 535nm against the blank.

Calculation:

MDA concentration (unit/mg protein) = $\frac{A \times V \times 1000}{M \times v \times y}$ (unit/mg protein)

Where:

A = absorbance

V = Total volume of reaction mixture

M = Molar extinction co-efficient

v = volume of sample

y = mg of protein used

3.7.2 Determination of Glutathione peroxidase (GPx) activity

Glutathione peroxidase was estimated by the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting to a light brown coloration, which was read at 430nm.

Procedure

To 0.2ml of serum, 2.5ml of phosphate buffer (pH 7.4), 2.5ml of H2O2 and1.5ml of pyrogallol were added. The reaction was allowed to stand for 30min at room temperature. The colour developed was read at 430nm.

Calculation:

$$\text{Enzyme activity} = \frac{OD/min \times vt}{E \times Vs \times Y} \quad (U/mg \text{ protein})$$

Where:

OD = absorbance

Vt = total volume of reaction mixture

E = Molar extinction co-efficient

Vs = volume of sample

Y = mg of protein used

CHAPTER FOUR

RESULTS

Effect of Ethanol leaf extract of *Acalypha wilkesiana* on the Malonialdehyde and Glutathione Peroxidase of 1,2-Dimethylhydrazine exposed Wistar rats

GROUPS

Parameters	Control	Group 2 (Xeloda 500mg)	Group 3 DMH extract	Group 4 200mg	Group 5 400mg	Group 6 800mg
MDA (mole/mg protein)	0.140 ± 0.015	0.188 ± 0.022	0.187 ± 0.019	0.228 ± 0.017[a]	0.211 ± 0.040	0.234± 0.044[a]
GPX (unit/mg protein)	0.006 ± .001	0.004 ± .000	0.003 ± .000	0.008 ± .001[c]	0.005 ± .000	0.004± 0.001

Data are expressed as mean ± SEM (n=4). Values with alphabetical superscripts are significantly different from groups with corresponding alphabet in caps lock ($p < 0.05$). Group 1= normal control; Group 2 = tumour control; Group 3 = 500 mg/ kg bwt Xeloda treatment; Group 4 = 200 mg/kg ethanol extract treatment; Group 5 = 400 mg/kg ethanol extract treatment; Group 6 = 800 mg/kg ethanol extract treatment

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 DISCUSSION

In the world of alternative medicine and disease management, the safety of medicinal plants is a significant concern. Despite the common belief that medicinal plants are free from adverse effects, it is scientifically important to assess the safety and potential toxic effects of these plant compounds on relevant organs and tissues (Christapher *et al*., 2017; Abraham *et al*., 2021). Research has focused on experimental designs aimed at evaluating the bioactive compounds present in medicinal plants to ensure their safety (Christapher *et al*., 2017; Erhirhie *et al*., 2022).

The use of medicinal plants in place of synthetic pharmaceuticals for the treatment of various ailments has increased recently. Their phytochemical components—tannin, cardiac glycosides, flavonoids, saponins, alkaloids, sugars, phlobatannins, terpenes, and steroids—make this approach feasible. This approach allows for the potential identification of novel crude pharmaceuticals derived from locally available medicinal plants in addition to serving as a potential substitute for synthetic drugs.(Nadeem *et al*., 2010).

Acalypha wilkisenia, also known as the copperleaf plant, has been traditionally used in various cultures for its medicinal properties. The plant is rich in bioactive compounds, including flavonoids, tannins, saponins, and alkaloids, which exhibit significant antioxidant and anti-inflammatory effects (Olubodun 2021) These properties suggest that *Acalypha wilkisenia* could play a role in mitigating oxidative stress and potentially in cancer prevention and treatment. Colorectal cancer is a major public health challenge globally, characterized by high incidence and mortality rates. One of the key factors contributing to the development and progression of

colorectal cancer is oxidative stress. The chemical 1,2-Dimethylhydrazine (DMH) is a well-known carcinogen used to induce colon tumors in experimental animal models. DMH induces oxidative stress by generating free radicals, leading to oxidative damage and the formation of colon tumors. This work focuses on the influence of ethanol leaf extract of *Acalypha wilkisenia* on oxidative stress indices, specifically malondialdehyde (MDA) and glutathione peroxidase (GPx), in Wistar rats exposed to DMH. MDA is a biomarker for lipid peroxidation, while GPx is an important enzyme in the antioxidant defense system. Understanding the impact of *Acalypha wilkesiana* on these markers could contribute to developing novel antioxidant therapies for colorectal cancer.

5.1.1 Effect of Ethanol Leaf Extract of *Acalypha wilkisenia* on Malondialdehyde (MDA) Levels

The induction of colon tumors by 1,2-Dimethylhydrazine (DMH) resulted in a non-significant increase in malondialdehyde (MDA) levels compared to the normal control group ($p > 0.05$). MDA is a marker of lipid peroxidation, indicating oxidative stress. Treatment with 200 mg/kg and 800 mg/kg body weight of ethanol leaf extract of *Acalypha wilkesiana* significantly elevated MDA levels compared to the normal control ($p < 0.05$). This finding may indicate a pro-oxidant effect at these doses, potentially due to increased metabolic activity or an imbalance in the extract's antioxidant components at higher concentrations. Conversely, the group treated with 400 mg/kg body weight did not show a significant difference in MDA levels when compared to the normal and tumorigenic controls ($p > 0.05$), suggesting a potential dose-dependent effect where this particular dose may effectively balance the antioxidant and pro-oxidant activities of the extract.

5.1.2 Effect on Glutathione Peroxidase (GPx) Levels

Glutathione peroxidase (GPx) is a crucial enzyme that protects cells from oxidative damage by reducing lipid hydroperoxides and hydrogen peroxide. In the DMH-induced tumor group, GPx activity showed a non-significant reduction compared to the normal control ($p > 0.05$), indicating a possible impairment in the antioxidant defense mechanism due to DMH exposure.

The administration of 200 mg/kg ethanol leaf extract led to a significant increase in GPx activity compared to the tumor control and Xeloda-treated groups ($p < 0.05$). This suggests that at this dosage, Acalypha wilkesiana effectively enhances the antioxidant defense, potentially offering protection against DMH-induced oxidative damage. However, treatments with 400 mg/kg and 800 mg/kg body weight of the extract resulted in non-significant changes in GPx activity compared to both the normal and tumorigenic controls ($p > 0.05$). This indicates a dose-dependent effect, with the moderate dose (200 mg/kg) being most effective in upregulating GPx activity, while higher doses may not offer additional benefits and could have neutral or inhibitory njeffects due to complex metabolic and enzymatic interactions.

5.1.3 Comparative Analysis with Xeloda Treatment

Xeloda (capecitabine), a chemotherapeutic agent, served as a positive control. The group treated with 500 mg/kg of Xeloda did not show significant changes in MDA or GPx levels compared to the tumor control, indicating that while Xeloda is effective in its chemotherapeutic action, it may not significantly modulate oxidative stress markers within the study's scope**.**

5.2 Conclusion

The study demonstrates that the ethanol leaf extract of *Acalypha wilkisenia* has a complex, dose-dependent effect on oxidative stress markers in DMH-induced colon tumors in Wistar rats. Specifically, the extract showed significant modulation of MDA and GPx levels, suggesting its potential as an antioxidant therapy. The results highlight that a moderate dose (200 mg/kg) is most effective in enhancing the antioxidant defense system, as indicated by the significant increase in GPx activity. In contrast, higher doses may not provide additional benefits and could potentially exert pro-oxidant effects.

These findings provide valuable insights into the potential use of *Acalypha wilkisenia* in managing oxidative stress and supporting cancer therapy. Further research is needed to explore the underlying mechanisms of these dose-dependent effects and to extend the study period to assess long-term impacts on oxidative stress and tumor progression. The study underscores the importance of dosage optimization in the therapeutic application of plant extracts and paves the way for developing novel antioxidant therapies for colorectal cancer based on traditional medicinal plants like *Acalypha wilkisenia***.**

This suggests that while DMH exposure did elevate oxidative stress, it did not significantly increase MDA levels within the study period.

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APPENDIX

Colon Cancer Induction

After the animals had acclimatized, the carcinogen (1,2 Dimethylhydrazine) was administered three times a week to the DMH groups through the subcutaneous route using an insulin syringe (1ml). The Dimethylhydrazine was prepared by dissolving it in normal saline.

DOSE CALCULATION

DMH (mg) Calculation

After the animals had acclimatized, the carcinogen (1,2-dimethylhydrazine) was induced subcutaneously three times a week using a 1ml insulin syringe prepared in a 10ml buffer (saline solution) to the DMH groups.

If 1000g (1kg) body weight rat should be induced subcutaneously with 40mg/kg body weight DMH in normal saline. An animal that weighs Y(g) should be induced with X.

40mg/kg = 1000g

Xmg/kg = Weight of rat / Y(g)

Therefore,

$$X \text{ mg/kg} = \frac{\text{Weight of rat / Y(g)} \times 40\text{mg of DMH}}{1000}$$

Where X is the DMH dose (mg) of each rat and Y is the weight of each rat

DMH (ml) Calculation

The DMH (ml) was the dose administered to each rat in the DMH group.

The total DMH (mg) gotten from all DMH groups was summed up and dissolved in 10ml of normal saline.

The administered dose of DMH (Xml) for each rat in the DMH group was calculated using the expression;

Xml = DMH (mg) of each rat x 10ml of normal saline

Total DMH (mg)

Extract (mg) Calculation

After the animals acclimatized, the ethanol leave extract of A. wilkesiana was orally administered daily via a gavage at doses of 200, 400, and 800 mg/kg body weight with a 5ml syringe prepared with distilled water to the extract groups.

If 1000g (1kg) body weight rat should be induced orally with 200mg of ethanol leave extract of A. wilkesiana. An animal that weighs Y(g) should be induced with X

200mg = 1000g

Xmg = Weight of rat / Y(g)

Therefore,

Xmg = Weight of rat / Y(g) x 200mg of extract

1000

Where X is the extract dose (mg) of each rat and Y is the weight of each rat

If 1000g (1kg) body weight rat should be induced orally with 400mg of ethanol leave extract of A. wilkesiana. An animal that weighs Y(g) should be induced with X

400mg = 1000g

Xmg = Weight of rat / Y(g)

Therefore,

Xmg = Weight of rat / Y(g) x 400mg of extract

1000

Where X is the extract dose (mg) of each rat and Y is the weight of each rat

If 1000g (1kg) body weight rat should be induced orally with 800mg of ethanol leave extract of A. wilkesiana. An animal that weighs Y(g) should be induced with X

800mg = 1000g

Xmg = Weight of rat / Y(g)

Therefore,

Xmg = Weight of rat / Y(g) x 800mg of extract

1000

Where X is the extract dose (mg) of each rat and Y is the weight of each rat

Extract (ml) Calculation

The extract (ml) was the dose administered to each rat in the extract group.

The total extract (mg) gotten from all extract groups was summed up and dissolved in 50ml of distilled water.

The administered dose of extract (Xml) for each rat in the extract group was calculated using the expression;

Xml = Extract (mg) of each rat x 50ml of distilled water

Total Extract (mg)

Standard Drug (mg/m2) Calculation

One tablet of Xeloda Capecitabine (500mg) was crushed and dissolved completely in distilled water.

The recommended dosage for a physiological adult who weighs 70,000g (70kg) is 1250mg/m2. Therefore, an animal weighing Y(g) should be administered X

1250mg/m2 = 70,000g

Xmg/m2 = Weight of rat / Y(g)

Therefore,

Xmg/m2 = Weight of rat / Y(g) x 1250mg/m2

70,000g (70kg)

Where X is the standard drug dose (mg/m2) of each rat and Y is the weight of each rat

Standard Drug (ml) Calculation

The standard drug (ml) was the dose administered to each rat in group 2.

The total standard drug (mg) from group 2 was summed up and dissolved in 2ml of distilled water.

The administered dose of standard drug (Xml) for each rat in group 2 was calculated using the expression;

Xml = Standard drug (mg) of each rat x 2ml of distilled water

12mg