ANTI INFLAMMATORY AND APOPTOTIC PROPERTIES OF LEAF EXTRACTS OF *ACALYPHA WIKESIANA* IN 1,2 DIMETHYLHYDRAZINE INDUCED COLON TUMOUR IN WISTAR RATS

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

MARCH, 2026

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A THESIS WRITTEN IN THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES AND SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCES IN MEDICAL BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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CERTIFICATION

We the undersigned certify that Odiana Osamudiame Rebecca carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Master of Science Degree (M.Sc.) in Medical Biochemistry.

Signed:

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_____	_____
External Examiner	Date

DEDICATION

This work is dedicated to all cancer survivors, to those who have lost their lives to cancer, to those who lost their loved ones to cancer and to those providing any form of care to cancer patients.

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ABSTRACT

Acalypha wikesiana, a medicinal plant contains various phytochemicals which enable it perform its beneficial activities. It has gained attraction for its anti inflammatory and apoptotic properties. Colon cancer is a life threatening disease caused by several factors like genetics, environmental exposure and the damaging condition of the digestive tract. And as such 1, 2 dimethylhydrazine, an effective chemical substance was used to induce cancerous tumors in the colon and rectum. The aim of this study is to evaluate the anti inflammatory and apoptotic properties of *Acalypha wikesiana* on a DMH induced colon cancer. The sum of 46 healthy male rats were assembled into nine. The groups are a control group, a 1,2 dimethylhydrazine induced colon cancer group, which served as the standard drug group. The other groups were also induced with 1, 2 dimethylhydrazine but were treated with different doses of *Acalypha wikesiana* extracts. The ninth group was the negative control group exposed only to 1,2 dimethylhydrazine. Results indicated significantly reduced tumor size and incidence. Reduced Proinflammatory markers level suggested an anti inflammatory effect of *Acalypha wikesiana*. In conclusion, it was indicated that *Acalypha wikesiana* ethanol extracts has an anti inflammatory and apoptotic properties which to a large extent contribute to its protective effects against 1,2 dimethylhydrazine induced colon cancer.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Cancer is a very serious health challenge in the health system as regards to it's management and/or cure. Colon cancer is the most common type of gastrointestinal cancer and the third most leading cancer in males and the second most leading cancer in females (Bray *et al*., 2018). Colon cancer is a multi-factorial disease that encompasses genetic factors, environmental factors and the inflammatory conditions of the digestive tracts.

1,2-Dimethylhydrazine (DMH) is a laboratory chemical and a good carcinogen that can cause tumour in the colon and rectum of rats and mice. It is injected in these animals subcutaneously or intraperitoneally. The effectiveness and potency of 1,2-Dimethylhydrazine (DMH) as a carcinogen was confirmed by several laboratories. (Bray *et al*.,2018). DMH induced tumours closely mimic human CRC genetic mutations profile induced APC and KRAS (Liu *et al.*, 2019).

Drugs used in the treatment of cancer may be effective however, a major drawback with these anticancer drugs is that they become toxic to the cells they were supposed to protect. Due to the cytotoxicity it exhibits, there has been a quest and search for the use of plants in handling medical complications. Furthermore, scientists and other herbal pactritioners have been on the search for affordable, effective and a readily accessible anticancer agent. Some of the available anticancer drugs like taxol, topotecan, and irinotecan are of plants derivatives.

Acalypha wilkesiana commonly known as ‘copperleaf', or ‘Irish petticoat’ or Jacob's coat’ is of south pacific islands origin, and belong to the family *Euphoribaceae*. *Acalypha wilkesiana* is both an ornamental and medicinal plant. (Omage *et al*., 2019). Igwe *et al*., 2016 reported that seeds of *Acalypha wilkesiana* have been used along with other plants by some traditional

practitioners in Southwest Nigeria as a powder mixture to treat patients with breast tumour and inflammation. The seeds of *A. wikesiana* can be toxic to cancerous cells in the breast thereby damaging or even killing (apoptosis) cancer cells present there. In addition, it has been reported that this plant possesses antibacterial and anti fungal properties (Katibi *et al.*, 2016, Omage *et al.*, 2018, Igwe *et al*., 2016).

In Oyelami experiments, he showed that this plant is potent against Tinea pedia, Candida interior, Pityriasis and 73.3% effective in mycoses among the 32 affected patients he used. (Oyelami *et al*., 2023). The leaves of this plant have also been used in diabetes and hypertension management (Omage *et al.*, 2018). In fact, it has also been reported recently that the crude leaf extracts and fractions of this plant exhibit antiparasitaemic and analgesic properties (Oyelami *et al*., 2023). Studies on *A. wilkesiana* has found it to be useful in the treatment of diarrhea, gastrointestinal disorders, blood stream infections, pneumonia, bone and joint infections *(*Katibi *et al.,*2021). It has anti inflammatory powers that can be beneficial for curing bloating, pain, appendix problems .

There is a limited study of this plant as an anticancer agent, it is necessary that this plant is further studied to harness its anticancer activity as the result of the investigations will be of great benefits to everyone especially as most part of this plant ranging from its seed, shoot, leaves has vital pharmacological value. An earlier study has reported the cytotoxic effect of *Acalypha wilkesiana* seeds on breast cancer (Suzuki *et al.,* 2018). The main sites of breast cancer metastases include the lung (Suzuki *et al.,* 2018; National Cancer Institute, 2021), and brain for HER-2 positive breast cancer (Suzuki *et al.,* 2018). Since respiratory failure, an increased intracranial pressure and haemorrhage are the major common leading cause of metastatic malignancy death, it could be used as an adjunct treatment against brain and lung cancers.

1.2 Justification

From time in memorial, medicinal plants have been used for preventing, managing and even treating certain diseases with the efficacy of these drugs not doubted. But of recent, the quest for the use of more medicinal plants has increased and this is largely due to their high photochemical properties and less toxicity they provide. The medicinal efficacy and use of *Acalphya wilkesiana* is well documented in different literature as stated above and more research work need to be done as regards the effectiveness of this medicinal plant on colon tumour. Hence the purpose of this study which is to investigate the anti inflammatory properties and apoptotic properties of leaf extracts of Acalphya wilkesiana in 1,2 Dimethylhydrazine induced colon tumour in Wistar rats.

1.3 General Objectives/ Aim of Study

There is an interestingly and increasing quest among researchers to search for new drugs for the treatment and management of cancer from natural resources, especcially plants. This study aimed

1.3.1 Specific Objectives/ Aim of Study

to investigate the anticancer properties of *Acalypha wilkesiana* extracts in colon tumor .

- To ascertain the anti inflammatory properties of *Acalphya wilkesiana* in colon tumour.
- To ascertain the apoptotic properties of the leaves of *Acalphya wilkesiana* in colon tumour.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

Medicinal plants are plants that have properties that help in healing or curing an illness. Medicinal plants, also known as medicinal herbs, have similar properties as conventional pharmaceutical drugs. They exhibits same medicinal value as the conventional pharmaceutical drugs (if not more). Medicinal plants have long been discovered and has been in use in traditional medical settings from time immemorial. As humans have used them throughout history to prevent, cure or even lessen the symptoms of an illness. According to World Health Organization (WHO) it is estimated that almost 80% of the people living in the underdeveloped nations depend on traditional and complementary medicines for their basic health care (Gershenzon 2022). In India, about 7000-7500 species of plants have medicinal usage in folk and documented systems of medicine (Pandely *et al*., 2018). it is believed that as many as 25,000 different formulations are prepared from these plants as a cure against various diseases and disorders (Pandely *et a*l., 2018).

Generally, medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine. (Ahn 2017). From time immemorial, herbs like rosemary, curry, thyme, basil, tumeric, peppermint etc have been used as spices because of their high essential oil content which helped them to keep food free from microbial infections while also adding taste to the food. The medicinal efficiency of these herbs have kept them relevant over the years as they have remain an important ingredient in out foods. These medicinal plants are favorite among many societies mainly because they are readily available around the communities, inexpensise and are potentially effective when compared to modern drugs which are not

readily available, they are not affordable and many times these modern drugs have some side effects that could counter it's original purpose.

The pharmaceutical industry on the other hand has keep on harnessing the use of these medicinal plants in its drug discovery efforts. Of the 1073 small-molecule drugs approved in the period 1981 to 2010, over half were either directly derived from or inspired by natural substances. (Atanasov *et al*., 2015). Among cancer treatments, of 185 small-molecule drugs approved in the period from 1981 to 2019, 65% were derived from or inspired by natural substances. (WHO 2005). Over the years, more drugs including anti-cancer drugs have been discovered from plants through vigorous researches.

2.2 Safety and toxicity of medicinal plants

2.2.1 Safety

Many a times, the mode of usage (dosage) of medicinal plants turned healing substances are usually given by traditional practitioners within their communities and sometimes they are taken by individuals without proper prescription. Therefore, the assessment of efficacy and safety of some of these traditional medicine were based on long historic use and the informal knowledge passed on from one generation to the other. (Chan 2015). This make it very necessary that a reliable research be done to investigate the effectiveness, potency and safety of these medicinal products. Plant medicines can cause adverse effects and even death, which could either result from the side-effects of the active substances present in the plant, or by adulteration or contamination, by overdose, or by inappropriate prescription (Chan 2015). Some of these effects are known while some are yet to be scientifically identified. There have been recorded cases where the negative effects ascribed to a particular medicinal plant used have been reported in both humans and animals.

In many countries, there is little regulation of the use of plants as medicine, but the World Health Organization (WHO) has been coordinating a network called the International Regulatory Cooperation for Herbal Medicines to try to improve the quality of medical products made from medicinal plants and the claims made for them. (Giovanni 2016). And as revealed in 2015, only about 20% of countries had well-functioning regulatory agencies, while 30% had none, and around half had limited regulatory capacity. (Chan 2015).

2.2.2. Toxicity

Toxicity is the level to which a substance can be harmful or cause injury to an organism. It also deals with the effect of substances on the general makeup and build of an organism, such as the cell or an organ of the said organism. Although medicinal plants are generally considered to be safe, they are not entirely free of side effects or toxicity (Boukandou Mounanga *et al*., 2015). Plants used as medicine may contain several substances (bio-active compounds) that are different, these differences may have unresolved complexities on the structure of living organism thereby destroying the cells and other vital organ. There are several toxic compounds in plants, some of which are pyrrolizidine alkaloids, furan derivatives, epoxy-diterpenoids etc, many of which are genotoxic, cytotoxic, neurotoxic etc, all of these toxicities affect the overall functioning of the human body. Therefore, medicinal plants must be used with caution because they might cause different toxicity profiles (acute toxicity, cytotoxicity, cardio-toxicity, genotoxicity, hepatotoxicity among others. (Mounanga *et al*., 2015). Researches done from previous studies indicated that some plants used for medicinal purposes can damage vital organs in the body. For instance, the medicinal plant *Aphania senegalensis* is hepatoxic in rats and *Herniaria cinerea* is toxic for the gastrointestinal tract (Rita 2018), *Durio zibethinus* and *Juniperus communis* both exhibit nephrotoxicity and hepatotoxicity (Edorh et al., 2015)

The toxicity of medicinal plants is not the same in every cases, it may depend on the chemical composition and structure of the said plant. Plant products may interact with conventional drugs due to the fact that they may provide an increased dose of similar compounds, and because some phytochemicals interfere with the body's systems that metabolize drugs in the liver including the cytochrome P450 system, making the drugs last longer in the body and have a cumulative effect. (Rita 2018). Toxicity of medicinal plants can also depend on the total amount consumed over a period of time or the period of treatment, the specific part of plant used, and the user's body reaction. Studies in these areas are thus warranted (Mounanga *et a*l., 2015).

The frequent use of Combination therapy, irrespective of its additional medicinal strength may pose a potential harm especially when the interaction between all the bioactive present becomes antagonistic. Though studies from Siegenfelg *et al.*, 2020 and De Wet *et al*., 2016 has explained further on the potency of combining two or more active plants to get a better result, however, scientifically, not much been done to ascertain how safe or less toxic it is to ingest or use combined active substances from two or more plants. To this regards, medicinal plants should not be used carelessly but with caution.

2.2.2.1 Genotoxicity and Carcinogenicity Evaluation

Genotoxicity is the ability of a chemical substance to cause harm or alterations to the genetic materials within a cell. These alterations or damages can result to genetic mutations, chromosomal aberrations and a high tendencies of getting cancer. These toxic substances stimulate damages to the genetic material in various cells by interacting with the DNA sequence and structure and these damages can result to mutations which may lead to malignancies. It may also lead to several chromosomal aberrations like chromatid breaks, isochromatid breaks, gaps, chromosomal fragments, exchanges and sister chromatid unions; even in case of the change in DNA structure (Lazaro *et al.,* 2015). the consequences of such

DNA impairment could be establishment of and/or predisposition to diseases, increased morbidity/mortality, changes in heritable characteristics and impaired reproductive capacity (Sponchiado *et al*., 2016)

Genotoxicity assessment is important for the safety evaluation of herbal medicinal products because there are several examples of plants with phytometabolites that present genotoxic/carcinogenic properties. Wang *et al.,* recently performed a bio assay where they evaluated the hepatocellular carcinogenicity risk in mice associated with long-term consumption of *Ginkgo biloba* leaf extracts, they found considerable evidence connecting ginkgo extracts to oncogenic events (Wang *et al*., 2017). Their findings were supported by Zhang *et al*., 2015. Also, several researches carried out by different people at different times have showed that some plants used for medicinal purposes were potentially genotoxic (Rita 2018; Kigen *et al*., 2016; Paul *et al.,* 2015; Shin *et al* 2021 and Sponchiado *et al.*, 2016).

To this regards, The International Conference on Harmonisation has recommended a chain of tests for genotoxicity testing. Some of the indicators of genotoxicity to be analysed during testing are: mutagenic potential (the ability to induce gene mutations), primary DNA damage, and chromosomal damage (structural and numerical aberrations) (Baldrick 2021). Apparently, it is not hidden that plants can be genotoxic, as there are visible evidence that genotoxicity and carcinogenicity are associated with some medicinal plants, therefore genotoxicity as well as carcinogenicity has to be well studied, more researches should be done to duly ascertain its safety so that no harm will be done to the users of these products.

2.3 Challenges of using medicinal plants

Using medicinal plants as for the treatment and management of diseases for people living in developed, developing and underdeveloped countries has not come without some hassles, fears and challenges. One of the challenges facing the use of plants as medicine is the lack of

in depth knowledge in traditional medical practices and the herbs used to treat diseases. This commonly happens among many contemporary practitioners because they operate based on the information they have sourced from indirect sources like online or literatures on herbal medicines. Traditional medicine are increasingly been used outside the confines of traditional culture and far beyond geographical areas without proper knowledge of their use and the underlying principles. (Pan *et al*., 2020). For example, maceration is the known aged method of extraction as commonly used in a traditional setting. Plant materials were macerated either dried or fresh, they were placed in boiling water to produce decoctions. However this method of extraction is almost been replaced with the use of highly efficient and sophisticated technology tools which could lead to reduced medicinal efficiency of a particular plant. Specie replacement with ineffective or injurious substances and adulteration with conventional medicine is another challenge of using medicinal plants. Several researches done has confirmed the drawbacks linked with the poor quality of the medicinal products obtainable for sale in the open market are related to specie substitution and adulteration of medicinal plants. Newmaster et al (2013). tested several herbal products marketed in North America and found a considerable rate of product substitution, contamination, and use of fillers not listed on the label. In some cases, contamination and substitutions can cause important adverse reactions for consumers. For example, *Datura stramonium* is a commonly used plant species in Ayurvedic medicine. If adulterated with *Brugmansia arborea*, an anticholinergic toxidrome can appear, medicinssible serious outcomes to the user . (Kerchner *et al.*, 2020 and Tankeu *et al*., 2016).

Also, inaccurate identification and mislabeling of medicinal plants is another huge challenge as it can lead to adverse reactions among users. Morphological, chemical, microscopic and other innovative methods should be employed to authenticate the raw herbal material and also the final marketed products. (Han *et al*., 2016). It is important to note that analysis based

on morphological method alone should not be relied on as phenotypic and genetic variations can affect the morphology of the plants and cause misidentification (Liu *et al.*, 2019). To curtail and finally curb this challenge, the most commonly used binomial names for medicinal plants should be used in order to vividly differentiate and identify plants with similar or closely related names or features. Because common names are mainly used, *Heliotropium europaeum* (heliotrope), which contains potent hepatotoxic pyrrolidine alkaloids, is often confused with *Valerian officinalis* (garden heliotrope), known to contain valepotriates with sedative and muscle relaxant properties. (Xiao *et al*., 2022). Therefore, it's very imperative to always state the specific scientific name of the plant used, the part of the plant used for clarity purposes.

Getting the right or definite dosage to be administered is another challenge of using plants as medicine, the lack of an indefinite dose can lead to overdose among the users. (York *et al.,* 2021). Education, urbanization, religious beliefs, as well as social status has made some elites and educated people to hold some reservations or prejudices towards the use of plants for medicinal purposes. These has created doubts about the benefits of these plants in maintaining as well as restoring health. The mass media is not left out as it has given a negative impression about the outcome from the use of herbal medicine rather than identifying the causes of these events which may relate to a variety of issues(Gowthaman *et al*., 2021).

2.4 *Acalypha wikesiana*

Acalypha wilkesiana is an evergreen shrub commonly known as copper leaf or Jacob's coat. This shrub can grow to 3 metres (9.8 feet) high and 2 metres (6 feet 7 inches) across. It has a 3.9- 7.9 inches long and 5.9 Inches wide heart shaped leaves that comes in a variety of mottled colour combination of brown/copper, pink, orange, green, gold, yellow,

red/burgundy and variegated white. The leaves are simple with an alternate leaf arrangement and serrated margin. They may be flat, large and broad with teeth around the edge. It has a closely arranged crown, with an erect stem. It has many branches. Both the branches and the leaves are covered in fine hairs which makes it feel prickly and rough. Male and female flowers are both present in the same plant. The Male flowers spikes long and hanging while the female flowers spike short. Locally in Nigeria, it is known as Jinwinni in Hausa, Jiwene in Igbo and Aworoso in Yoruba, the three major languages in Nigeria.

Figure 1: *Acalphya wilkesiana*

2.4.1.Taxonomy of *Acalphya wilkesiana*

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopside

Order : Malpighiales

Family : Euphobiaceae

Genus : Acalpha

Specie : *A. wikesiana*

The binomial name of this plant is Acalypha wikesiana.

2.4.2 Distribution of Acalphya wilkesiana

Acalypha wilkesiana is a tropical and subtropical plant which grows in rainforest, dry rainforest and vine thickets of the Pacific Islands. This plant is native to the South Pacific, specifically Fiji and nearby islands from which it spread to other parts of the world including the tropics of Africa, Asia and America. The plant prefers light, moist, well drained soil and is suited to a protected shady position. It can be damaged by both drought and frost. It needs a minimum temperature above 10 °C (50 °F). It is best suited to hardiness zones.

2.4.3 Cultivation and propagation

Acalypha wikesiana is cultivated outdoors, it does well in a richly nourished , moist, and fast-draining soil. *A. wilkesiana* cultivated indoors do better in a soilless potting mix, with the medium constantly moist, but not saturated. If kept indoors, it should be watered daily with an exemption to the winter period, the plant should be sparsely watered during winter. Also when grown indoors, it must be maintained in a warm, humid, bright environment for optimal growth. Propagation can be done by stem cuttings (woody, semi woody or herbaceous) at any time of year.

2.4.4 Diseases affecting *Acalypha wikesiana*

The plant is mainly affected by insects like mealy bug, white flies, scale insects, spiders, mites. These insects are disastrous in the sense that they cause a decline in the growth of these plants. They also cause fungal contamination.

2.2.5 Phytochemical studies of *Acalypha wikesiana*

The plant *A. wikesiana* contains various phytochemicals which enable it perform it's beneficial activities as related to it's medicinal value. Flavonoid , tannin, saponins, terpenoids,

glycosides, alkaloids, are some of the phytochemicals found in these plants. Individually, these phytochemicals have great potential health benefits.

Specifically, quercetin has potential anticancer properties which include antiproliferative, growth factor suppression, and antioxidant (Xuet *et al.,* 2019). Its antiproliferative properties is done through reducing intracellular ROS levels in HepG2 cells. Quercetin has potent anti carcinogenic properties and known to contribute as apoptosis inductor whereby it decreases the growth of tumour in and brain, liver, colon, and other tissues and inhibits the spread of malignant cells.(Suzuki *et al.,*2018). It also has an anti metastatic, anti antigenic properties against colon cancer. Not limited to anti cancerous activities alone, Quercetin can inhibits gastric acid secretion and lipid peroxidation of gastric cells thereby serving as gastroprotective agents, It has a favorable anti-ulcer activity due to its free-radical scavenging properties or its increased gastric mucus production. (Ruhee *et al*.,2020). It is known to exhibit anti bacterial effects against almost strains of bacteria particularly respiratory, urinary, dermal or gastrointestinal systems. (Sun *et al*., 2014). It also has anti inflammatory properties. Kaempferol is another flavonoid compound that has been proven to stop the growth of cancer cells in the colon or rectum by reducing the levels of proteins that promote cancer cell growth. It can make the cancerous cells present in the colon or rectum to be more sensitivite to chemotherapy drugs. So, kaempferol, if used as an adjuvant therapy may improve the effectiveness of cancer treatment. Apigen, also a flavonoid has been shown to have anti-inflammatory, antioxidant, and anticancer properties. It has been shown to reduce the growth and spread of colorectal cancer cells in laboratory studies.

Luteolin has beneficial properties such as anti-inflammatory, anti-allergic, anti-cancer, antioxidant activities. It stops cell invasion, metastasis, transformation, all of which are the critical events associated with carcinogenesis. It has the potential to inhibit angiogenesis, by putting an end to transcription factors, cell cycle arrest and inducing cell death.

Trepenoids are antibacterial in nature, they disrupt multiplication and development in microbes, and also interfere with their physiological and metabolic activities They also perform other biological activities such as anticancer (Potočnjak *et al.*, 2018), anti-allergic, antibacterial (Guimarães *et a*l., 2019), and antioxidant (Wang *et al.,* 2019**).** Sheikh *et al* 2017 studied the effect of citral on the proliferation of human colorectal cancer HCT116 and HT29 cells. They found that citral decreased the expression of Bc-2 and Bcl-xL while inducing p53 protein phosphorylation and Bax expression. (Sheikh *et al*., 2017). Citral also eincreased mitochondrial-mediated cell death through augmentation of intracellular ROS and attenuated the total GSH levels.

Tanins on the other hand**,** stops the proliferation of microbes by denaturating enzymes involve in its metabolism, it has antiviral, antibacterial, anti-parasitic and anticancer potentials.

2.4.6 Medicinal efficacy of *Acalphya wilkesiana*

Acalypha wikesiana is a potent plant with many medicinal efficacies and health benefits that cut across different diseases and illnesses. Researches done on the health benefits of *Acalypha wikesiana* on cancer is quite limited, however it showed great potential in respect to its anti inflammatory, anti oxidant and apoptotic properties. The anti inflammatory properties of *A. wikesiana* inhibits the spread of cancer by stopping the production of cytokines, thereby preventing and cutting off oxygen supplies and nutrients to the tumor. Its antioxidant activity protects against DNA damage that can lead to cancer. It's has the ability to induce apoptosis by causing single strand and double strand DNA breaks. It also Inhibit cancerous growth and proliferation. Thereby slowing down the progression of colorectal cancer. Inflammatory responses that turns on when it shouldn't or that doesn't shut off can damage healthy tissues and lead to mutations, as well as DNA damage are two key risk factors for colorectal cancer.

Therefore, the anti inflammatory and antioxidant effects of *A. wilkesiana* may help reduce the risk of developing the disease.

In addition, it has been reported that extractss of the leaf of this plant possesses antibacterial and antifungal properties (Omage *et al.*, 2018), antimicrobial properties (Katibi *et al*., 2021). Extracts from the plant are used in medicines, especially in Ayurveda, to create curative ointments for bruises, wounds, scrapes, etc. The leaves crushed in water, filtered to remove undone particles, and the resulting filter (juice) can be drunk as a means for treating diarrhoea and dysentery. The leaves of the plant have also been used in diabetes and hypertension management (Katibi *et al.*, 2021). It has also been reported recently that the crude leaf extracts and fractions of this plant exhibit antiparasitaemic and analgesic properties (Egwim *et al.,* 2017). Chopped pieces of the dried stem and root in past studies were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Igwe *et al.*, 2016). The leaves of this plant are eaten as vegetables in the management of hypertension, in Southern Nigeria (Omage *et al.,* 2019). The fresh shoots can be squeezed in water, the filter ate (juice)can be ingested to regulate menstruation. It can also be applied externally on the skin to treat issues of the skin such as rashes. The leaves can be steamed and used to massage swelling or bath people with fever. Also, the plant has anti-inflammatory powers that can be beneficial for curing bloating, pain, appendix problems.

In addition to its medicinal uses, *A. wikesiana* has been known and grown for its non medicinal values some of which include:

- Planted for decoration purposes and to add a splash of color to gardens.
- Grown as dense ornamental hedges and used as natural fences to gardens.
- Kept in balconies to make the place look colourful and fresh.
- The leaves can be used as natural pesticides and insecticides.

2.5 1,2 Dimethylhydrazine

1,2-Dimethylhydrazine is an effective carcinogen for inducing tumour of the colon and rectum in animals like rats and mice. Dimethylhydrazine occurs as 1,2-dimethylhydrazine and 1,1-dimethylhydrazine isomers. Both liquids are clear and has no colours. DMH is mainly administered through subcutaneous injection thereby causing 100% epithelial dysplasia and precancerous lesions. Other route of administering DMH is through intraperitoneal injections and through a single injection intrarectal. It can be injected subcutathrouly at a dose rate of 20 mg/kg body weight, only once in a week for 20 weeks, this is capable of inducing colon adenomas and adenocarcinoma in about 60% of male rats.

2.5.1 Metabolism of 1,2 Dimethylhydrazine

1,2 Dimethylhydrazine and its metabolite, azoxymethane (AOM), are pro carcinogens that has to be metabolically activated to form DNA reactive products. The initial oxidation of DMH involves its conversion into azomethane, which subsequently transforms into azoxymethane (AOM). This compound then undergoes hydroxylation in the liver to become methylazoxymethanol (MAM)), a reactive metabolite of 1,2 Dimethylhydrazine and azoxymethane, which readily yields methy methyldiazonium ion that can alkylate macro molecules in the liver and colon. The pathway starts with glucuronic acid conjugation and biliary excretion. As the glucuronides gets to the colon, it undergoes hydrolysis by bacterial enzymes to produce active carcinogen in the colonic lumen. Hydroxylation ococcur by cytochrome p40 dependent pathway. MAM is chemically unstable and spontaneously decompose to form formaldehyde, water, and nitrogen. During the decomposition process, methyldiazonium ion is formed, which generates a reactive carbonium ion capable of alkylating macro molecules (DNA, RNA, or protein) by enzymic and non-enzymic process in

the colon. Mispairing of the DNA occurs due to the ablation of the oxygen atoms present in the nitrogenous bases. This has b

een suggested to be critical in mutagenesis and carcinogenesis .

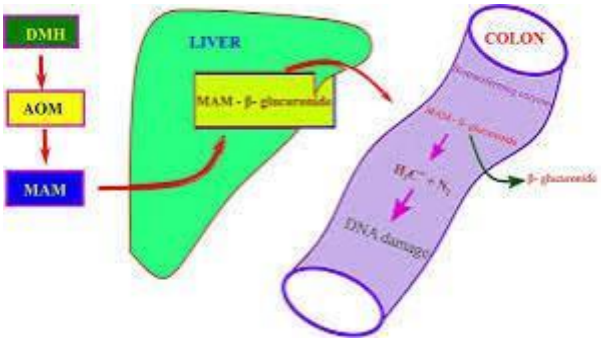


FIGURE 2: Transport of DMH from the subcuteanous site to the colon (Toxical Research Cambridge 2020)

2.6 Cancer

Cancer as defined by the National Cancer Institute is a collection of diseases in which abnormal cells can grow, divide and spread to nearby tissues. With this definition, it means that, cancer can occur in any part of the body and then spread to other part from which it did not originally start from. The spread of cancer in the body is through the blood and lymph systems. As the second leading cause of death globally next only to cardiovascular diseases in developed and developing countries, cancer should be seen as a medical emergency. Globally, the figures for cancer incidence and cancer-related deaths are rising. In 2015 alone, cancer was responsible for 8.8 million deaths and as at 2020, it was estimated that there were 10 million deaths from cancer worldwide (Ferlay *et al.,* 2021). Also, it was estimated that nearly 1 in 6 deaths is due to cancer globally (WHO 2018). Generally, cancer cells evolve from normal cells due to DNA damage. While the body can often repair damaged DNA,

cancer cells fail in this regard. Cancer usually manifests as a solid tumour, with the exception of certain cancers, like leukaemia (blood cancer). Over 100 types of cancers affect humans among which are colon, breast, lung, skin, prostate, bladder, lymphoma, leukaemia, melanoma, brain tumour, cervical, endometrial, anal, gall bladder, myeloma, bone tumour cancers. The most common cancer diagnosed in both sexes is lung cancer (11.6% of the total cases), followed by breast cancer in women (11.6%) and prostate cancer in men (7.1%). (Douaiher *et a*l., 2017). Colorectal cancer is third most commonly diagnosed cancer and second ass regards to its mortality rate (9.2%). It is estimated that by the year 2035, the total number of deaths from rectal and colon cancer will increase by 60% and 71.5%, respectively (Douaiher *et a*l., 2017). These figures may not be the same in all countries as these differences is majorly due to each country's level of economic development. Therefore, the disease is widely recognized as a marker of the country's socioeconomic development [Bray *et al.*, 2018]. Cancer is caused by several multi-factorial, activities which could be Inherited or otherwise. Inherited cancers can result from DNA passed down from parents, however, environmental exposures, such as smoking, excessive intake of alcohol can damage a person's DNA.

2.6.1 Colon cancer

Colon cancer otherwise known as colorectal cancer is a cancer caused by uncontrollable and progressive cell growth in the colon, rectum, or appendix. Colorectal cancer is the third most popular occurring cancer in men and the second most commonly occurring cancer in women in 2020 (Ferlay *et al*., 2021). Colon cancer is multi factorial disease, caused by genetic factors, environmental exposure and the inflammatory condition of the digestive tract. Some of the symptoms of colon cancer are narrowing or blockages in the bowel, abdominal pain, iron deficiency anaemia, rectal bleeding.

There are five stages of colon cancer, the first stage is the stage 0, where the cancer is in its early stages, it is still within the inner lining of the colon, it hasn't spread yet to other parts of the body. At stage 1; the cancerous cells has grown from the inner lining of the colon into the wall of the intestine though they haven't spread beyond the muscular coat or into the lymph nodes. In stage 2, the cancerous cells from the inner lining of the colon spreads into the wall of the intestine but hasn't spread to nearby lymph nodes. At stage 3, the cancer has spread into the lymph nodes. The lymph nodes further enables it's spread to other parts of the body and in stage 4, the cancer has advanced and metastasized to other parts of the body such as the liver, lungs, ovaries etc.

2.6.2 Risk factor of colon cancer

Multiple factors have been implicated in the development of colorectal cancer, among which include:

- **Genetics and personal medical history :** A person who comes from a family with a history of colorectal cancer has the chances and there's a higher risks of that person to develop colorectal cancer. This statement is supported by previous studies which indicated that people with one affected first-degree relatives (parents, siblings and children) have on average, two times higher risk of colorectal cancer in comparison to those with no family history. (Win *et al* 2016; Thelin *et al*., 2015). Inflammatory bowel disease (Crohn's disease and ulcerative colitis) are risk conditions for the development of colorectal cancer. These diseases affect the immune system of the gastrointestinal tract, and causes uncontrollable inflammation that could lead to tumour growth. The risk of CRC increases with the duration of IBD and the anatomic extent and severity of the disease. In addition, those who have had cholecystectomy are a at high risk of developing colon cancer.

- **Age** : Age is a contributory risk factor for colorectal cancer. It is prevalent among people above 50 years of age. However, it can still occur among people who are not up to 50 years of age.
- **Dietary pattern**s: ,When it comes to colon cancer and cancer in general an individual's dietary pattern plays a contributory factors. Studies have shown that regular consumption of red and processed meat is an important risk factor for the development of colorectal cancer (Rawla *et a*l., 2018). The presence of heterocyclic amines (HACs), polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs) harmful substances that may be produced during high-temperature or open-fire cooking of meat (such as pan-frying, grilling and roasting). These substance are considered genotoxic because they have the potential to cause point mutations (deletion, insertion or substitution) thereby triggering the process of carcinogenesis.
- **Overweight and obesity**: Being overweight is a risk factor for colon cancer as the adipose tissue won't be able to regulate energy intake and inflammatory responses off the body. The accumulation of excess fat can cause alternations in the adipose tissue hormone and cytokine secretions. Which in turn will inhibit apoptosis and exhibit exhibit mitogenic effects on epithelial cells.
- **Gut microbiome**: A healthy gut microbiota aids in maintaining the integrity of the intestinal barrier, it protects against pathogens and pathogenic activities in the gut. Reverse is the case in an unhealthy gut. The integrity of the gut is compromised, pathogens proliferates uncontrolably. Also composition of the gut changes thereby resulting in changes in the functions of the gut microbiome which can lead to the initiation and progression of cancerous cells in the colon.
- **Unhealthy lifestyle** such as smoking, lack or reduced physical activity, alcohol intake are all risk factors to colon cancer. The smoke from tobacco contains a mixture of several

chemicals, of which some are well known carcinogens that are known to cause damages to the DNA. Safe to say that smokers are supscitable to various types of cancer in comparison to non smokers. Production of reactive oxygen species (ROS) and nitrogen species during the oxidative metabolism of ethanol, the production of mutagenic acetaldehyde, tumour suppressor genes inactivation, reduction in folate concentration and impairment of retinoic acid metabolism [Keum *et al*., 2019; Rossi *et al.,* 2018] are some of the mechanisms through which alcohol intake can lead to colon cancer. Regular physical exercises have shown to improve the immune system, reduce stress levels, regulate hormonal levels, and even prevent obesity which by extension will protect against cancer.

- **Gender** also as act a predisposed factor for colon cancer as men are at higher risks of getting colon cancer compared to women. This is buttressed by the American Cancer Society, which states "that men have 30% higher risk of developing colorectal cancer when compared to women" this is mainly due to the differences in the exposure to risk factors such as alcohol and tobacco, dietary patterns and sex hormones. As men are likely to smoke and take alcohol than women.

2.6.3 Detection and screening of colon cancer

Early detection and screening are key to managing colorectal cancer. There are many methods to screen and perform early diagnosis, they include:

- Colonoscopy, a camera is attached to a long flexible tube and it is passed into the gut of the person. This method can be used to take out adenomas and early cancer.

- Sigmoidoscopy, an endoscopic examination of the rectum and distal colon is carried out. They are examined for polyps, abnormal areas and cancer. Sigmoidoscopy is less comfortable and it requires little intestinal preparation.

- Colon capsule endoscopy, a wireless camera which has the size of a poll is swallowed, it moves along the gastrointestinal tract and take images of its surrounding to expose any abnormalities.
- DNA Stool testing, an invasive method is used to check out for the presence of molecular debris which might be mutant DNA and occult blood in stool samples.

2.6.4 Management and treatment

There are several options of treatment for colorectal cancer and cancer in general. The type of treatment plan used depends on the stage, type, location, and the patient's state of health and choice. Many a times, 2 different treatment can be used together. Treatment and management of colon cancer may involve the following:

a) Targeted therapy

Targeted therapy targets the body's genes, proteins and any tissues that will help cancer cell to die. Cells grow and multiply. Targeted therapy uses medicines that will attack and block certain chemicals in cancer cells, targeted therapy is usually combined with chemotherapy and it is typically used for people with advanced colon cancer.

b) Radiation therapy

Radiation therapy make use of powerful and high energy beams from protons or from X-rays to kill cancerous cells in any area it is found in the body. Radiation therapy is capable of shrinking a large cancer before surgery thereby making it easy to remove. It works by damaging the DNA of tissues affected by cancer, and by extension apoptosis (a programmed cell death).

c) Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs as part of a standardized regimen. (Steele *et al.*, 2022). Chemotherapy medicines can be alkylating agents or anti metabolite. The drugs are given to disrupt the cell division process. Chemotherapy for colon cancers are usually given after surgery has been done or if the cancerous cells has spread to the lymph nodes. Chemotherapy as a treatment plan is used to shrink large cancerous cells to enable easy removal. These Cytotoxic drugs are typically administered intravenously, while some can be taken orally. Combination therapy has an advantage in the statistics of survival and response to the tumour and in the progress of the disease. (Bray 2018) than administering a single cytotoxic drug.

d) Immunotherapy

Immunotherapy is a treatment plan used to kill any cancer cells that may have invaded the immune system. Medicines that will help the body's immune system locate and kill cancerous cells are administered. Immunotherapy are usually reserved for advanced colon cancer.

e) Surgery

Laparoscopy , endoscopic mucosa resection , polypectomy can be done at the early stages of colorectal cancer. Partial colectomy can be used to remove only the parts of the colon affected while also reconnecting the healthy lymph nodes at an advanced cancer stage.

2.6.5 Standard drug

Capecitabine(Xeloda), a pro drug form of 5- Fluoruracil (5-FU) that gets changed into 5-FU once it gets to the tumor. It is taken orally after which it gets hydrolyzed in the liver and tissues, it form the active moiety (fluorouracil), and inhibits thymidylate synthase, which in turn blocks methylation of deoxyuridylic acid to thymidylic acid. This step interferes with the synthesis of DNA and RNA in cancerous cells. Most often in the treatment of colorectal cancer, Xeloda is usually given in combination with another chemotherapy drug, like

oxaliplatin. It is administered for 14days at 2500 mg/m2 followed by 7 days off, in a cycle that is repeated every 21 days. Duration of treatment may depend on the patient's response to the drug.

CHAPTER THREE

3.0 MATERIALS AND EQUIPMENTS

3.1 Materials and Apparatus

The materials used include the following:

- *Acalypha wikesiana*
- Wistar Rats
- Cages to house the rats
- Plain Containers
- EDTA Containers
- Lithium Heparin containers
- Universal Bottles
- Micro Centrifuge Tubes
- Beakers (100ml, 250ml and 500ml)
- Conical Flakes
- Measuring Cylinder
- Round Bottom Flakes
- Test Tubes
- Test Tube Racks
- Micro Pipette
- Concentrating Jars

- Glass Jar
- Cuvette
- Dissecting Set
- Syringe (2ml and 5ml)
- Lancet
- Oral Gavage
- Nose Mask
- Cheese Cloth
- Cotton Wool
- Aluminium Foil
- Steel Plates
- Ceramic Plates
- Methylated Spirit
- Gloves
- Funnel Sieve
- Masking Tape
- Spatula
- Mortar and Pestle
- Scissors and Blades
- Blender

3.1.2 Equipment / Instruments

They include the following:

- pH Meter
- Centrifuge (Sigma, Germany)
- Grinder (Waring, USA)
- Spectrophotometer UV-Vis: Biochrom Libra S12 (USA).
- Dissecting Set [TecmelTecmel, USA]
- Sensitive Electronic Weigh Balance (Buchi, USA)
- Freeze drier (Christ Alpha, Germany).
- Refrigerators such as Hisense refrigerator (Model: REF302DR) and Haier Thermocool Chest Freezer (Model: HTF-319H)

3.1.3 Chemicals and Reagents

All chemicals and reagents that were used in this study were of analytical grade (BDH, MERCK, MAY AND BAYER). They include:

- 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB)
- Phenylhydrazine hydrochloride (New Jersey, USA)
- Trichloroaceticacid (Merck, Germany)
- 1,2 dimetylhydrazine (Sigma Aldrich, Germany) DPX mountant (BDH, USA)
- Methyline blue (BDH, USA)
- Ethylene diamine tetraacetic acid (EDTA)

- Reduced glutathione (GSH) standard
- Phosphoric acid (H3PO4),
- Potassium chloride (KCl),
- Potassium hydroxide (KOH)
- Sodium carbonate (Na2CO3),
- Sodium citrate
- Sodium chloride (NaCl),
- Sodium dihydrogen phosphate (NaH2PO4),
- Chloroform
- Hydrochloric acid (HCl),
- Potassium permanganate (KMnO4)
- Pyrogallol (May and Bayer, England).
- Hydrogen peroxide (H2O2),
- α-tocopherol (vitamin E) (Merck, Germany)
- Petroleum ether
- Sulphuric acid (H2SO4) (BDH, England).
- Formalin
- Diphenylcarbazone indicator
- Absolute ethanol
- Epinephrine (adrenaline),

- Distilled water (Trigas, UNIBEN),

3.2 Methods

3.2.1 Collection of plants

Fresh *Acalypha wilkesiana* leaves were purchased from gardens within and outside Benin, Edo State, Nigeria. The leaves were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The herbarium specimen for archives was deposited at the University of Benin's Departmental Herbarium under the herbarium code UBH- A549. The leaves were properly washed, air dried and ground into fine powder using an electric blender. The powdered leaf extracts were weighed in order to determine the appropriate weight of the plant as well as to calculate the percentage yield after extraction has been done.

3.2.2 Plant extraction

Preparation of ethanol extract

Three hundred grams of the ground leaves were immersed in 800 ml of 95% ethanol for a duration of 72 hours. To guarantee adequate mixing of the contents of the vessel, the mixture was periodically stirred using a magnetic stirrer. A sintered funnel—the equivalent of four bandage folds or a sheet of cheese cloth—was then used to filter the material. After being weighed, the extract (filtrate) was concentrated using a rotary evaporator.

Aqueous Extract

Three hundred grams of the powdered leaves was soaked in 800ml distilled water for 72 hours while stirring at intervals. After 72 hours,it was filtered with chis cloth with cotton wool on it. It was soaked for another 48 hours and 24 hours respectively with the same procedure carried out. This was done in order to allow exhaustive extraction. The collected

filtrate was freeze dried to remove excess water and concentrate the extract. This process was done by using a rotary evaporator at 60 degrees.(Omage *et al.,* 2018).

After extraction, a total of four thousand three hundred grams (4,300g) of powdered Acalypha wilkesiana sample was used and it yielded a percentage of 10.5

To calculate the percentage yield;

the weight of the dried extracted sample will be divided by the weight of the powdered plant sample, then thee resulting value should be multiplied by 100. The ethanol leaf extract recorded 450g of dried Acalypha wilkesiana sample. Therefore,

% yield = 450g/4300g × 100 = 45000/4300 = 450/43 = 10.5

3.2.3 Experimental animals

The experimental procedures performed on the animals were approved by the Animal Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria. The use of rats for the study was in accordance to the Ethical Guidelines Involving Whole Animal Testing of the Animal Ethics Committee, Faculty of Basic Medical Sciences, University of Benin.

A total number of forty-six (46) male wistar rats of three weeks old were purchased from the Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin. They weighed 90g on an average at the onset of this experiment. The Wistar rats were kept in a clean and serene cage in the animal house of the University of Benin's Department of Biochemistry.

They were fed with just grower mash, a normal poultry fed and water for a period of two weeks pending when the acclimatization period will be over. After acclimatization, the wistar rats were divided into nine groups, each group was kept in a separate cage from the others. Group 1 consisted of 6 rats while group 2, 3, 4, 5, 6, 7, 8 and 9 had five wistar rats each. The

animals were weighed on a weighing balance in order to establish their various body weight and to easily identify each rat, they were stained with gential violet on different part of their bodies such on the Head, Tail, Back, Neck, Right hand, Right leg, left hand, left leg , abdomen.

3.2.4 Experimental design and feeding protocol

The 46 Male rats were divided into 9 groups designated as Group 1, Group 2, Group 3, Group 4, Group 5, Group 6, Group 7, Group 8 and Group 9 respectively. Each group received different doses of the leaf extracts with some members of the group getting induced with some carcinogen.

Group 1: Those in this group received nothing but water and food. This is the control group.

Group 2: Those in this group were induced with 1, 2 dimethylhydrazine subcutaneously for six weeks. The DMH was administered three times per week bringing it to a total of 18 doses by the end of the first six weeks. After the first six weeks, treatment with Xeloda Capecitabine, a standard drug was administered orally using a gavage for another six weeks. This is the positive control / treatment group.

Group 3: Those in this group were induced with DMH then the *Acalypha wikesiana* extracts at low doses of 200mg.

Group 4: Those in this group were induced with DMH then the *Acalypha wikesiana* extracts at medium doses of 400mg.

Group 5: Those in this group were induced with DMH, then *Acalypha wikesiana* extracts at high doses of 800mg.

Group 6: The rats in this group were given low doses(200mg) of *Acalypha wikesiana* extracts and then they were induced

Group 7: The rats in this group were given medium doses (400mg) of *Acalypha wikesiana* extracts and then they were induced

Group 8: The rats in this group were given high doses (800mg) of *Acalypha wikesiana* extracts and then they were induced

Group 9: The rats in this group were induced with DMH, three times per week, for six weeks. After which the administration of DMH stopped after 18 doses. This is the negative cancer control.

3.2.5 Administration

Induction of DMH: The 1,2 Dimethylhydrazine (carcinogen) was administered sub-cutaneously using a 1ml insulin syringe, prepared in a buffer (Saline solution (9g NaCl in 1000ml of distilled water) to the DMH groups after a period of two weeks to enable the experimental animals acclimatize.

Administration of *Acalypha wikesiana*: The ethanol leaf extract was orally administered daily using a gavage with the 5ml syringe, prepared with distilled water to the extract groups after the acclimatization period.

Preparation of Standard Drug To Be Administered One tablet of Xeloda Capecitabine (500mg) was ground into powder afterwards it was dissolved completely in distilled water. The volume of drug to be administered depends on the weight of the wistar rats.

3.2.6. Animal sacrifice

At the end of the research, the animals were euthanized in a chloroform chamber.

Collection and preparation of blood sample: A 5ml syringe was used to draw blood samples from the abdominal aorta and heart, the drawn blood was placed in a sample bottles including the EDTA bottles. The blood in the EDTA bottles was thoroughly mixed with the

EDTA in the bottle, this was done to ensure that the blood does not coagulate. The drawn blood samples were centrifuged for ten minutes at 3000 rpm to produce a clear serum, the serum was frozen at -4°C until the time needed for biochemical analysis.

3.3. Phytochemical analysis of the extract

Qualitative Analysis

The bio-active components of *A. wilkesiana* were qualitatively analyzed using different methods suitable for each. The alkaloid content was determined using Dragendorrf method. Flavonoids content was determined by the method of Sodium hydroxide. For the determination of anthraquinones, the modified Borntrager test was employed. For the determination of saponin, the emulsifying and frothing test was used. The tannin and phenol contents were determined by the ferric chloride and lead acetate methods and the quinonechlorimide method respectively.

Quantitative analysis

Determination of anthraquinone content: For 16 hours, 5 ml of the plant extract was soaked in 50 ml of distilled water. The suspension was then heated in a water bath for one hour at 70oC. After cooling the suspension, 5 ml of 50% methanol was added and filtered. The clear solution was then measured using a UV Spectrophotometer at 450 nm and compared to a standard solution containing 1 mg/100 ml purpurin and having an absorption maximum at 450 nm (Nneoyi-Egbe *et al.*, 2020).

Determination of total phenolic content: The Folin-ciocalteau method was modified. To 1 ml of leaf extract, approximately 2.5ml of 1% Folin-ciocalteau reagent and 2% sodium carbonate solution were added. The resulting mixture was incubated at room temperature for 30 minutes. The Phenolic content of the resulting mixture was measured spectrophotometrically at 765 nm using Gallic acid as a standard (Aiyegoro and Okoh, 2010).

Determination by total flavonoids: In the assay of total flavonoid content, the aluminum chloride procedure was used with little modification. Approximately 1 mL of the leaf extract was mixed with 3 mL of methanol, 0.2 mL of 10% AlCl3, 0.2 mL of 1 m potassium acetate, and 5.6 mL of distilled water for 30 minutes at room temperature. This was followed by a 420nm colometric reading with quercetin as the standard (Aiyegoro and Okoh, 2010).

Determination of Total alkaloids: The total alkaloids content was calculated using a UV-spectrometer (Patel *et al.,* 2015). 5 ml of the plant extract was dissolved in 2N HCl and then filtered. After that, one milliliter (1 ml) of the filtrates was transferred to a separating funnel and washed with 10 ml chloroform. 0.1N NaOH was used to adjust the pH of the buffer solution to neutral. As a result, 1 mL of the resulting solution was transferred to a separating funnel, and 5 mL of bromocresol solution and 5 mL of phosphate buffer were added. The mixture was vigorously shaken, and the resulting complex was fractionated using chloroform. The fractions were collected in a 10 ml volumetric flack and diluted to volume with chloroform. The complex's absorbancein chloroform was measuredspectrophotometrically at 470 nm (Ganapaty *et al*., 2013).

Determination of total saponins: A conical flask was filled with 50 cm^3 of aqueous ethanol after transferring ten milliliters (10ml) of the sample. The mixture was then heated in a water bath for 4 hours at 55^oC with continuous stirring. The resulting mixture was filtered, and the residue was extracted again witsh 100 mL of 20% ethanol (Obadoni and Ochuko, 2002).

Determination of tannins: 0.5g of extract/sample was added to 10ml of freshly made 10% KOH in a beaker and shaken to dissolve. The presence of tannin was indicated by the presence of a dirty precipitate.

Determination of terpenoids:5ml of each extract/sample was mixed in 2ml of chloroform, and 3ml of conc. H2SO4 was carefully added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids .

3.4. Histopathological Examination

After collecting blood on the final day, the animals were sacrificed to isolate the colon for examination. Each rat's colon were histologically examined in detail. They were also dehydrated with increasing concentrations of isopropyl alcohol (80 - 100%). After being examined, these organs were immediately stored in 10% formalin in normal saline. Using a Leica rotary microtome, paraffin sections of five micrometer thickness were cut from the paraffin-embedded organs (Bright B5143 Huntington, England). Routine hematoxylin and eosin staining of the slice was immediately followed by deparaffinizing, hydrating, staining, rinsing, and clearing in a xylene solution (Olayode et al., 2020). The prepared slides were

examined under a light microscope, and photomicrographs were taken using a Leica DM750 Camera Microscope (X 100).

3.5. Determination of pro-inflammatory cytokines/ inflammatory cytokines and mediators

Pro inflammatory cytokines and mediators such as TNF, IL-6, were determined or quantitated by the commercially enzyme-linked immunosorbent assay (ELISA) based on the manufacturer's instructions.

Also, Inflammatory cytokines like tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were estimated using enzyme-linked immunosorbent assay (ELISA) kits using the manufacture's instructions.

3.6. Apoptosis

The colorectal tissues with tumour lesion were excised and processed in paraffin blocks, it was cut into 4 micron thickness and then prepared for histopathology. After which the level of apoptosis markers were estimated using the available kits and following the manufacture's instructions.

CHAPTER FOUR

4.0 RESULTS

4.1 Concentration of Interleukin 6 in rats exposed to DMH.

Results showed that rats exposed to DMH expressed a significantly higher concentration of IL6 relative to the normal control ($P<0.05$). Upon administration of the standard drug, there was a significant reduction in the concentration of IL6 relative to DMH control ($P<0.05$). However, this concentration was significantly higher when compared with the normal control ($P<0.05$). Furthermore, administration of the extract pre- and post- DMH exposure significantly reduced the concentration of IL6 in a dose dependent manner relative to DMH control ($P<0.05$), while the concentration of IL6 remained significantly higher than normal control in a dose dependent manner ($P<0.05$). This result is shown in Figure 1.

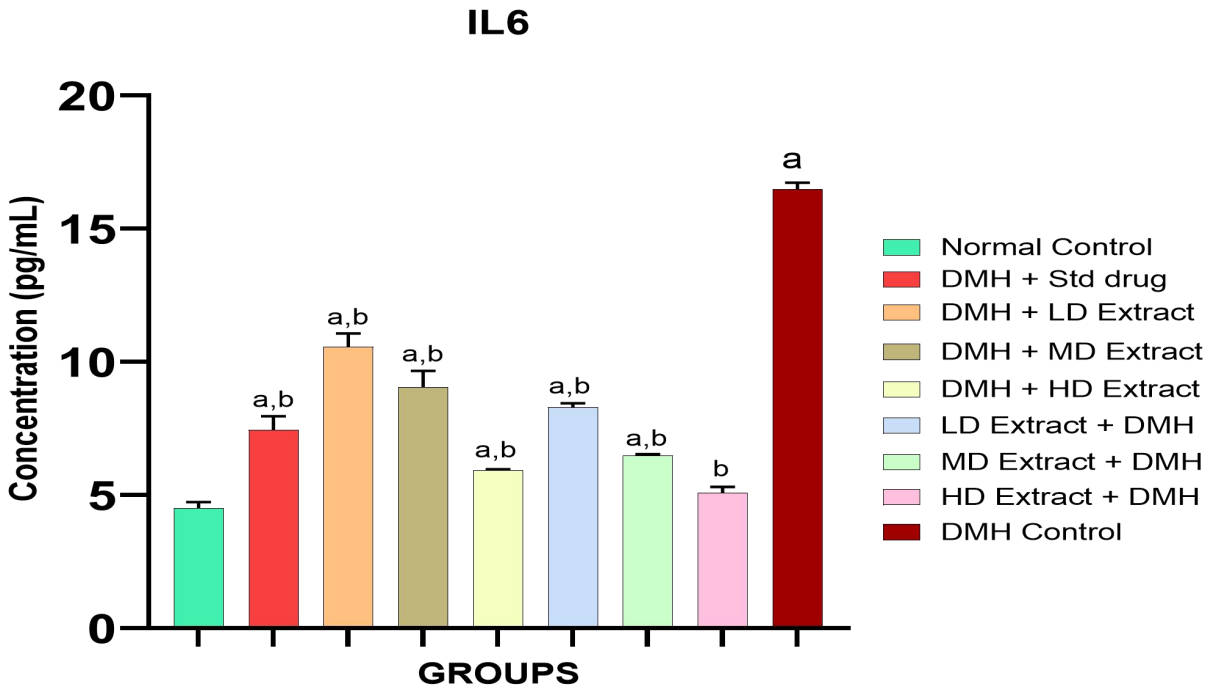


Figure 1: Concentration of Interleukin- 6 in rats exposed to DMH. Results were analyzed with one-way ANOVA followed by Fisher's LSD test. Values are expressed as mean ± SEM, a= $P<0.05$ compared with normal control, b= $P<0.05$ compared with the DMH control, IL6= Interleukin 6.

4.2 Concentration of Tumor Necrosis Factor Alpha (TNF-α) in rats exposed to DMH.

Figure 2 showed that rats exposed to DMH expressed a significantly higher concentration of TNF-α relative to the normal control (P<0.05). Upon administration of the standard drug, there was a significant reduction in the concentration of TNF-α relative to DMH control (P<0.05). However, this concentration was significantly higher when compared with the normal control (P<0.05). Furthermore, the administration of the extract pre- and post- DMH exposure significantly reduced the concentration of TNF-α in a dose dependent manner relative to DMH control (P<0.05), while the concentration of TNF-α remained significantly higher than normal control in a dose dependent manner (P<0.05).

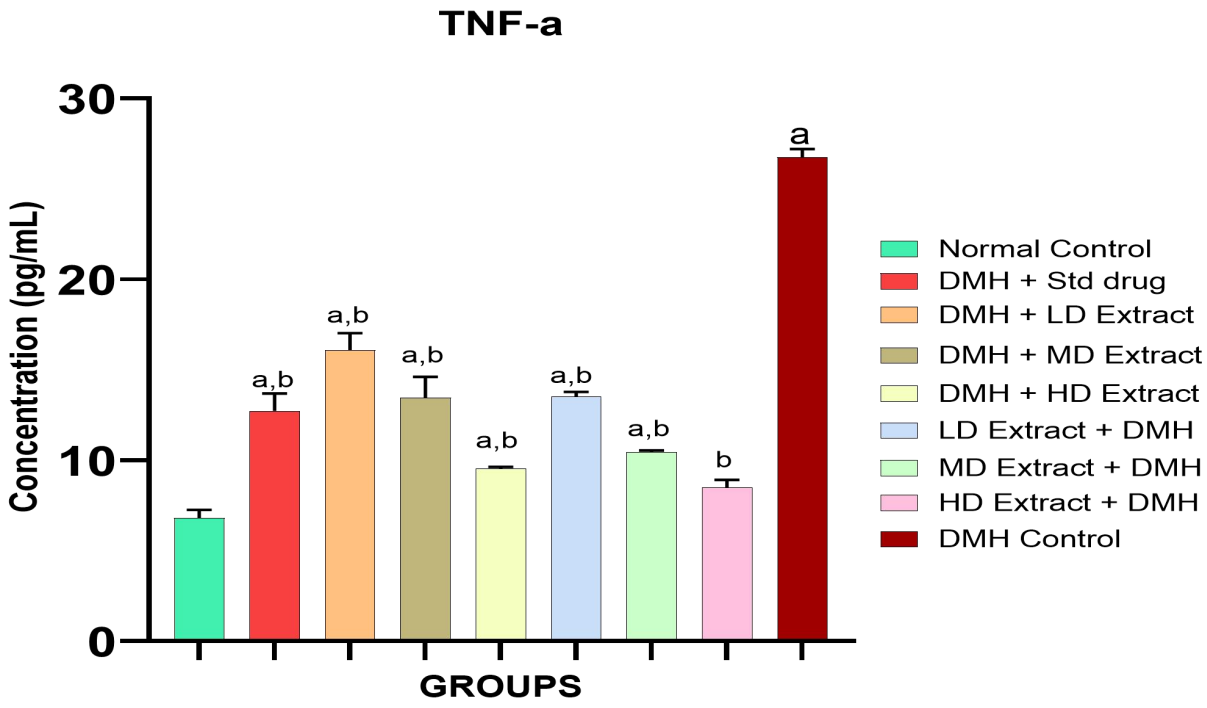


Figure 2: Concentration of TNF-α in rats exposed to DMH. Results were analyzed with one-way ANOVA followed by Fisher's LSD test. Values are expressed as mean ± SEM, a= P<0.05 compared with normal control, b= P<0.05 compared with the DMH control, TNF-α= tumor necrosis factor alpha

4.3 Concentration of P53 in rats exposed to DMH.

The concentration of P53 as shown in Figure 3 revealed that rats exposed to DMH expressed a significantly lower concentration relative to the normal control ($P<0.05$). Upon administration of the standard drug, there was a significant elevation in the concentration of P53 to near normal ($P>0.05$) relative to DMH control ($P<0.05$). Furthermore, administration of the extract pre- and post- DMH exposure significantly elevated the concentration of P53 in a dose dependent manner relative to DMH control ($P<0.05$) but lower, with an exemption of the groups administered mid and high dose of the extract post-DMH exposure when compared with the normal control ($P<0.05$). While the concentration of P53 was near normal in the mid-extract group ($P>0.05$), that of the high extract group was significantly higher than normal control ($P<0.05$).

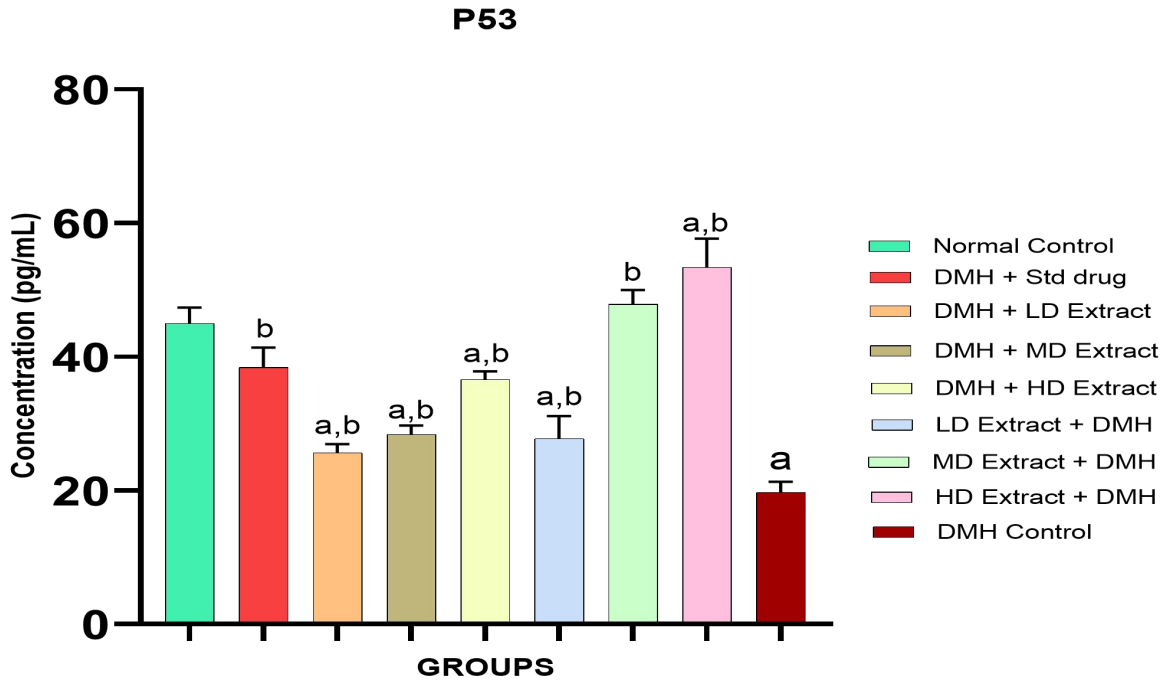
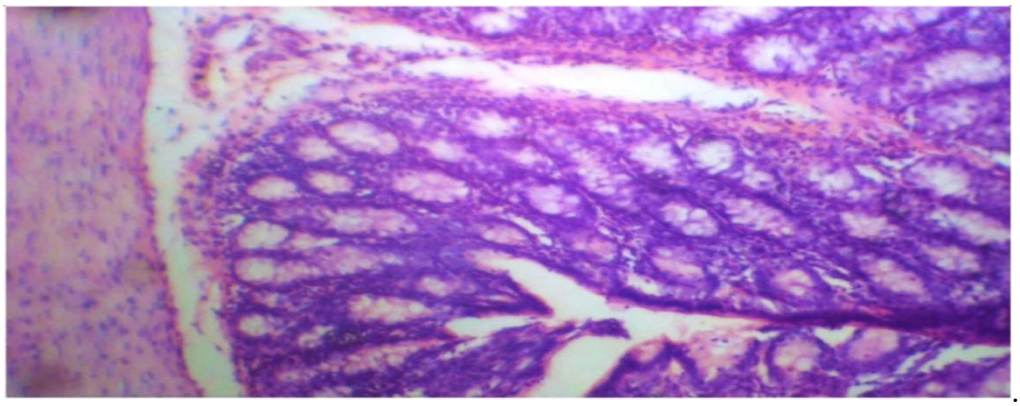
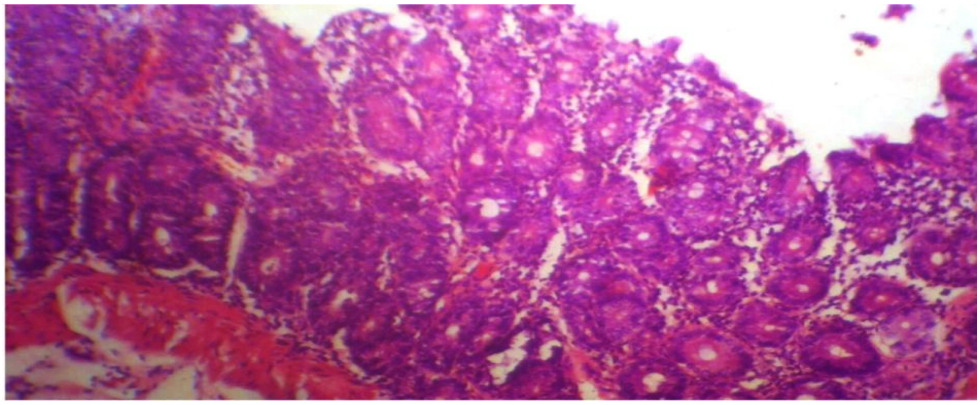


Figure 3: Concentration of P53 in rats exposed to DMH. Results were analyzed with one-way ANOVA followed by Fisher's LSD test. Values are expressed as mean ± SEM, a= $P<0.05$ compared with normal control, b= $P<0.05$ compared with the DMH control, P53= tumor suppressor protein.

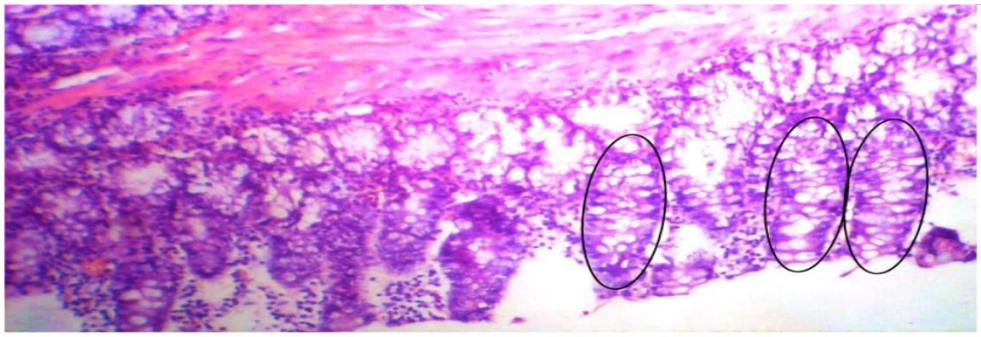
COLON



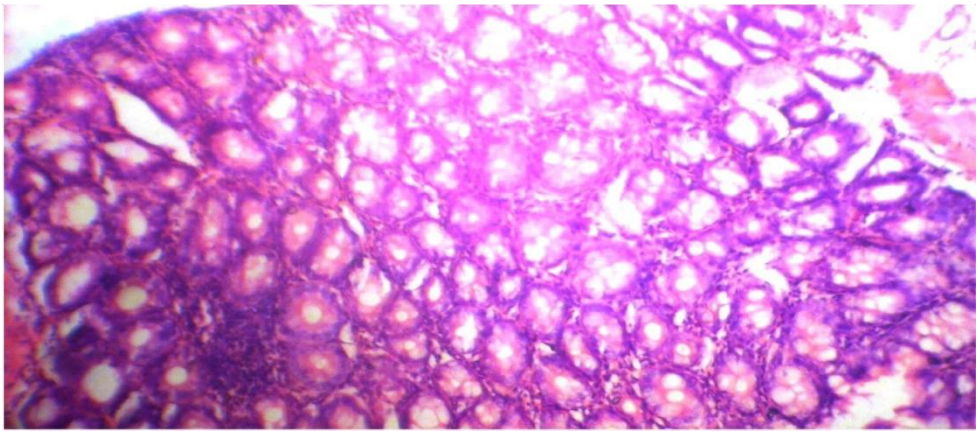
Photomicrograph of a section of the colon of the control group(**group 1)** showing normal histo-architecture with normal mucosal gladular structure; the crypts are mostly short and unserrated (H&E; 100X).



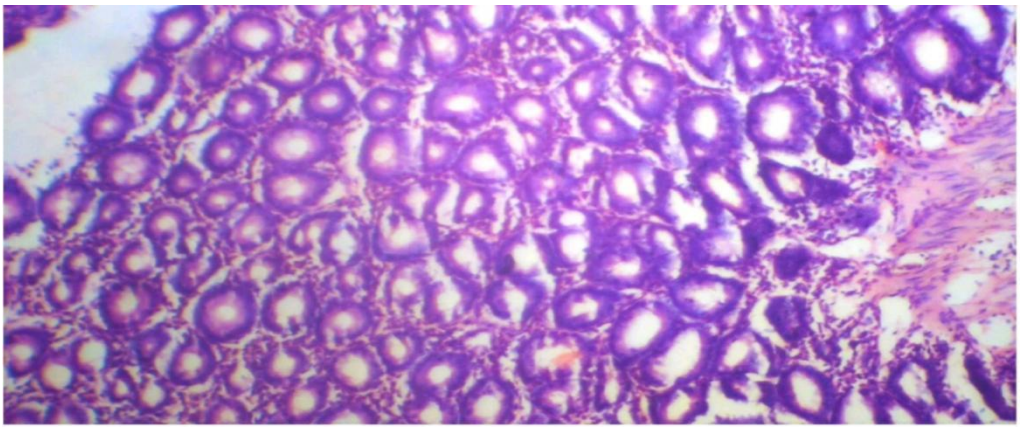
Photomicrograph of a section of the colon of the DMH+xeloda group**(group 2)** showing histo-architecture similar to the control group with short and unserrated crypts (H&E; 100X)



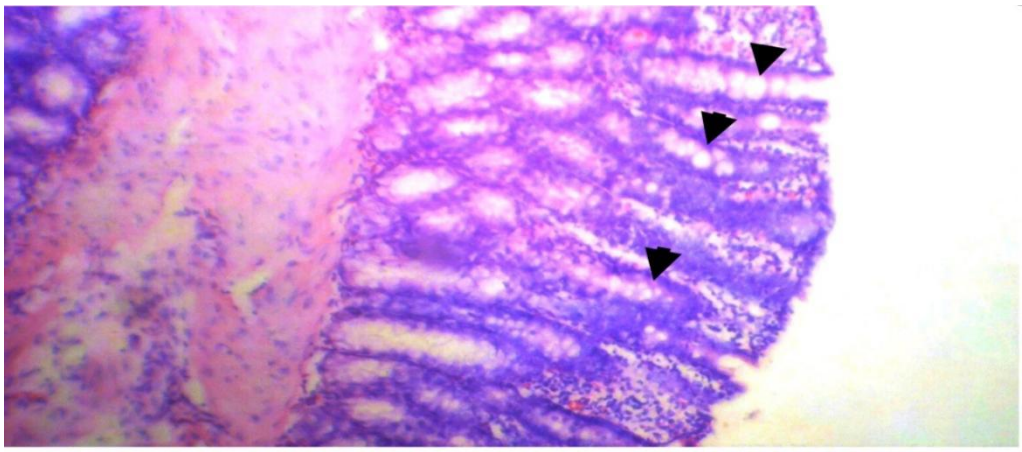
Photomicrograph of a section of the colon of the **group 3** induced with DMH before low dose of extract showing mild hyperplastic polyps with serrations (encircled) (H&E; 100X)



Photomicrograph of a section of the colon of the **group 4** induced with DMH before medium dose of extract showing histo-architecture similar to the control group with short and unserrated crypts (H&E; 100X)
DMHB4MD



Photomicrograph of a section of the colon of the **group 5** induced with DMH before high dose of extract showing histo-architecture similar to the control group with short and unserrated crypts (H&E; 100X)



Photomicrograph of a section of the colon of the DMH-induced **group 9** showing hyperplastic polyps with elongated crypts and serrated architectures (black arrow heads) in the upper part of the crypts (H&E; 100X)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The use of plants as medicine to treat several ailments has been practiced from time immemorial, however its demand has been on the increase in recent times, this is largely due to its very active phytochemicals, less toxicity, availability and affordability. This study was done to investigate the anti inflammatory and apoptotic properties of *Acalypha wikesiana* extracts in 1, 2 Dimethylhydrazine induced colon tumour in Wistar rats.

Interleukin-6, is a cytokine released by monocytes macrophages, B cells, T cells etc. As a pro-inflammatoy cytokine and an anti-inflammatory myokine, interleukin 6 plays a central role in inflammation by stimulating inflammatory responses to other cytokines (IL-10, IL-11, TNF-beta) thus mediating its anti inflammatory properties. And as a pro-inflammatory cytokine, it exert its actions by stimulating the release of acute-phase proteins, activating immune cells, and promoting the production of other inflammatory mediators. It plays a role in the development and progressiveness of colon cancer by promoting the proliferation, survival, and metastasis of tumour cell. The metastasis of tumor cells is as a result of IL-6 ability to induce epithelial-mesenchymal transition (EMT), i.e the ability of cancer cells to lose their cell-to-cell adhesion and become very mobile, this mobility enables them to spread.

The mechanism by which IL-6 act on cells is by activating signaling pathways like Signal Transducers and Activators of Transcription (STAT3), which is known to promote tumor cell proliferation in various cancer. (Masjedi *et al.,* 2018). As explained by Wang et al., IL-6 signals through a cell-surface type I cytokine receptor complex consisting of the ligand-

binding IL-6Rα chain (CD126), and the signal-transducing component gp130 (also called CD130). (Wang *et al.*, 2019).

Also, IL-6 can induce cancer stem cell expansion and create an immunosuppressive environment that further favors tumour growth. Studies have shown that IL-6 can also protect cancer cells from therapy-induced damage and apoptosis. (Wang *et al.,* 2019.) IL-6 can also suppress the immune system, making it easier for tumors to evade detection and destruction.

Extracts of *Acalypha wikesiana* inhibit cancerous growth and proliferation. As seen in figure 1, the results showed that rats exposed to DMH expressed a significantly higher concentration of IL-6 in comparison normal control ($P<0.05$). But when given the standard drug, there was a significant reduction in the concentration of IL-6 relative to DMH control ($P<0.05$). However, this concentration was clearly higher than the normal control ($P<0.05$). Furthermore, administration of the extract pre- and post- DMH exposure significantly reduced the concentration of IL-6 in a dose dependent manner relative to DMH control ($P<0.05$), while the concentration of IL-6 remained significantly higher than normal control in a dose dependent manner ($P<0.05$). The phytochemicals present in the plant extracts play a key role in this positive results. Specifically, quercetin which has anticancer benefits among which are its ability to inhibit proliferation, suppress growth factors and having antioxidant property as well as the ability to induce apoptosis, all these enable the plant extracts to decrease the growth and spread of tumour in the colon. In addition to quercetin, another phytochemical that enhances the functions of Acalypha wikesiena in relation to colon cancer is Kaempferol. This flavonoid inhibit the proliferation of colorectal cancer cells by reducing the levels of proteins that promote cancer cell growth. Also, Apigen, another flavonoid has been shown to have anti-inflammatory, antioxidant, and anticancer properties. It has been

shown to reduce the growth and spread of colorectal cancer cells in laboratory studies. (Keum *et al.,* 2019). These phytochemicals worked as a unit to reduce the levels of IL-6.

Furthermore, *Acalypha wikesiana* has shown potentials in combating colon cancer by influencing Tumour Necrosis Factor (TNF) levels. TNF-α, a procytokine, is a signaling protein produced by various immune cells like macrophages, dendritic cells, etc. It acts by binding to its receptors TNFR1 AND TNFR2. Under certain conditions, TNF-α plays a complex role in inflammation and immune response by having the ability to induce apoptosis in tumor cells (Alotaibi ***et al.***, 2021; Mercoglianoet *et al.*, 2021 and Mozooni *et al.,* 2024). However, it can promote cell proliferation; promote tumour growth thereby enhancing tumour invasiveness [Li *et al.,* 2017) in other cells.

Studies have shown that *Acalypha wikesiana* extracts can reduce the production of TNF-α and other inflammatory mediators in LPS-stimulated macrophages. This effect is linked to the extract's ability to modulate the TLR/MAPK/NF-κB signaling pathway, which is crucial in inflammation. Inflammations are triggered by various inflammatory cytokines and mediators, released from pro-inflammatory cells such as macrophages (Kim *et al.,* 2017). In the body, Macrophages act as defense mechanisms against pathogens, they stimulate the host immune system to react against any infection-related inflammation (Le *et al.,* 2018). As inflammation progresses, macrophages accumulate around the site of inflammation by invading agents (He *et al.*, 2018).

As shown in figure 2; rats exposed to DMH expressed a significantly higher concentration of TNF-α relative to the normal control ($P<0.05$) upon administration of the standard drug, there was a significant reduction in the concentration of TNF-α relative to DMH control ($P<0.05$). However, this concentration was significantly higher when compared with the normal control ($P<0.05$). Furthermore, the administration of the extract pre- and post- DMH exposure as

seen in group 3 t0 8 significantly reduced the concentration of TNF-α in a dose dependent manner relative to DMH control ($P<0.05$), while the concentration of TNF-α remained significantly higher than normal control in a dose dependent manner. This effect can largely be due to the flavonoids, tannins (phytochemicals) present in Acalypha wikesiana.

p53 is a protein that suppresses tumours. Its activities stops the formation of tumours . p53 is known as the "guardian of the genome.". It is significant in regulating cell division and cell death. As a transcription factor, it regulate the output of many biological processes by binding to specific DNA sequences. In the case of colon cancer, when damages to the DNA is detected or when other stress signals are detected, p53 can trigger DNA repair, cell cycle arrest, or apoptosis by transactivates numerous target genes involved in this processes. p53 transactivates MDM2, thereby targeting p53 for proteasomal degradation, a negative feedback loop is created. The interaction of p53–MDM2/MDM4 is blocked, thereby bringing stability to p53 either in a problematic cell or in an unstressed cell. Furthermore, p53 can promoting autophagy, cellular differentiation, and ferroptosis, it can stop invasion and metastasis. All these p53-regulated responses contribute to tumour suppression. (.Sabapathy *et al.,* 2017). As seen in group 3, 4 and 5, p53 was elevated in group 5 where the rats received a high dosage of the plant extracts. Even in group 8 where a high dose of the extract were administered before been induced with carcinogen, p53 was still high.

5.2 Conclusion

In summary, *Acalypha wikesiena* has shown great potentials from the study carried out in the sense that it was able to decrease the levels of IL-6 and TNF and well as increase the level of p53. Inflammatory responses that turns on when it shouldn't or that doesn't shut off can damage healthy tissues and lead to mutations, as well as DNA damage are two key risk factors for colorectal cancer. The anti inflammatory properties of *Acalypha wikesiana* inhibits

the spread of cancer by stopping the production of cytokines, its antioxidant activity protects against DNA damage that can lead to cancer. It has the ability to induce apoptosis. It also inhibit cancerous growth and proliferation. Thereby slowing down the progression of colorectal cancer. Therefore, the anti inflammatory and apoptotic effects of *Acalypha wilkesiana* may help reduce the risk of developing the disease and in the management of the disease.

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