EFFECT OF ACALYPHA WILKESIANA LEAF EXTRACT ON THE MCV, MCH AND MCH STATUS OF WISTAR RATS EXPOSED TO 1,2 DIMETHYLHYDRAZINE

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SCHOOL OF BASIC MEDICAL SCIENCE
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MAY, 2024.

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

MAY, 2024

CERTIFICATION

This is to certify that this project work was carried out by OSAZUWA OSAMEDE with matriculation number BMS1606131, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, in partial fulfillment of the requirement for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

…… ……

Prof. S. OLUBODUN **Date**
(Project Supervisor)

…… **……**

Dr. F.E. OLUMESE **Date**
(Head of Department)

…… **……**

Examiner **Date**

DEDICATION

I dedicate this work to God Almighty who sustained me all through my time in the University of Benin and gave me the wisdom and enablement to successfully carry out this research and my lovely parent Mr. and Mrs. Victor Osazuwa for their immeasurable support and contributions to my academic success so far, to my lovely siblings and mom for all their encouragements, advice and keen interest towards my success guidance.

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My profound gratitude goes to God Almighty whose grace sustained me all through my years of study in the University of Benin. I am very grateful to my wonderful Lecturers and supervisors Prof. S. Olubodun for his immense contributions towards the success of this research. I wish to acknowledge also with unreserved thanks to my course advisor and lecturers and Staff of Medical Biochemistry Department, University of Benin, Benin City.

I deeply appreciate my lovely parents Mr. and Mrs. Victor Osazuwa for their immeasurable support and contributions to my academic success so far, and my lovely siblings and friends for all their encouragements and advice.

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ABSTRACT

This study evaluates the hematoprotective effects of ethanol leaf extract of *Acalypha wilkesiana* on Wistar rats exposed to 1,2-dimethylhydrazine (DMH), a carcinogen. The experiment involved nine groups of rats with varying treatments: normal control, positive control with DMH and Xeloda (Capecitabine), and several groups receiving different doses of *Acalypha wilkesiana* extract both before and after DMH exposure. Hematological parameters analyzed included Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). Results demonstrated significant changes in these parameters among treated groups. Rats treated with *Acalypha wilkesiana* extract exhibited increased MCV, MCH, and MCHC levels compared to controls, indicating potential hematoprotective properties. Specifically, groups receiving the extract showed improvement in red blood cell indices affected by DMH, suggesting its efficacy in mitigating DMH-induced hematological damage. These findings highlight the potential therapeutic application of *Acalypha wilkesiana* in managing hematological disorders caused by carcinogenic agents. Further research is necessary to elucidate the mechanisms of action and optimize dosage for clinical use. Overall, this research opens promising avenues for utilizing natural remedies to enhance patient well-being and combat hematological abnormalities.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The use of medicinal plants in traditional medicine has gained significant interest due to their potential therapeutic effects and fewer side effects compared to synthetic drugs. One such plant, *Acalypha wilkesiana*, commonly known as copperleaf or Jacob's coat, has been widely used in folk medicine for its antimicrobial, anti-inflammatory, and anticancer properties (Akinmoladun et al.,

2010). *Acalypha wilkesiana* belongs to the Euphorbiaceae family and is native to the Pacific Islands but is also cultivated in tropical and subtropical regions worldwide.

The hematological system, comprising blood and its components, plays a crucial role in maintaining homeostasis and overall health. Hematological parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are critical indicators of the functional status of red blood cells and are often used to diagnose and monitor various blood disorders (Hoffbrand et al., 2016). Changes in these parameters can provide valuable insights into the impact of therapeutic agents on the blood system.

1,2-Dimethylhydrazine (DMH) is a potent chemical carcinogen widely used in experimental models to study colon carcinogenesis. Exposure to DMH induces oxidative stress and generates reactive oxygen species (ROS), leading to DNA damage and subsequent development of colon tumors (Rao et al., 2006). The deleterious effects of DMH are not limited to the colon; they also

extend to various organs and systems, including the hematopoietic system. DMH exposure can result in hematological abnormalities, highlighting the need for effective interventions to mitigate these effects.

Recent studies have explored the potential of natural products to counteract the adverse effects of chemical carcinogens. *Acalypha wilkesiana*, with its rich phytochemical composition, including flavonoids, tannins, and saponins, presents a promising candidate for such interventions. However, there is limited research on its specific effects on hematological parameters in the context of chemical-induced toxicity.

1.2 PROBLEM STATEMENT

Despite the widespread use of *Acalypha wilkesiana* in traditional medicine and its reported therapeutic properties, there is a paucity of scientific evidence regarding its effect on hematological parameters in animal models exposed to chemical carcinogens. Understanding the potential hematoprotective effects of *Acalypha wilkesiana* leaf extract on the MCV, MCH, and MCHC status of Wistar rats exposed to 1,2-dimethylhydrazine is essential for developing effective strategies to mitigate hematological damage caused by chemical exposure.

1.3 AIM OF STUDY

The aim of this study is to investigate the effect of *Acalypha wilkesiana* leaf extract on the hematological parameters, specifically MCV, MCH, and MCHC, of Wistar rats exposed to 1,2dimethylhydrazine.

CHAPTER TWO

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

There has been a noticeable increase in the use of herbal medicine over modern medicine over the past twenty years. Moreover, there is still a tremendous lap of proper research data in the field of traditional herbal medicine, this is to say that amongst large varieties of this medicinal plants, only a few of them has been properly researched. Though other medicinal plants are known but they are phytochemical compositions still remain unknown due to lack of research (Bandaranayake, 2006).

Several studies have shown that some of this already researched plants can be multifunctional but have not been exhaustively and extensively researched to be able to ascertain all their phytochemical constituents, anti-oxidant properties and possible pharmacopoeia characteristics. The contributions of plants in some industries are remarkable as it serves as a source of raw materials in the production of cosmetics, fine chemicals and drugs. Medicinal plants play a vital role in the discovery and development of a new drug. Plants are not indispensable in health care as they are seen as the best hope and source for save future medicine and this calls for an urgent need to research, discover and develop new therapeutic agents with little or no side effects from plants (Abayomi *et al.,* 2013).

Acalypha wilkesiana ointment has been used to treat skin fungal infection and disorders. In Southern Nigeria, the leaves of this plant are eaten as vegetables in the management of hypertension, diabetes mellitus, gastrointestinal disorders, hypertension, malaria and skin infections, (Akinyemi, 2005) and they also possess antihypertensive, diuretic, hypoglycemic and hypolipidemic activities (Ikewuchi, 2009).

Fig 2.1 *Acalypha wilkesiana*

2.2 History of *Acalypha wilkesiana*

Acalypha wilkesiana belongs to the family *Euphorbiaceae* and is native to the south pacific islands. It is widely cultivated in the tropical and subtropical countries. *Acalypha wilkesiana* is also identified with other scientific names such as *A. amentaceae* and *A. tricolor*, while its common names are 'copperleaf', 'Joseph's coat', 'fire dragon', 'beef steak plant' and 'matchme-if-you-can' (Christman, 2004). The Hausas of Northern Nigeria call it "Jiwene" and

"Jinwinini", while the Yoruba of Southern Nigeria call it "aworoso". It is native to Fiji and nearby islands in the South Pacific, and is a popular outdoor plant that provides color throughout

the year, although it is also grown indoors as a container plant. It is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches near the base and can get up to 3.1m tall with a similar spread. Its leaves measuring 12.7 - 20.3cm long are alternate, oval shaped, serrate and multi-colored, with small inconspicuous flowers measuring 10.2 - 20.3cm that hangs in catkin-like racemes beneath its foliage. Many cultivars are available which demonstrate different leaf forms and colors: *A. wilkesiana* 'Godseffiana' has narrow, drooping, green leaves with creamy-white margins, 'Marginata' has coppery-green leaves with pink or crimson margins, 'Macrophylla' has larger leaves, variegated with bronze, cream, yellow and red, while 'Musaica' has green leaves that are mottled with orange and red (Gilman, 1999; Christman, 2004).

2.2.1 Botanical Description of the Plant

The genus includes annual or perennial herbs, shrubs, and small trees. The leaves are alternately arranged, undivided, mostly stemmed and fixed. The leaves are complete or more commonly serrated or spinous, pinnate or palmately veined, with several types of inflorescences. Male inflorescences are densely flowered, with multiple flowers at each node, supported by small bracts. Female inflorescences, on the other hand, are generally pointed, racemes or panicles, with 1 to 3 (-5) flowers per node, usually supported by large bracts.

Hermaphrodite inflorescences are usually present in female flowers at proximal nodes and male flowers at distal nodes. The flowers are unisexual, with pointed tips that lack discs. The male flowers are very small, short-stemmed, and globular in bud, with sepals divided into four small sepals. The 4-8 (~16) stamens are in slightly raised receptors, devoid of filaments and fused at the base. Anthers with fissures or drooping anthers, unilocular, more or less elongated, later wormlike. Female flowers are generally sessile or semi-sessile, and some species have stalks.

Calyx composed of 3 (4-5) small sepals fused at base. The carpel ovary is often mucinous, pubescent, or papillary on the surface. Oocytes individually for each cell.

Seeds are often small, ovate or elliptical, usually nodular, smooth, or foveated, and the endosperm present in a whitish color. The embryo is straight and the cotyledons are broad and flat. Atypical female flowers are present in some species and are generally terminal (sometimes central or basal) of the inflorescence. Shrimp, long-stalked or stalkless. Calyx like normal female flowers.

Several research on *A. wilkesiana* has indicated and subsequently identified the presence of 3 flavonoids (artemetin, luteolin, vitexicarpin), 5 carotenoids and 12 simple terpenes (Onyeike *et al.*, 2010). In a study carried out by Anokwuru *et al*., 2012, the leaves of *A. wilkesiana* were found to be rich in antioxidants and could be used as a possible anti-denaturing agent.

2.3 Phytochemicals

Acalypha wilkesiana has a wide range of therapeutic benefits because of the abundance of bioactive chemicals it contains. The pulverized dried leaves of A. wilkesiana were subjected to phytochemical analyses, which found the presence of tannin, cardiac glycosides, flavonoids, saponins, alkaloids, a high concentration of carbohydrates, a moderate concentration of phlobatannins, and a negligible amount of terpenes and steroids. Chloride, sodium, potassium, calcium, iron, magnesium, zinc, copper, and manganese were all somewhat abundant according to the elemental analysis. Therefore, it can be said that *A. wilkesiana* includes therapeutically useful compounds for both disease prevention and treatment (Madziga H. A. *et al.,* 2020).

2.4 Medicinal benefits

The medicinal herb *Acalypha wilkesiana* 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. wilkesiana*.

a. Remedy for skin infection

According to reports, *A. wilkesiana* has antimicrobial effects on *Yersinia enterocolitica, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, and Klebsiella aerogenes*. It also has antibacterial effects on *Staphylococcus aureus*. Skin problems and gastrointestinal disorders are both known to be brought on by these germs. A. wilkesiana ethanol extracts were used for in vitro antibacterial screening by Gotep *et al.,* 2010 because of the plant's reputed application in treatment for digestive illness. The results of this investigation indicate that the plant *A. wilkesiana's* ethanol extract has a range of antibacterial properties. Results indicate that the herb has the claimed therapeutic impact on diseases related to the skin and gastrointestinal tract (Forcados, *et al*., 2016)

b. Cardiovascular diseases

Acalypha wilkesiana is claimed to be effective in the treatment of cardiovascular disorders and diabetes. Ikewuchi and Ikewuchi looked into this claim and used rats to test how the plant extract administration affected their blood sugar and cholesterol levels. According to several reports, the aqueous extract of A. wilkesiana reduced blood sugar and cholesterol levels. This supports its application in the management of illnesses with a cardiovascular component (Forcados *et al*., 2016).

c. Anti-oxidant activity

A. wilkesiana enhanced animal antioxidant capacity and decreased lipid oxidation mediated by reactive oxygen species (Ogbuehi *et al.,* 2014). Igwe *et al.,* (2016) reported that ethanolic leaf extracts of *Acalypha wilkesiana* contain pharmacologically useful active phytochemicals that affect progesterone receptors, glucocorticoid receptors, androgen and estrogen receptors with mild antioxidant and atherosclerotic effects and may also be beneficial in the treatment of pregnancy especially Synchronize estrus related. According to Kingsley *et al.,* (2013), the antioxidant activity of *Acalypha wilkesiana* leaves is due to the presence of abundant flavonoids that help protect against free radical damage.

d. Anti-microbial and antifungal activity

In addition to its use as a vegetable, *Acalypha wilkesiana* is said to have antibacterial and antifungal properties (Akinde, 1986). Ethanolic extracts from *A. wilkesiana* exhibited antibacterial activity and were active against Gram-positive bacteria (Staphylococcus aureus and Gram-negative bacteria E. coli, K. pneumonia and Shigella) (Awe and Eme 2014). In vitro antibacterial screen using ethanol extracts of *A. wilkesiana* found ethanolic plant extracts to be effective against Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa, and reported that they have different antibacterial activities against Klebsiella aerogenes (Gotep, 2010). According to Olaribigbe and Sorbari (2014), *Acalypha wilkesiana* cream containing cetomacrogol showed the greatest antimicrobial properties, whereas the emulsifying ointment showed the least antimicrobial properties. This is due to the inhibitory effect of the paraffin contained in the formulation. As a result, *A. wilkesiana*

leaves have antimicrobial activity to treat skin infections, as claimed by traditionalists, and can be used in formulating herbal skin creams to treat some skin infections.

e. Anti-obesity effect

Polyphenols, one of the constituents of *Acalypha wilkesiana* leaf extract, have been shown to have potent anti-obesity activity (Rayalam *et al.,* 2008) and inhibitory activity against pancreatic lipase, leading to weight loss (Thielecke and Boschmann, 2009; Nakai *et al.,* 2005). These components may affect body weight through alterations in body fat metabolism and oxidation, or through increased metabolic rate, which may be the mechanism for the anti-obesity effects of *Acalypha wilkesiana* leaf extract (Iyamu *et al*., 2014).

f. Anti-diabetic activity

Al-Attar (2010) conducted a physiological study on the effect of *Acalypha wilkesiana* leaf extract on streptozotocin-induced experimental diabetes in male mice and reported its antidiabetic properties. Dietary leaf extract supplements lowered blood sugar levels and improved lipid and electrolyte profiles in experimental diabetic mice. In vivo studies have shown antidiabetic properties of *A. wilkesiana* and *Acalypha indica L*. (Ikewuchi *et al.,* 2011; Itankar *et al.,* 2011), but the active substance is still unknown.

g. Anti-hypertensive activity

Ikewuchi *et al.,* (2008) studied the effects of *Acalypha wilkesiana* on plasma sodium and potassium concentrations in normal rabbits and found it to have antihypertensive properties due to the presence of a phytochemical, 2-butenyl propionate which may also

help control renin-dependent hypertension (Igwe *et al.,* 2016). According to Kingsley *et al.,* (2013), the cardiotonic effects of steroids, which are abundant in leaves, directly affect myocardial contraction and thus may be beneficial in the treatment of hypertension.

h. Anti-cholesterol and anti-arrhythmic activity

The use of *A. wilkesiana* was used in the treatment of diabetes and cardiovascular disease in research by Ikewuchi and Ikewuchi (2010), who used a rat model to examine the effects of plant extract administration on blood glucose and cholesterol levels. They reported that an aqueous extract of *A. wilkesiana* was effective in lowering blood cholesterol and blood sugar levels, explaining its use in treating cardiovascular disease. Cardiac glycosides are used therapeutically in the treatment of heart failure due to their antiarrhythmic effects. This is one of the main advantages associated with the use of *A. wilkesiana* leaves as cardiac glycosides are known to function by inhibiting the Na+/K+ pump which increases sodium ion levels within muscle cells and increases calcium ion levels. This inhibition increases the amount of Ca2+ ions available for myocardial contraction, improves cardiac output, and reduces cardiac stretching, therefore, it's use in the treatment of congestive heart failure and cardiac arrhythmias. The presence of alkaloids in *A. wilkesiana* leaves indicates their use as a source of substances that are precursors of neurotransmitters which act on nervous system signaling that directly influences the constriction of blood vessels in the cardiovascular system. The effects of these alkaloids on the cardiovascular system are useful in treating cardiovascular disease, hypertension, and serve as a source of precursors for the synthesis of psychotropic drugs (Kingsley *et al.,* 2013).

i. Hypnotic and anticonvulsant effect

The presence of acetophenone has hypnotic, sedative and antispasmodic effects. This compound can be used to induce sleep (hypnosine) or as an anesthetic prior to treatment or surgery to fix reflexes and also acts as a sedative by suppressing convulsions and depressing the central nervous system. (Igwe *et al.,* 2016).

j. Anti-cancerous activity

The beta-carotene found in *A. wilkesiana* leaves acts as an antioxidant that helps strengthen the immune system against cancer, cataracts, and the harmful effects of radiation. Basil *et al.,* (2007) and Lim *et al.,* (2011 & 2013) demonstrated that the combination of *A. wilkesiana* ethyl acetate extract and a-tocopherol has potent cytotoxicity against human brain and lung cancer cell lines making it effective. Extracts of *A. wilkesiana, A. alopecuroidea Jacq*. and *Australis L*. has shown anticancer properties against various cell lines in vitro (Madlener *et al.,* 2010; Shin *et al.,* 2012).

2.5 Toxicology and Toxicity

Toxicology is the branch of science that deals with the adverse effects of chemical, physical or biological agents on humans, animals, and the environment (Curtis, 2010). This branch of science studies toxic substances with respect to their; sources, properties, mechanism of toxicity, toxic effects, detection, clinical manifestations and management. Any substance can be toxic if introduced in a dose capable of disrupting the normal physiological homeostasis of the exposed body. Thus a remedy can become a poison if there is a significant alteration in the dose. The term

"LD_{50}" refers to the dose of a toxic substance that kills 50 percent of a test population. How well patients recover from their sicknesses/diseases depends on whether the drugs are safe or not

(Curtis, 2010). Toxicity is the degree to which a substance can damage an organism. It refers to the effect a substance exerts on whole organism such as an animal, bacterium, or plant, as well as the effect on substructures of the organism, such as a cell (cytotoxicity) or an organ such as the kidney (nephrotoxicity). The central dogma of toxicology is that effects are dose-dependent; even water can lead to water intoxication when consumed in too high a dose, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect. The fact that toxicity is species-specific makes cross-species analysis problematic. Newer paradigms and metrics are evolving to bypass animal testing, while maintaining the concept of toxicity endpoints (Philomena, 2011; Ernst *et al*., 2007).

2.5.1 Factors Influencing Toxicity

Factors that influence the toxicity of a substance are route of administration (whether the toxin is applied to the skin, ingested, inhaled, or injected), time of exposure (a brief encounter or long term), number of exposures (a single dose or multiple doses over time), physical form of the toxin (solid, liquid or gas), genetic makeup of an individual and an individual's health status.

2.6 1,2-dimethylhydrazine (DMH)

1,2-dimethylhydrazine (DMH) is a member in the class of hydrazines. It’s a strong DNA alkylating agent, normally present in cycads. DMH is broadly utilized as a carcinogen to induce colon cancer in animal models.

In this convention, colon disease is prompted or induced in mice through a series of injections with 1,2-dimethylhydrazine. Mice will grow essentially colon cancers beginning at around 90 days (3 months) after the first injection. Tumors in the lung, uterus, and intestine may likewise be seen, as well as lymphomas. Investigation of DMH-induced colon carcinogenesis in rat models

gives the information to see the biochemical, atomic, and histological mechanisms of various phases of colon carcinogenesis. The procarcinogen DMH, after a progression of metabolic reactions, at long last arrives at the colon, there creates an ultimate carcinogen and reactive oxygen species (ROS), which further alkylate the DNA and start the developement of colon carcinogenesis. The preneolpastic lesions and histopathological observations of DMH-induced colon tumors might give a typical understading about the sickness in rodents and human beings. In addition, this review examines about the activity of biotransformation and antioxidant enzymes involved in DMH intoxication. This understanding is fundamental to precisely distinguish and decipher modifications that happen in the colonic mucosa while evaluating normal or pharmacological mixtures in DMH-actuated creature colon carcinogenesis.

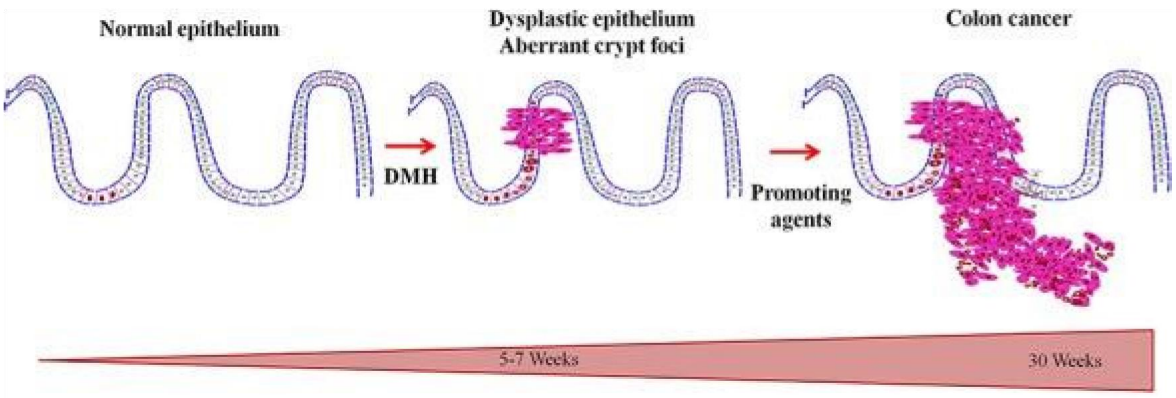


Fig 2.2 DMH injections induced the mutation in normal epithelium, later multi-step conversion of mutated epithelium to malignant one by 30 weeks (Venkatachalam *et al.,* 2020)

2.6.1 Role and History of DMH

DMH and its metabolite, azoxymethane (AOM), are procarcinogens that require metabolic initiation to shape DNA-receptive products. The alkylating agents DMH and AOM start their mutagenic movement through the methylation of guanine in DNA at N-7 position. The alkylated

guanine is matched with thymidine rather than cytosine by giving a proton, which leads to the modification of bases. Further ensuing replication, mispairing of guanine to thymine and cytosine to adenine, happens, which leads to mutations in DNA. Metabolism of these procarcinogenic compounds involves various metabolic enzymes, including xenobiotic-metabolizing enzymes, several N oxidation hydroxylation stages are processed by these enzymes, including the formation of ultimate carcinogen methylazoxymethanol (MAM). MAM is a reactive metabolite of DMH and AOM, which readily yields methyldiazonium ion that can alkylate macromolecules in the liver and colon (Fiala *et al*., 1984), demonstrated by different studies (Fiala *et al*., 1987; Notman *et al*., 1982).

MAM is a substrate of the nicotinamide adenine dinucleotide (NAD)- dependent dehydrogenase present in the colon and liver, proposing that the active metabolite of MAM might be the corresponding aldehyde (Zedeck MS *et al*., 1979), and these metabolites of CYP2E1 are moved to the colon by means of the bile or bloodstream. The principal pathway includes the hepatic conversion of DMH to AOM and azoxymethanol which subsequently undergoes glucuronic acid conjugation and biliary excretion (Fiala *et al*., 1977); notwithstanding, the toxicity of azoxymethanol doses influences the liver, cell membranes, and other organelles, which is supported by the release of aspartate and alanine amino transferases and alkaline phosphatase (Parthasarathy *et al*., 1986; Skakun *et al*., 1992). The glucuronides arrives at the colon, and it further goes through hydrolysis by bacterial enzymes to produce active carcinogen in the colonic lumen (Weisburger *et al*., 1971).

Cycasin, and a β-d-glucosyloxyazoxymethane isolated from crude material and the first metabolite of aglycone cycasin (MAM) are responsible for the tumors in the intestinal tract (Fiala, 1975).

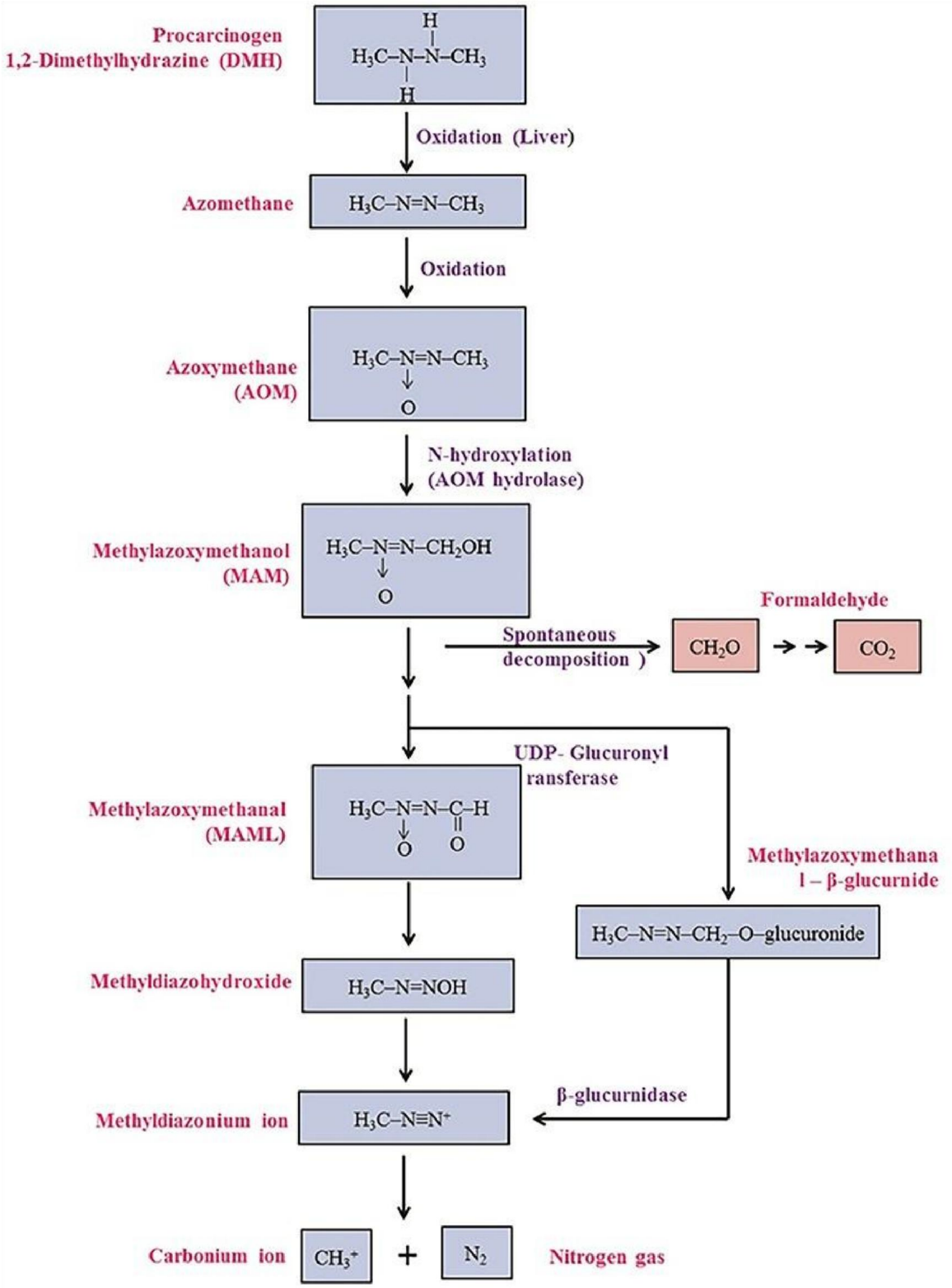


Fig 2.3 Metabolism of DMH (Venkatachalam *et al.,* 2020)

2.6.2 Routes and Dosage of DMH Administration to Rodents

The main route of administration of DMH is subcutaneous injection (Aranganathan *et al*., 2013). Despite the fact that it is a successful technique to induce tumor, intraperitoneal injections likewise prevailed to create tumors in the colon (Ghadi *et al*., 2013), while single injection intrarectal exposure of DMH also produced tumors in the colon of germ-free mice (Reddy *et al*., 1976; Chan *et al*., 1976).

The subcutaneous injection of DMH causes 100% epithelial dysplasia and precancerous lesions, found in a 12-week study (karaca *et al*., 2010). DMH causes a wide range of tumors in the GI tract of C57BL/6 mice, and most of tumors tracked down in small intestine and colon in the respective studies (Rowlatt *et al*., 1969; Newmark *et al*., 2001). Despite the fact that most of experimental colon cancer study carried out in rats, the high frequency of tumor in lower part of colon, a histopathological proof of multiple adenomas and subsequent progression of adenocarcinoma likewise approves the significance of mice in pathogenesis of colon cancer (Chang *et al*., 1978; Nambiar *et al*., 2003).

In animal studies, repeated exposure of DMH produces colon tumors, which shows the pathological features that are similar to sporadic forms of human colon cancer (Fiala *et al*., 1976). In the vast majority of the experiments, carcinogen DMH was injected subcutaneously. The subcutaneous site of injections does not possess the enzymes able to metabolize or react with DMH. Consequently, subcutaneously administered DMH is released into the circulation gradually, and afterward it arrives at the liver and gets metabolized into different intermediates (Fiala *et al*., 1977). Metabolic activation of DMH is displayed in Fig. 3.

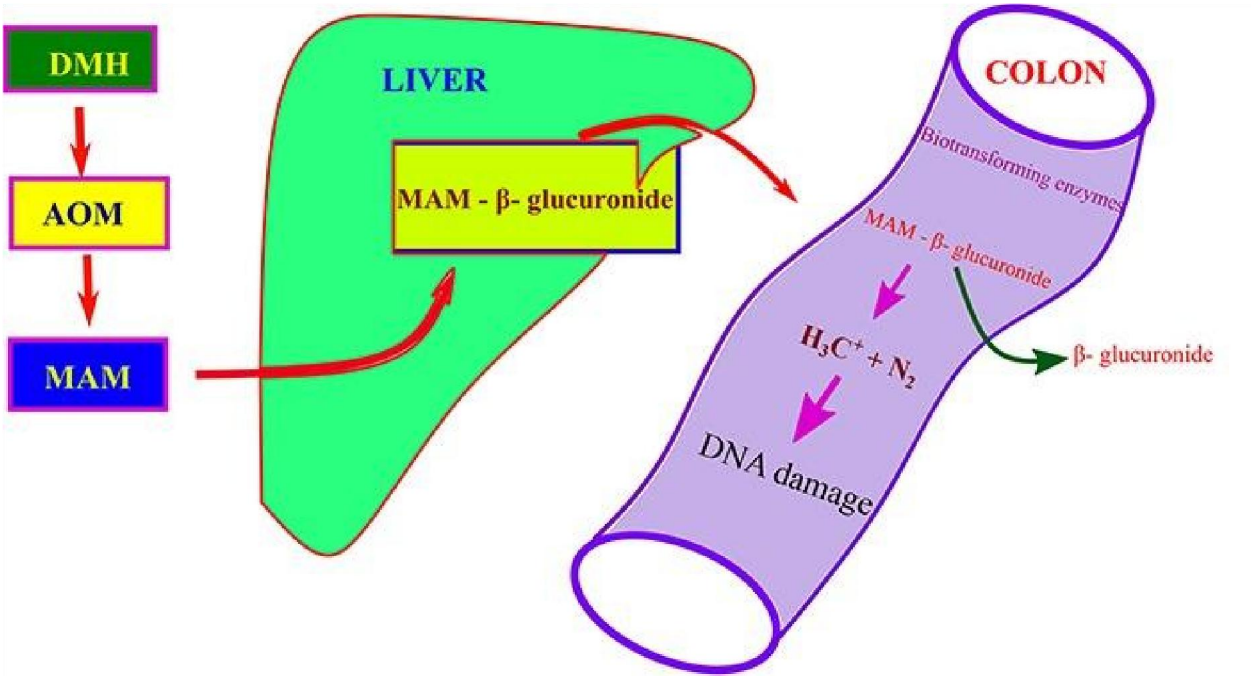


Fig 2.4 Transport of DMH from subcutaneous site to colon through glucoronidation (Venkatachalam *et al.,* 2020)

Colorectal cancer is the third most commonly diagnosed cancer and fourth most causes of cancer related mortality worldwide (Favoriti *et al*., 2016). The emerging nations additionally represent a disturbing expansion in the rate of incidence due to adaptation of the Westernized lifestyle (diet and reduced physical activity), rural populations and poor socioeconomic status (Goss *et al*., 2014; Karthi *et al*., 2016). The chemopreventive methodologies utilizing dietary supplements and pharmacologically active compounds act as a successful therapy against colorectal cancer compared with other treatments (Manju *et al*., 2010; Aranganathan *et al*., 2013). The procarcinogenic 1,2-dimethylhydrazine (DMH) is widely used to induce tumorigenesis in

experimental rodent models (Pozharisski *et al*., 1975; Manju *et al*., 2005; Sreedharan *et al*., 2009). It mimics human colon carcinoma in epithelial origin, colonic mucosa anatomy, morphology, histology and tumorigenic characteristics, subsequently, fills in as an ideal trial

model for chemoprevention studies (Nirmala *et al*., 2011; Muthu *et al*., 2013). DMH induction makes epithelial cells go through pathogenesis from preneoplastic lesion aberrant crypt foci (ACF) into adenomas and malignant adenocarcinomas. The macroscopic preneoplastic lesions appear after the exposure of DMH for two weeks, recommending that ACF are precursors of colorectal cancer (Kilari *et al*., 2016; (Ansil *et al*., 2013). Epidemiological studies propose that rodents fed with high fat eating diet (HFD) prompts an increase in the number of carcinogen induced aberrant crypt foci (Lasko *et al*., 1995). High fat diet intake in DMH induced tumor model could advance the formation of colonic neoplasm through inflammation, metabolic dysfunctions and increased cell proliferation (Zhu *et al*., 2014).

2.7 XELODA CAPECITABINE

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a prodrug, that is enzymatically converted to fluorouracil (antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. Capecitabine is indicated as treatment for a variety of cancer types. For colorectal cancer, capecitabine is indicated as a single agent or a component of a combination chemotherapy regiment for the adjuvant treatment of stage III colon cancer and treatment unresectable or metastatic colorectal cancer. It can also be used as a part of a combination chemotherapy perioperative treatment of adult locally advanced rectal cancer.

Capecitabine is a fluoropyrimidine carbamate belonging to a group of antineoplastic agents called antimetabolites, which kill cancerous cells by interfering with DNA synthesis (McKendrick and Coutsouvelis 2005; Longley *et al.,* 2003).

It is an orally administered systemic prodrug that has little pharmacologic activity until it is converted to 5-fluorouracil (5-FU) by enzymes that are expressed in higher concentrations in

many tumors. Capecitabine was designed specifically to overcome the disadvantages of 5-FU and to mimic the infusional pharmacokinetics of 5-FU without the associated complexity and complications of central venous access and infusion pumps (McKendrick and Coutsouvelis 2005). Particularly, since the enzymes converting 5-FU into active metabolites exist in the gastrointestinal tract, infusion of 5-FU can have gastrointestinal toxicity while also losing efficacy (Shimma *et al.,* 2005). Since capecitabine can be transported intact across the intestinal mucosa, it can be selectively delivered 5-FU to tumor tissues through enzymatic conversion preferentially inside tumour cells (Shimma *et al.,* 2005). 5-FU exerts its pharmacological action through the inhibition and interference of 3 main targets: thymidylate synthase, DNA, and RNA, leading through protein synthesis disruption and apoptosis (Longley, 2003; Zhang, 2008). Populationbased exposure-effect analyses demonstrated a positive association between AUC of 5-FU and grade 3-4 hyperbilirubinemia.

2.7.1 Mechanism of Action of Capecitabine

Capecitabine is a fluoropyrimidine anticancer drug. After absorption, it is metabolized in the liver to the intermediate 5'-deoxy-5-fluorouridine, which is subsequently converted into 5-fluorouracil (5-FU) by intracellular thymidine phosphorylase. 5-FU exerts cytotoxic effects on the cell by direct incorporation into DNA and RNA as well as by inhibiting thymidylate synthase. Since thymidine phosphorylase is present at 3-10 fold higher concentration in cancer cells compared normal cells, capecitabine's cytotoxic effect is selective for cancer cells.

Capecitabine undergoes metabolism by carboxylesterase and is hydrolyzed to 5'-DFCR. 5'DFCR is subsequently converted to 5'-DFUR by cytidine deaminase. 5'-DFUR is then hydrolyzed by thymidine phosphorylase (dThdPase) enzymes to the active metabolite fluorouracil. Fluorouracil is subsequently metabolized by dihydropyrimidine dehydrogenase to 5-fluoro-5, 6-dihydro-

fluorouracil (FUH2). The pyrimidine ring of FUH2 is cleaved by dihydropyrimidinase to yield 5-fluoro-ureido-propionic acid (FUPA). Finally, FUPA is cleaved by β-ureido-propionase to α-fluoro-β-alanine (FBAL).

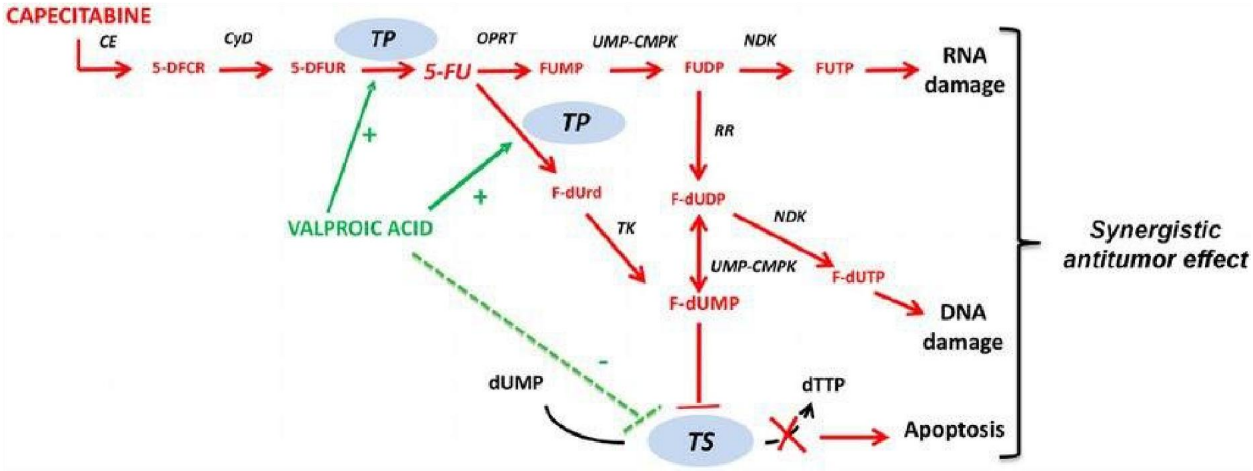


Fig 2.5 Mechanism of action of capecitabine and proposed synergetic interaction with valproic (Walko and Lindley, 2005)

2.8 THE COMPLETE BLOOD COUNT

The human body is primarily made up of water and cells. Many of the cells group together to form the skin, muscles, bones and organs, such as the heart, lungs, kidneys, etc. Such cells are stationary, staying in one place within the body. Some very special and important cells, however, move throughout the body by traveling (circulating) in the blood. These circulating cells provide oxygen to all of the stationary cells in the body, help fight infection throughout the body, and help stop bleeding after an injury. Information about these cells can provide important clues about the overall health of the body (Lewandrowski *et al.* 2016).

The complete blood count, or CBC, is a lab test that provides information about these circulating cells. First, a sample of your blood is collected and sent to the lab. A lab instrument then

automatically counts the number of each type of circulating cell. If results from the automated instrument are outside specified limits, a medical technologist will examine the cells closely so complete information about the cells can be provided.

Results from the CBC test can help:

- Provide basic information about your health
- Detect a health condition before you have any symptoms
- Confirm that a health condition exists
- Identify the causes of your symptoms
- Find out if your medicine is working
- Rule out a disease
- Establish a baseline that can be used for comparison with future test results

CBC test results are usually reported along with a reference range of expected or desired values to help guide your doctor in interpreting them. Reference ranges reflect the numeric values found in healthy people; however, a small number of healthy people (5%) have values that are higher or lower than the ones shown in the reference range. Thus, values higher or lower than those in the reference range might or might not indicate a medical condition.

In addition to the reference range, your doctor will consider other factors when interpreting your CBC test results. These factors include your personal and family medical history, results from a physical exam, and other test results. Your doctor will also consider factors that might cause an incorrect test result such as improper sample collection or handling. Therefore, it's important that you talk with your doctor about the meaning of your test results (Walls *et al.* 2017).

2.8.1 Red Blood Cells (RBCs)

RBCs play a vital role in transporting oxygen from the lungs to the rest of the body. These ovalshaped cells contain hemoglobin, the protein that binds oxygen while it is being carried to all the stationary cells in the body (cells in the skin, muscle, bone and organs). The chemical process that converts the nutrients found in food into energy requires oxygen. All the stationary cells require energy to function; thus, they need oxygen and are dependent on the RBCs to transport it (Heireman *et at.,* 2007).

Hemoglobin (Hb or Hgb) is an iron-rich protein that carries oxygen and makes the blood red. Since hemoglobin is contained only in the RBCs, a low number of RBCs leads to low levels of hemoglobin. However, if there is something wrong with the RBCs, hemoglobin levels can be low even when the RBC count (i.e. number of RBCs) is within the reference range. So a CBC test report includes the number of RBCs, the amount of hemoglobin, and other measurements related to the RBCs (Heireman *et at.,* 2007).

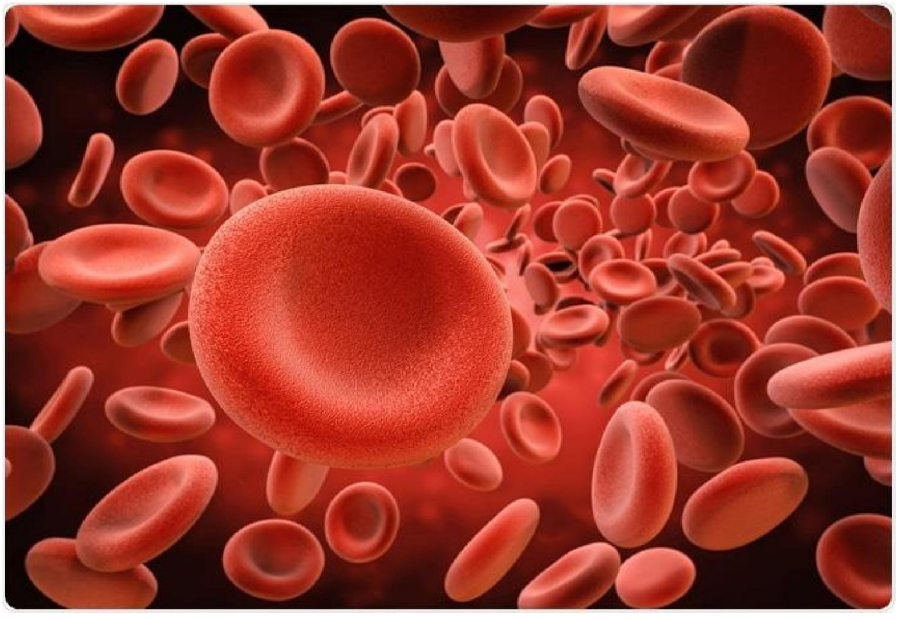


Fig 2.6 Red blood cells (Heireman *et at.,* 2007)

2.8.2 RBC Measurements

The hematocrit reflects the amount of space in the blood that is occupied by RBCs. Hematocrit measurements are affected by the number of RBCs and by the size of the RBCs.

The mean corpuscle (cell) volume (MCV) is a measurement of the average size of the RBCs. Small-sized RBCs result in a lower MCV, while larger RBCs result in a higher MCV.

The mean corpuscular hemoglobin (MCH) reflects the average amount of hemoglobin in a person's RBCs. RBCs with more hemoglobin result in a higher MCH and vice versa.
The mean corpuscular hemoglobin concentration (MCHC) is a measurement of the average amount of hemoglobin in the RBCs compared to the average size of the RBCs. Put another way, the MCHC is the ratio of the MCH to the MCV.

The red cell distribution width (RDW) reflects the degree of variation in size of the RBCs. Not all the RBCs are the same size; some are larger and some are smaller. The RDW measurement is affected by the size of the smallest RBC and the size of the largest RBC.

In patients with anemia, hemoglobin levels are low and the patient may be frequently tired and have little energy. This is because there is not enough hemoglobin to carry oxygen to the stationary tissues; thus, there is not enough oxygen available to convert nutrients into energy. The RBC count, hematocrit level, MCV, MCH and MCHC might also be low in patients with anemia (Hurley, 2007).

Low RBC counts, hemoglobin and hematocrit levels can be caused by other things too, such as a lot of bleeding or malnutrition (not enough nutrients in the food eaten). Kidney disease, liver disease (cirrhosis), cancer, and medications used to treat cancer can also cause low levels (Wan *et al.,* 2019).

An increased RBC count and increased levels of hemoglobin and hematocrit may be caused by dehydration (not enough water in the body) or by some diseases (Hurley, 2007).

2.9 HEMATOLOGICAL PARAMETERS: MCV, MCH, AND MCHC

The hematological parameters Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are vital indicators of erythrocyte morphology and function. MCV reflects the average volume of red blood cells (RBCs) and is a critical parameter in the diagnosis of anemia and other hematological disorders. MCH measures the average amount of hemoglobin per RBC, while MCHC indicates the concentration of hemoglobin in a given volume of packed RBCs. Alterations in these parameters often signify underlying pathological conditions and can provide valuable diagnostic insights (Hoffbrand et al., 2016).

- **Mean Corpuscular Volume (MCV)**

The Mean Corpuscular Volume (MCV) refers to the average volume of a red blood cell (RBC). It is typically measured in femtoliters (fL). MCV is an essential parameter in the complete blood count (CBC) and is used to classify anemia and other hematological disorders based on RBC size.

Significance:

MCV provides valuable information about the size of red blood cells, which can help in the diagnosis and classification of anemia.

An MCV value outside the normal range may indicate the presence of underlying health conditions such as iron deficiency anemia, vitamin B12 deficiency anemia (pernicious anemia), or folate deficiency anemia.

Understanding MCV variations can aid healthcare professionals in determining the underlying cause of anemia and guiding appropriate treatment strategies.

Effects on the Body:

Macrocytic Anemia: A high MCV (> 100 fL) indicates macrocytic anemia, characterized by the presence of larger-than-normal RBCs. This condition may result from vitamin B12 or folate deficiency, bone marrow disorders, or liver disease. Macrocytic anemia can lead to symptoms such as fatigue, weakness, and shortness of breath due to reduced oxygen-carrying capacity of RBCs.

Microcytic Anemia: Conversely, a low MCV (< 80 fL) indicates microcytic anemia, characterized by smaller-than-normal RBCs. Common causes of microcytic anemia include iron deficiency, chronic diseases, and thalassemia. Microcytic anemia can result in symptoms similar to macrocytic anemia, including fatigue and weakness, as well as pale skin and cold hands and feet.

❖ **Mean Corpuscular Hemoglobin (MCH)**

The Mean Corpuscular Hemoglobin (MCH) refers to the average amount of hemoglobin (Hb) per red blood cell. It is typically measured in picograms (pg). MCH provides information about the hemoglobin content of individual RBCs and is used in conjunction with other hematological parameters to assess various types of anemia.

Significance:

MCH helps in evaluating the amount of hemoglobin available in each red blood cell, which is crucial for oxygen transport and delivery to tissues.

Abnormal MCH values can indicate underlying anemia or other hematological disorders, guiding further diagnostic investigations and treatment decisions.

Monitoring MCH levels over time can help assess the response to treatment and the progression of hematological conditions.

Effects on the Body:

- Normochromic Anemia: When MCH values fall within the normal range, it suggests the presence of normochromic anemia, where the amount of hemoglobin in RBCs is adequate. Normochromic anemia can result from various causes, including chronic diseases, kidney disorders, and hemolytic anemias. Symptoms may include fatigue, weakness, and shortness of breath, depending on the underlying cause and severity of anemia.

- Hypochromic Anemia: A low MCH (< 27 pg) indicates hypochromic anemia, characterized by RBCs with reduced hemoglobin content. Hypochromic anemia is

commonly associated with iron deficiency, where insufficient iron leads to decreased hemoglobin synthesis and smaller, paler RBCs. Symptoms of hypochromic anemia may include fatigue, pale skin, brittle nails, and difficulty concentrating.

❖ **Mean Corpuscular Hemoglobin Concentration (MCHC)**

The Mean Corpuscular Hemoglobin Concentration (MCHC) refers to the average concentration of hemoglobin in a given volume of packed red blood cells. It is typically expressed as a percentage. MCHC provides insights into the concentration of hemoglobin within RBCs and aids in the diagnosis and classification of anemia.

Significance:

MCHC helps assess the degree of hemoglobin saturation within red blood cells, which influences their oxygen-carrying capacity.

Abnormal MCHC values can indicate various types of anemia and guide further diagnostic evaluations and treatment decisions.

Monitoring MCHC levels is essential for evaluating the response to treatment and managing hematological disorders effectively.

Effects on the Body:

- Normochromic Anemia: A normal MCHC (32-36 g/dL) suggests normochromic anemia, where the hemoglobin concentration within RBCs is within the normal range. Normochromic anemia may result from conditions such as chronic inflammation, kidney

disease, or certain genetic disorders. Symptoms and complications of normochromic anemia vary depending on the underlying cause and severity of anemia.

- Hypochromic Anemia: A low MCHC (< 32 g/dL) indicates hypochromic anemia, characterized by RBCs with reduced hemoglobin concentration. Hypochromic anemia is commonly associated with iron deficiency anemia, where inadequate iron leads to decreased hemoglobin synthesis and paler RBCs. Symptoms of hypochromic anemia may include fatigue, weakness, shortness of breath, and pale skin.

CHAPTER THREE
MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Apparatus

The apparatus used during the research study were procured from a registered vendor and were at experimental standard at the point of purchase. They include:

1. *Acalypha wilkesiana*	Edo, Nigeria
2. Aluminium foil paper	
3. Beakers (250ml, 100ml, 50ml)	Pyrex, England
4. Cages	
5. Cardboard papers	
6. Ceramics plate	
7. Cheese cloth	
8. Conical Flask	Pyrex, England
9. Cotton wool	Fantastik, England
10. Cover slip	Pyrex, England
11. Cuvette	Pyrex, England
12. Detergent	
13. Dissecting set	Tecmel Tecmel, USA
14. EDTA container	Fantastik, England
15. Feed	Edo, Nigeria
16. Filter paper	Fantastik, England

17. Glass slide	Pyrex, England
18. Gloves	Fantastik, England
19. Masking tape	
20. Measuring cylinder	Pyrex, England
21. Measuring Cylinders	Pyrex, England
22. Micro pipettes	Microlux, England
23. Micro-centrifuge tubes	Pyrex, England
24. Microplate washer	Oatek, China
25. Mortar and pestle	Merck, Germany
26. Multi-channel micro pipette	Microlux, England
27. Nose mask	Fantastik, England
28. Oral gavage	
29. Petri dish	Pyrex, England
30. Plain tube	Fantastik, England
31. Round bottom flask	Pyrex, England
32. Scissors	Tecmel Tecmel, USA
33. Separating funnel	Fantastik, England
34. Spatula	Pyrex, England
35. Syringe (1ml and 5ml)	Pyrex, England
36. Test tube racks	Pyrex, England
37. Test tubes	Pyrex, England
38. Universal bottles	Fantastik, England
39. Wistar rats	

3.1.2 Equipment

Major equipment used include:

1. Centrifuge [Tecmel Tecmel, USA]
2. Electric grinder
3. Freeze drier: Water extracts and fractions isolated with a high-water content was dried using a freeze drier [Christ Alpha, Germany].
4. Grinder: The dried plant materials were grounded with a heavy-duty grinder (Waring, USA) into powder form.
5. Haier Thermocool Chest Freezer [Model: HTF-319H]
6. Hisense refrigerator (Model: REF302DR),
7. Incubator [Tecmel Tecmel, USA]
8. Oven [Tecmel Tecmel, USA]
9. Rotary evaporator: Extracts and other forms of liquid samples were concentrated using rotary evaporator [Buchi, USA] at the range of room temperature to not more than 40ºC.
10. Sensitive electronic balance [TYPE: LAC214C, 704010]
11. Spectrophotometer UV-Vis: Total phenolic contents were conducted using a UV-Vis spectrophotometer from Biochrom Libra S12 [USA].
12. Water bath [Tecmel Tecmel, USA]

3.1.3 Reagents

All the chemicals and reagents used in this study were of analytical grade. They include;

1. 1,2-dimethylhydrazine — Sigma, Germany
2. 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB) — Merck, Germany
3. α-tocopherol (vitamin E) — Merck, Germany
4. Absolute ethanol — BDH, England
5. Chloroform, hydrochloric acid (HCl) — May and Bayer, England
6. Diphenylcarbazone indicator — Merck, Germany
7. Distilled water — Trigas, UNIBEN
8. DPX mountant — BDH, England
9. Epinephrine (adrenaline) — BDH, England
10. Ethanol
11. Ethylene diamine tetraacetic acid (EDTA) — BDH, England
12. Formalin — BDH, USA
13. Gentian Violet
14. Hydrogen peroxide (H_2O_2) — BDH, England
15. Methylated spirit — Ivee Pharmaceutical Limited
16. Methyline blue — BDH, England
17. Normal Saline — Ivee Pharmaceutical Limited
18. Petroleum ether — BDH, England
19. Phenylhydrazine hydrochloride — [Fisher Scientific Company, New Jersey, USA]
20. Phosphoric acid (H_3PO_4) — May and Bayer, England
21. Potassium chloride (KCl) — May and Bayer, England

22. Potassium hydroxide (KOH) May and Bayer, England
23. Potassium permanganate ($KMnO_4$) May and Bayer, England
24. Pyrogallol May and Bayer, England
25. Reduced glutathione (GSH) standard Merck, Germany
26. Sodium carbonate (Na_2CO_3) May and Bayer, England
27. Sodium chloride (NaCl) May and Bayer, England
28. Sodium citrate May and Bayer, England
29. Sodium dihydrogen phosphate (NaH_2PO_4) May and Bayer, England
30. Sulphuric acid (H_2SO_4) BDH, England
31. Thiobarbituric acid (TBA) Merck, Germany
32. Trichloroaceticacid (TCA) Merck, Germany
33. Vitamin E [Nature Made, USA] was purchased from a Pharmacy shop within University of Benin premises.

3.2 METHODS

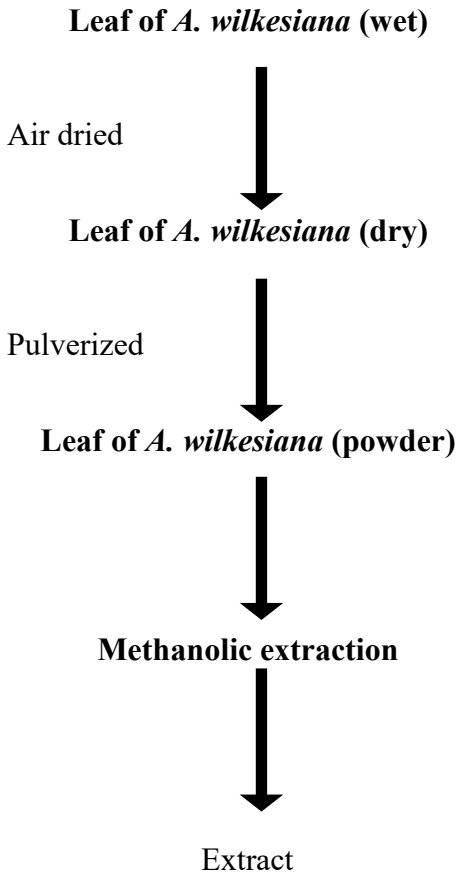
3.2.1 Plant Collection and Identification

Between March and May 2020, fresh *Acalypha wilkesiana* leaves were collected from the University of Benin's campus in Benin City, South-Western Nigeria. A Plant Biologist from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin identified and authenticated the leaves. 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 40 C, and pulverized with an electric grinder.

3.2.2 Extraction of Plant

The pulverized plant sample was weighed in order to determine the appropriate weight of the plant and to calculate the percentage yield after extraction. Four thousand three hundred grams (4,300g) of powdered *Acalypha wilkesiana* sample was used in total. The ground plant material was macerated in 6,400ml of methanol (methanol extract) in a glass container covered with foil paper for 3 days (72 hours). It was constantly stirred, and at the end of the third day, it was filtered through two layers of cheesecloth and then with filter paper to remove all residues. A rotary evaporator at 40°C was used to concentrate the filtrate, which was then freeze-dried using a freeze dryer and weighed.

Schematics for the extraction of *Acalypha wilkesiana*



3.2.3 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:

$$\% \text{ yield} = \frac{\text{weight of dried extracted sample}}{\text{weight of the pulverized plant sample}} \times 100$$

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

$$\% \text{ yield} = 450\text{g}/4300\text{g} \times 100$$

$$= 45000/4300$$

$$= 450/43$$

$$= \mathbf{10.5}$$

3.3 Preparation of Animals used

69 Male Wistar rats of 3 weeks weighing an average of 90g were obtained from the Department of Animal Environmental Biology, Faculty of Life Sciences, University of Benin, Benin city, Edo State, Nigeria. The animals were maintained under controlled conditions and 12-hour light-dark cycles.

The animals were acclimatized for fourteen days (2 weeks) before the commencement of the study. They were housed in cages and given free access to food and water. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria).

The animals were divided into nine groups. Six of these groups had 8 rats while the remaining three groups had 7 rats. The animals were then stained using gentian violet in various body parts for purpose of identification. The stained parts include head, back, left leg, left hand, right hand, right leg and tail.

3.4 Experimental Design

The animals were separated into nine groups. Group 1,2 and 8 had seven animals while Group 3,4,5,6,7 and 9 had eight animals. The experimental design of each group are as follow:

Group 1: Served as the normal control group. In this group, no administration was given.

Group 2: Served as the positive control/treatment group. In this group, DMH was administered subcutaneously three times a week. After 18 doses of DMH, the standard drug (Xeloda

Capecitabine) was then orally administered daily via a gavage for the next six weeks.

Group 3: DMH + Low dose of extract (200mg)

Group 4: DMH + Medium dose of extract (400mg)

Group 5: DMH + High dose of extract (800mg)

For the animals in group 3 to 5, DMH was administered subcutaneously three times a week. After 18 doses of DMH in six weeks, the administration was then switched to ethanol leave extract of *A. wilkesiana* which was orally administered daily via a gavage at doses of 200, 400, and 800 mg/kg body weight for a period of 6 weeks.

Group 6: Low dose of extract (200mg) + DMH

Group 7: Medium dose of extract (400mg) + DMH

Group 8: High dose of extract (800mg) + DMH

For the animals in group 6 to 8, ethanol leave extract of *A. wilkesiana* was orally administered daily via a gavage at doses of 200, 400, and 800 mg/kg body weight for a period of 12 weeks . After the first 6 weeks of the experiment, there was a simultaneous administration of DMH subcutaneously three times a week for another six weeks.

Group 9: Served as negative control/DMH group. In this group, DMH was induced subcutaneously three times a week for a period of 6 weeks. After 18 doses, all adminstrations ceased. No form of treatment was given.

After 12 weeks, the animals were monitored for an extra week and then fasted for 12 hours before euthenization.

The use of rats for the study was according to the Ethical Guidelines Involving Whole Animal Testing of the Animal Ethics Committee, Faculty of Life Sciences, University of Benin

3.5 Euthanization and Collection of Tissue Samples

At the end of the animal study, animals were fed and monitored for one week and euthanized in a chloroform chamber. The liver, lungs, heart, pancreas, kidney and colon of the animals were excised via lateral dissection of the abdominal cavity and blood was collected from the cardiac puncture and abdominal aorta and all samples were preserved in containers under cold temperature using a freezer.

3.6 Preparation of Tissue Homogenate

Using a mortar and pestle and a 20% (w/v) cold normal saline solution, desired organ samples were homogenized. The supernatant was then obtained by centrifuging the homogenates at 3000 rpm for 10 min. When it came time for biochemical examination, the supernatants were then kept in a freezer temperature of -20 C until required.

3.7 Preparation of Blood Samples

The blood samples stood for 1 hour and a clear serum was produced and collected after centrifuging blood samples at 3000 rpm for 10 minutes, and it was kept in a freezer at -20 C until the required time for biochemical analysis.

3.8 BIOCHEMICAL ASSAYS

3.8.1 Histopathological Examination

After collecting blood on the final day, the animals were euthanized to isolate the liver, lungs, kidneys, heart, pancreas and colon for examination. Each rat's kidneys, heart, lungs, liver, pancreas and 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).

3.8.2 Haematological Indices

The following haematological parameters were performed on the blood sample: Pack cell volume (PCV), haemoglobin concentration, mean cell volume (MCV), Mean cell haemoglobin concentration, total differential white blood cell count, platelet etc using a standard haematology analyser.

3.8.3 Haematology Analyzer

This employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The Hb detector block measures the haemoglbin concentration using the non – cyanide haemoglobin method. Most electronic blood cell analyzers count blood cells by impedence.

3.8.4 Principle of Impedence Analyzer

Blood cells are diluted in a buffer electrolyte solution. A measured volume of the sample passes through an aperture tube between two electrodes. Interruption of the current by the non – conducting blood cells alters the electrical charge and a pulse is produced. The amplitude of each pulse is proportional to the volume of the ells which caused it. A threshold circuit ensures only those pulses that exceed the pre – set threshold levels are counted. The cell count is determined from the number of pulses obtained from a measured volume of blood (Cheesebrough, 2010). The analysis of the pulse height enables mean cell volume (MCV) to be measured and the haematocrit to be calculated from the MCV value and RBC count. In this analyzer, haematocrit is determined from voltage pulse data and the MCV calculated from the haematocrit value. The haemoglobin concentration is used to with the RBC count, MCV, and haematocrit, to calculate the MCH and MCHC values (Cheesebrough, 2010). More advanced analyzers in addition to determining haemoglobin, WBC, RBC, platelets, haematocrit, MCV, MCHC and MCH, also provide red cell distribution width (RDW), platelet distribution width (PDW) and a white cell differential width (WCDW).

CHAPTER FOUR

RESULTS

TABLE 1: Eethanol leaf extract of *Acalypha wilkesiana* on some Hematological Parameters in 1, 2-Dimethylhydrazine exposed Wistar Rats

			GROUPS	
Parameter	**CONTROL (A)**	**GROUP 2 (B)**	**GROUP 3 (C)**	**GROUP 7 (G)**
MCV (%)	5.806 ± 1.393	6.487 ± 5.526^{c}	6.792 ± 2.708^{a}	7.040x10^{1} ± 1.645x10$^{\circ a}$
MCH (%)	2.202x10^{1} ± 0.299^{b,c}	2.538x10^{1} ± 1.573x10$^{\circ a}$	2.460x10^{1} ± 1.053x10$^{\circ a}$	2.585 ± 0.742^{a}
MCHC (%)	3.798 ± 0.453 ^{b,c}	3.630 ± 0.601^{a}	3.622 ± 0.132^{a}	3.673 ± 0.381^{a}

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= 500 mg/ kg bwt standard vitamin E treatment; Group 3= anemic control; Group 4= 200 mg/kg bwt aqueous extract treatment; Group 5= 400 mg/kg bwt aqueous extract treatment; Group 6= 600 mg/kg bwt treatment; Group 7= 200 mg/kg ethanol extract treatment; Group 8= 400 mg/kg methanol extract treatment; Group 9= 600 mg/kg methanol extract treatment; Group 10= 500 mg/kg methanol extract control.

CHAPTER FIVE

DISCUSSION

The investigation aimed to evaluate the impact of ethanol leaf extract of *Acalypha wilkesiana* on selected hematological parameters in Wistar rats exposed to 1,2-dimethylhydrazine (DMH), a known carcinogen. The parameters assessed included Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). Results are presented in Table 4.1, comparing different treatment groups with controls.

MCV, indicative of the average volume of red blood cells, showed interesting variations across treatment groups. Compared to the control group (Group A), rats treated with 500 mg/kg bwt standard vitamin E (Group B) exhibited a slight increase in MCV, although not statistically significant. Notably, groups treated with ethanol leaf extract of *Acalypha wilkesiana* at 200 mg/kg bwt (Group G) demonstrated a significant elevation in MCV compared to both the control and vitamin E-treated groups. This observation suggests a potential stimulatory effect of *Acalypha wilkesiana* extract on erythrocyte volume, warranting further investigation into its underlying mechanisms.

MCH, representing the average amount of hemoglobin per red blood cell, exhibited notable variations among treatment groups. Rats in the anemic control group (Group C) displayed a significant decrease in MCH compared to the normal control group (Group A), indicative of reduced hemoglobin content per erythrocyte. However, treatment with ethanol leaf extract of *Acalypha wilkesiana* at both 200 mg/kg bwt and 400 mg/kg bwt (Group G) resulted in a marked increase in MCH compared to the anemic control group, suggesting a potential hematopoietic

effect of the extract. This finding underscores the therapeutic potential of *Acalypha wilkesiana* in ameliorating anemia-associated alterations in MCH levels.

MCHC, reflecting the concentration of hemoglobin within red blood cells, demonstrated intriguing trends across treatment groups. While the anemic control group (Group C) exhibited a significant decrease in MCHC compared to the normal control group (Group A), treatment with ethanol leaf extract of *Acalypha wilkesiana* at both 200 mg/kg bwt and 400 mg/kg bwt (Group G) resulted in a notable increase in MCHC levels. This observation suggests a potential role of *Acalypha wilkesiana* extract in restoring hemoglobin concentration within erythrocytes, highlighting its hematoprotective properties in the context of anemia-induced alterations in MCHC.

CONCLUSION

Investigating the effects of *Acalypha wilkesiana* leaf extract on hematological parameters in Wistar rats exposed to 1,2-dimethylhydrazine (DMH) provides valuable insights into its potential therapeutic applications. Through meticulous analysis of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC), significant alterations were observed in response to *Acalypha wilkesiana* treatment, indicating its hematoprotective properties. These findings underscore the importance of exploring natural remedies for mitigating hematological abnormalities induced by carcinogenic agents. Further research is warranted to elucidate the underlying mechanisms of action and optimize dosage regimens for potential clinical translation, offering promising avenues for combating hematological disorders and enhancing patient well-being.

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