AMELIORATING EFFECTS OF CHLOROFORM EXTRACT OF *Phyllantus amarus* AND *Piper guin*eense ON 1,2-DIMETHYLHYDRAZINE INDUCED CANCER ON WISTAR RAT.

BY
IYAYOMWANGBE OSARUMWENSE RACHEL
LSC2006806

DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF LIFE SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY

JANUARY, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR DEGREE (B.SC.) IN BIOCHEMISTRY.

JANUARY 2025

CERTIFICATION

This is to certify that this work was carried out by IYAYOMWANGBE OSARUMWENSE RACHEL with the matriculation number LSC2006806 in the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo state.

Dr. F.O. Omoregie
Project Supervisor Date

Dr.S.I Ojeaburu
Project coordinator

Prof.E.C onyeneke Date
Head of department

_____ _____
External supervisor Date

DEDICATION

This work is dedicated to God for his unending love and provisions for me throughout my days in school and to my dear mother, Mrs Beauty Omoruyi for her constant support.

ACKNOWLEDGMENT

My profound gratitude to God almighty for His infinite love and mercy towards me. I wish to specially appreciate my mother, Mrs Beauty Omoruyi for her unending support throughout my days in the University of Benin. To the Omoruyi Family in general I say a big thank you.
I also want to use this opportunity to thank Dr. Frank Omoregie for being the most understanding and supportive project supervisor. God bless you sir.

To all project group members, all friends who in one way or the other impacted my academic pursuit, I say a big thank you and I can't wait to watch us all achieve our goals.

TABLE OF CONTENT

ABSTRACT

Colorectal cancer is a major health concern worldwide, characterized by uncontrolled cell growth in the colon or rectum. While conventional treatments such as chemotherapy and surgery exist, they can be physically and emotionally taxing. This study investigates the ameliorating effects of chloroform extracts of *Phyllanthus amarus* and *Piper guineense* on 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in Wistar rats. The research aimed to determine the potential of these plant extracts in mitigating oxidative stress and tissue damage caused by DMH exposure. 18 male Wistar rats were divided into three groups: a control group, a DMH-only group, and a treatment group receiving DMH along with 250 mg/kg of the chloroform extract. DMH was administered orally using a dolphin's gavage for 8 weeks (twice a week).At the end of DMH administration, 250 mg/kg bwt of Chloroform fraction of P.amarus and P.guinensis were administered with the aid of a dolphin's gavage for 2 weeks (daily) treatment period. Biochemical markers such as liver and kidney function enzymes, oxidative stress indicators (superoxide dismutase, catalase, and malondialdehyde), and serum electrolytes as well as Tumor Protein 53(TP53)were analyzed. The results indicated a significant increase in oxidative stress markers in the DMH-only group, while rats treated with the plant extracts exhibited improved antioxidant enzyme activities and reduced markers of tissue damage. These findings suggest that Phyllanthus amarus and Piper guineense

possess potential chemoprotective properties against DMH-induced colorectal cancer through antioxidant mechanisms. Further research is necessary to explore their clinical applicability and potential therapeutic benefits in cancer prevention and treatment.

CHAPTER 1

INTRODUCTION

Over time, plants have been considered good sources of medicinal compounds and have been used in the treatment of different diseases and maintenance of health. Over 50% of all modern chemical drugs originate from plants and are used in herbal treatment due to their high pharmacological activity (Burton *et al*, 1983).

Colorectal cancer,a type of cancer that develops in the colon or rectum is one of the most prevalent and deadly forms of cancer worldwide(Alzahrani, Al-Doghaither, and Al-Ghafari, 2021). The cancer is characterized by uncontrollable growth of abnormal cells in the colon or rectum, leading to formation of tumors. This cancer can metastasize and spread to other organs (Sawicki *et al*., 2021).

Surgery, chemotherapy and radiation therapy have made significant steps in treating colorectal cancer, these treatments can be physically and emotionally taxing to patients (Mishra *et al.,* 2013). Resistance to these treatments can develop leading to limits in their effectiveness. As a result of this, there is a growing recognition of the importance of alternative treatments in the fight against colorectal cancer (Chong, 2014)

One of such avenue of exploration is the use of herbal remedies and natural compounda. Treatments like the chloroform extract of *Phyllantus* amarus and *Piper guineense* shows significant help in mitigating the side effects of conventional therapies, bolstering the body's defense against cancer and potentially inhibiting tumor growth (Bose, Banerjee, and Chattopadhyay, 2022).

AIMS AND OBJECTIVES

AIM OF STUDY

This study was aimed at examining and presenting an overview of the therapeutic and medicinal effects of chloroform extract of *Phyllantus amarus* and *Piper guineense* on 1,2-Dimethylhydrazine induced colon carcinogenesis in *Rattus norvegicus.*

OBJECTIVES

The objectives of this study are:

- To induce colorectal cancer in *Rattus norvegicus*
- To obtain chloroform extract of *Phyllantus amarus* and *Piper guineense* from dried leaves of plants
- To ascertain if *Phyllantus amarus* and Piper guineense can ameliorate 1, 2-Dimethylhydrazine induced colon carcinogenesis in *Wistar rat*

LITERATURE REVIEW

COLORECTAL CANCER

Cancer encompasses a broad range of diseases characterized by uncontrolled cell growth. These cancerous cells can spread to other parts of the body through metastasis, often leading to severe consequences (Son *et al*., 2007). Colorectal cancer, commonly referred to as colon cancer, is the most prevalent form of gastrointestinal cancer, occurring when cells in the large intestine and rectum grow uncontrollably. It typically begins as non-cancerous polyps in the lower digestive tract, which can later develop into malignant tumors. While many organs are susceptible to cancer, the liver, colon, and breast are among the most frequently affected. Colon cancer originates in the large intestine and impacts over a million people globally each year (Cherbuliez, 2013). In oncologic pathology, colorectal cancer ranks as the third most commonly diagnosed cancer (Smith *et al.*, 2004).

Despite being one of the primary causes of cancer-related mortality, the exact pathological mechanisms of colorectal cancer remain unclear (Son *et al.,* 2007). The disease progresses through multiple stages, influenced by both genetic and environmental factors. It begins with the transformation of normal colonic epithelium into dysplasia, advancing through a benign precursor stage known as a premalignant polyp, which may eventually develop into invasive cancer. In addition to genetic predisposition, dietary habits play a significant role in determining an individual's risk. A healthy diet, particularly one rich in fruits and vegetables, has been shown to lower the likelihood of developing colon cancer (Cherbuliez, 2013).

Risk Factors for Colorectal Cancer

Several factors contribute to the development of colorectal cancer, including genetic predisposition, lifestyle choices, and underlying medical conditions.

Inflammatory Bowel Disease (IBD)

IBD is the third most significant risk factor for colorectal cancer. It consists of chronic, incurable conditions that impact the gastrointestinal immune system, leading to persistent inflammation. The two primary forms of IBD are Crohn's disease and ulcerative colitis. While the exact cause remains unknown, it is believed that a combination of genetic, immune, and environmental factors plays a role in its development (Rawla *et al.,* 2019; Valle et al., 2019; Yang *et al.,* 2020).

Colon Polyps

Colon polyps are abnormal tissue growths that develop on the colonic mucosa, with some having the potential to become cancerous. They are classified into two main types: non-neoplastic (hamartomatous, hyperplastic, and inflammatory polyps) and neoplastic (adenomatous polyps). Adenomatous polyps are of particular concern due to their potential to develop into malignancies. Research suggests that approximately 95% of colorectal cancers originate from these polyps, but only about 5% progress to cancer. The transformation from an adenomatous polyp to invasive adenocarcinoma typically takes 5–15 years. The likelihood of malignant transformation increases with polyp size, severity of dysplasia, and advancing age. Since around 40% of individuals aged 50 and older have one or more adenomatous polyps,

early detection and removal are crucial in preventing cancer (Amersi *et al.,* 2005; Shussman and Wexner, 2014).

Diet High in Red and Processed Meat

A diet rich in red and processed meats significantly raises the risk of colorectal cancer. Studies indicate that consuming 100 grams of red meat daily increases the risk by 17%, while eating 50 grams of processed meat per day raises it by approximately 18% (Aykan, 2015; Aran *et al.,* 2016).

Diet Low in Fiber, Fruits, and Vegetables

A high-fiber diet has been shown to lower the risk of colorectal cancer by up to 50%. Fruits and vegetables are excellent natural sources of fiber and contain antioxidants and anti-inflammatory compounds that help protect against cancer development (Amersi *et al.,* 2005).

Overweight and Obesity

Excessive body fat is a well-established risk factor for colorectal cancer. Overweight men have a 50% higher risk, while overweight women face a 20% increased risk compared to individuals with a normal body weight. Additionally, the overall risk of colorectal cancer rises by 3% for every five kilograms of weight gain (Rawla *et al.,* 2020).

Cigarette Smoking

Smoking is strongly associated with an increased risk of colorectal cancer, with smokers being 2–3 times more likely to develop the disease than non-smokers. The risk also escalates with prolonged exposure to tobacco smoke. Furthermore, smoking is estimated to contribute to up to 12% of colorectal cancer-related deaths (Giovannucci, 2001; Thelin and Sikka, 2015).

Alcohol Consumption

Excessive alcohol intake is a major factor in colorectal cancer development. Regular heavy drinkers can increase their risk by up to 52% (Marley and Nan, 2015).

Screening for Colorectal Cancer

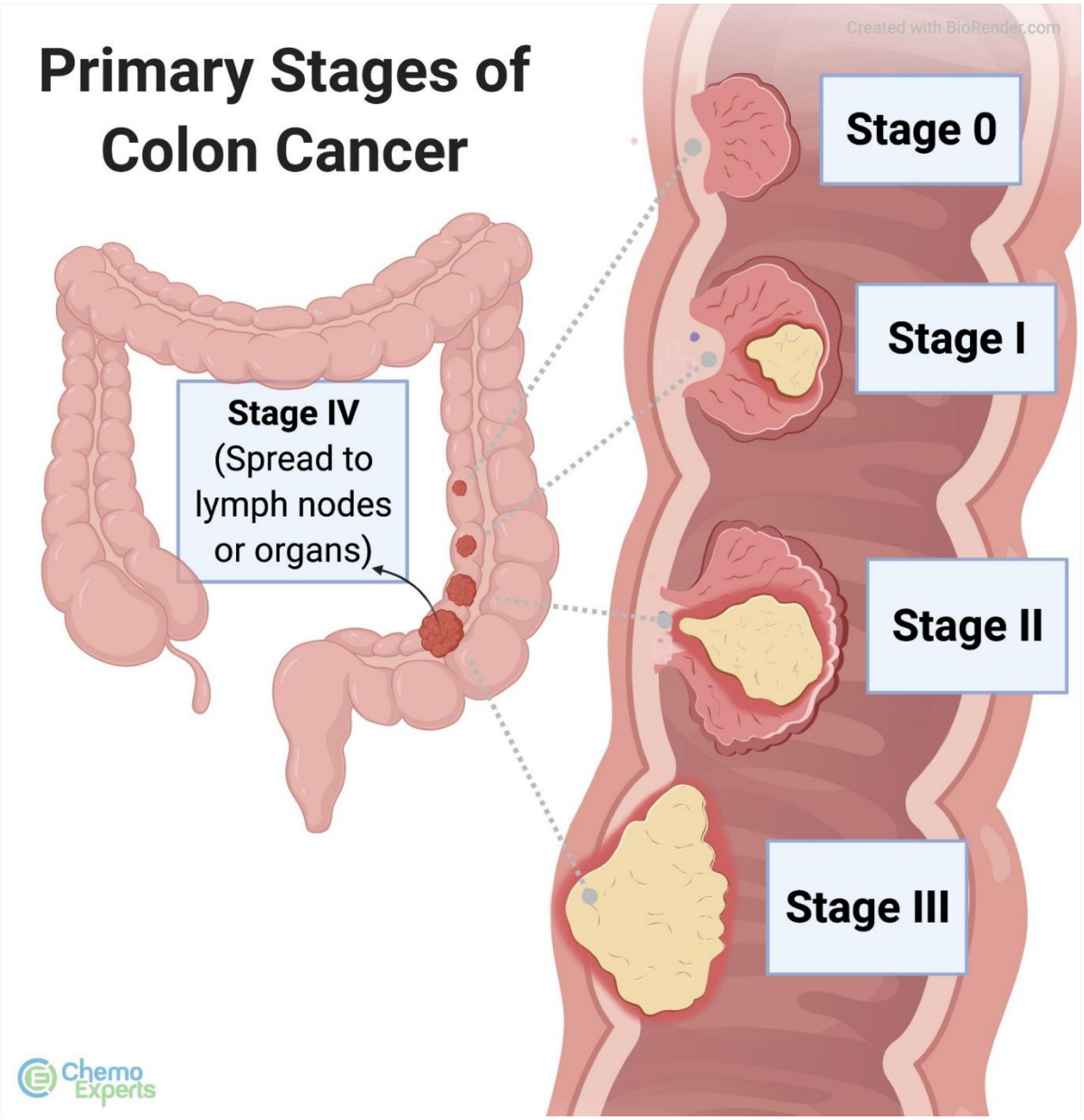
Colorectal cancer screening involves detecting precancerous polyps or early-stage cancer before symptoms appear, improving the chances of successful treatment. Screening methods include high-sensitivity fecal occult blood testing (FOBT), sigmoidoscopy, and colonoscopy (Torre et al., 2015).

Prevention and Control

Colorectal cancer prevention involves adopting a healthy lifestyle and undergoing regular screenings. Consuming fiber-rich foods, fruits, and vegetables, as well as maintaining adequate levels of vitamin D and calcium, can help reduce cancer risk. Regular physical activity, limiting alcohol intake, quitting smoking, reducing red meat consumption, and using anti-inflammatory medications may also help

prevent polyp formation and slow cancer progression. Additionally, genetic testing and environmental modifications play essential roles in colorectal cancer prevention and control (Breitkopf *et al.,* 2014). Treatment for colorectal cancer varies based on the patient's overall health, tumor size, and location.

- **Stage 0:** If the cancer is confined to a single polyp, it can be removed through colonoscopy.
- **Stages I–II**I: If the cancer has spread beyond a small polyp, a bowel resection may be required. Surgery typically involves a radical colectomy with a margin of at least 5 cm. Laparoscopic surgery has been proven as effective as traditional open surgery (Arribas-Martin *et al.,* 2014).
- **Metastatic Colorectal Cancer**:Patients with distant metastases may benefit from chemotherapy, including irinotecan in combination with fluorouracil and leucovorin, which has been shown to improve survival rates (Herbert e*t al.,* 2014). Fluorouracil remains the most widely used chemotherapy drug for colon cancer, significantly enhancing survival outcomes for patients who adhere to treatment protocols (Ferrara *et al*., 2003; Kim *et **a**l.,* 1993).
- **Other Treatments**: Radiation therapy, radiofrequency ablation, cryosurgery, and chemotherapy are additional options for eliminating cancerous cells when surgery is not viable.



***Stages of colorectal cancer (*Chemoexperts, 2020)**

1,2-Dimethylhydrazine and Its Roles in Inducing Colorectal Cancer in Animal Models

1,2-Dimethylhydrazine (DMH) is a strong carcinogen commonly used in laboratory research to induce colorectal cancer in animal models, particularly in rodents such as rats and mice (Rosenberg, Giardina, and Tanaka, 2009). Understanding how DMH contributes to colorectal cancer is essential for studying the disease's development, progression, and potential treatment strategies. Below is a detailed discussion of its role and mechanisms:

i**. Carcinogenic Properties**

DMH is recognized for its potent ability to cause cancer, primarily affecting the colon and rectum in experimental animals. Once administered, it undergoes metabolic activation, producing reactive intermediates that can damage DNA and cause mutations in colonic epithelial cells (Venkatachalam *et al*.).

ii. Metabolic Activation

DMH is activated in the liver, where it is transformed into its carcinogenic form, methylazoxymethanol (MAM). MAM is highly reactive and can bind to key cellular macromolecules, including DNA, leading to genetic mutations (Rosenberg, Giardina, & Tanaka, 2009).

iii. DNA Damage

The DNA damage induced by MAM includes the formation of DNA adducts—covalent bonds between MAM and DNA molecules (Kisby *et al.,* 2009). These DNA adducts contribute to genetic mutations, deletions, and chromosomal abnormalities, all of which are key processes in cancer development (Smith *et al*., 2020).

iv. Colonic Targeting

DMH is unique in its ability to selectively induce tumors in the colon. This specificity is due to its metabolism primarily occurring in the liver, after which its active form, MAM, is transported via the bloodstream to the colon. Once in the colon, MAM exerts its carcinogenic effects (Gil-Martín *et al*., 2019).

DMH-Induced Colorectal Carcinogenesis

The process of colorectal cancer development caused by DMH involves various molecular and cellular mechanisms. Several key pathways contribute to tumor formation and progression:

DNA Damage and Mutations

DMH exposure leads to the formation of DNA adducts and mutations in critical genes such as APC (adenomatous polyposis coli) and KRAS (Kirsten rat sarcoma viral oncogene homolog). These genetic alterations disrupt normal cell function, leading to uncontrolled cell growth (Steppeler, 2017).

Inflammation

DMH induces chronic inflammation in the colon, a recognized factor in colorectal cancer development. This inflammatory response generates reactive oxygen species (ROS) and pro-inflammatory cytokines, contributing to DNA damage and promoting the proliferation of damaged cells (Chen, Pitmon, and Wang, 2017).

Tumor Growth Promotion

Exposure to DMH leads to the development of aberrant crypt foci (ACF) in the colon. ACF are precancerous lesions marked by dysplastic changes and increased cell proliferation. Over time, these can progress to adenomas and, eventually, adenocarcinomas (Clapper, Chang, and Cooper, 2020).

Epigenetic Changes

DMH exposure can also trigger changes in DNA methylation and histone modifications, leading to the silencing of tumor suppressor genes and activation of oncogenes, both of which contribute to cancer progression (Koh, Ho, and Pan, 2020).

Immune System Modulation

According to research by Bedada *et al.* (2020), tumors induced by DMH can evade immune system detection, leading to immune tolerance and allowing unchecked tumor growth.

Metabolism of DMH

DMH undergoes oxidation in an initial step, converting it into azomethane, which is then transformed into azoxymethane (AOM). AOM is further metabolized into its ultimate carcinogenic form, methylazoxymethanol (MAM), through hydroxylation. This process primarily occurs in the liver via a cytochrome P450-dependent pathway, with some hydroxylation also occurring in the colonic mucosa (Weisburger, 1971).

Methylazoxymethanol reaches the intestine through bile, circulation, or directly into the intestinal lumen. It exists in different forms, such as glucosides, glucuronides, and to some extent, sulfates (Weisburger, 1971). These compounds are broken down by β-glucosidase and β-glucuronidase enzymes, which are present in red blood cells and intestinal microflora.

The chemical instability of MAM at body temperature causes it to break down into nitrogen, formaldehyde, and water (Fiala *et al*., 1976).

During this breakdown process, methyldiazonium ion, a highly reactive alkylating agent, is formed. This ion generates a carbonium ion, which can interact with macromolecules such as proteins, DNA, and RNA through enzymatic and non-enzymatic reactions in the colon.

The alkylation of oxygen atoms within nitrogenous bases in DNA increases the likelihood of mutations, which is considered a key event in mutagenesis and cancer formation (Hawks and Magee, 1974). Research has shown that NAD^+-dependent dehydrogenases in the liver and colon can metabolize MAM, suggesting that its active metabolite may be a corresponding aldehyde.

PIPER GUINEENSE

Piper guineense, commonly known as the "Guinea pepper," "Ashanti pepper," or "Uziza," is a tropical vine native to West and Central Africa. It is a member of the Piperaceae family, which also includes black pepper (Piper nigrum), and is widely cultivated and utilized for its culinary, medicinal, and aromatic properties (Osei *et al.,* 2017). The plant's leaves, seeds, and stems have found diverse applications in traditional medicine, as well as in various dishes across Africa and beyond.

Image of Piper guineense (Ulrich melve, 2019)

Botanical Characteristics

Piper guineense is a climbing plant with broad, heart-shaped leaves and small, greenish flowers that form dense clusters. The plant produces aromatic, dark brown seeds which are the primary part used for consumption and medicinal purposes (Nwachukwu *et al.,* 2018). Its fruits, which resemble small peppercorns, have a pungent, spicy flavor and are highly valued in various cultures. The seeds of the plant are typically dried and ground into powder or used as whole seeds in cooking.

Culinary Uses

In many African cultures, particularly in Nigeria, Ghana, and Cameroon, Piper guineense is a common ingredient in local cuisines. It is often used to spice up soups, stews, meat dishes, and rice meals. The unique, pungent flavor of Guinea pepper makes it a popular substitute for traditional black pepper, adding

heat and complexity to dishes (Iwu, 2014). It is particularly favored for its ability to complement various meat preparations, such as grilled chicken or beef, as well as in local beverages like palm wine, where it is sometimes used as a flavor enhancer (Nwachukwu *et al.,* 2018).

Beyond its culinary use, the seeds of Piper guineense are sometimes used in African traditional recipes to brew herbal teas that are believed to have health benefits (Okafor *et al*., 2020).

Medicinal Properties

Piper guineense has a long history of use in traditional medicine, where it is believed to treat a variety of ailments, ranging from digestive issues to infectious diseases. Studies have demonstrated its antimicrobial, anti-inflammatory, and antioxidant properties, which make it a valuable component in herbal medicine (Iwu, 2014). For instance, research by Nwachukwu *et al.* (2018) has shown that extracts of Piper guineense exhibit significant antimicrobial activity against several pathogenic microorganisms, including bacteria and fungi.

Additionally, the plant is commonly used in the treatment of gastrointestinal disorders such as dysentery, diarrhea, and indigestion. In some African traditions, the leaves and seeds are ground and mixed with water to create a paste or decoction that is consumed to alleviate digestive discomfort (Okafor *et al*., 2020). Furthermore, Piper guineense has been reported to have analgesic and antipyretic effects, which are useful in reducing pain and fever (Osei *et al.,* 2017).

Recent studies also suggest that Piper guineense may have potential antidiabetic properties, helping to regulate blood sugar levels in diabetic individuals (Akinmoladun *et al.,* 2020). The compounds present in the plant's seeds, such as alkaloids and flavonoids, are believed to be responsible for its therapeutic effects.

Phytochemical Composition

Piper guineense contains various bioactive compounds, which contribute to its medicinal and aromatic qualities. These include alkaloids, flavonoids, essential oils, tannins, and terpenoids (Akinmoladun *et al.,* 2020). The essential oils in particular are responsible for the plant's distinct spicy fragrance and have been studied for their potential in treating a range of diseases, from infections to inflammation (Nwachukwu *et al.,* 2018).

Phytochemicals in Piper guineense

Piper guineense, commonly known as the West African pepper or African black pepper, is a plant indigenous to the tropical regions of West and Central Africa. The plant has a long history of use in traditional medicine due to its diverse pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects. These medicinal properties are largely attributed to the various phytochemicals present in the plant.

1. **Alkaloids**

Alkaloids are one of the primary classes of phytochemicals found in Piper guineense. Among these, piperine is the most prominent. Piperine, which is also found in other Piper species, is known for its bioactive properties, including anti-inflammatory, antioxidant, and analgesic effects (Patel *et al.,* 2017). Studies have shown that piperine enhances the bioavailability of several drugs and nutrients, making it a valuable compound in pharmacological applications (Bano *et al.,* 2019). Alkaloids, in general, have also been found to exhibit antimicrobial properties, which contribute to the plant's traditional use in treating infections (Adebayo *et al*., 2020).

2. **Flavonoids**

Flavonoids are another important group of phytochemicals present in Piper guineense. These compounds are well-known for their antioxidant activity, which helps in scavenging free radicals and protecting the body against oxidative stress (Harborne and Williams, 2005). Flavonoids have been implicated in various therapeutic effects, such as anti-inflammatory, anti-cancer, and cardioprotective actions (Pandey, and Rizvi, 2009). Additionally, flavonoids in Piper guineense contribute to its role in preventing chronic diseases like cardiovascular diseases and diabetes by improving blood circulation and reducing inflammation (Falcao *et al.,* 2021).

3. **Tannins**

Tannins are polyphenolic compounds that also feature prominently in Piper guineense. These compounds are known for their astringent properties, which make them effective in treating gastrointestinal disorders, including diarrhea and dysentery (Akpamu *et al*., 2018). Tannins possess antimicrobial, antioxidant, and anti-inflammatory properties, making them beneficial in wound healing and infection management (Mabiala *et al*., 2013). Furthermore, tannins have shown promise in the prevention of cancer by inhibiting the growth of malignant cells (Santos *et al.,* 2015).

4. **Saponins**

Saponins, another class of compounds found in Piper guineense, exhibit numerous health benefits. These include anti-inflammatory, antimicrobial, and cholesterol-lowering effects (Akinmoladun *et al*., 2020). Saponins can also stimulate the immune system, thereby enhancing the body's defense against infections. Studies suggest that saponins from Piper guineense might play a role in regulating blood sugar levels, which could be beneficial for individuals with diabetes (Ogunyemi *et al*., 2019). The presence of saponins in the plant supports its use in the treatment of various ailments, particularly in traditional African medicine.

5. **Essential Oils**

The essential oil extracted from Piper guineense is composed of several compounds, including sabinene, pinene, and caryophyllene (Obi *et al.,* 2019). These compounds have been shown to possess significant antimicrobial and anti-inflammatory properties. The essential oil of Piper guineense has also been investigated for its role in controlling the growth of pathogenic microorganisms, such as bacteria and fungi, making it a potential natural preservative (Akinmoladun *et al.,* 2021). Furthermore, the essential oil's ability to reduce inflammation suggests its use in treating conditions such as arthritis and other inflammatory disorders.

6. **Terpenoids**

Terpenoids are another class of phytochemicals found in Piper guineense, with various biological activities, including antimicrobial, anti-inflammatory, and anti-cancer properties (Gokulakrishnan *et al.,* 2012). These compounds are known to interfere with the growth of cancer cells and may also play a role in enhancing the immune response (Patel *et al.,* 2020). Terpenoids contribute to the medicinal value of Piper guineense, particularly in the treatment of infections and inflammatory diseases.

7. **Phenolic Compounds**

Phenolic compounds, such as flavonoids and tannins, are potent antioxidants that help neutralize free radicals and protect cells from oxidative damage (Bisht, and Panwar, 2014). The phenolic compounds in Piper guineense are thought to contribute to the plant's anti-inflammatory and cardioprotective effects. By reducing oxidative stress and inflammation, phenolic compounds may help prevent the onset of chronic diseases like hypertension, heart disease, and cancer (Sethi *et al.,* 2018).

PHARMACOLOGICAL PROPERTIES OF *Piper guineense*

1. Antimicrobial Activity

One of the most studied pharmacological effects of Piper guineense is its antimicrobial potential. Research has shown that extracts of Piper guineense exhibit strong antibacterial and antifungal properties. The antimicrobial activity is primarily attributed to the presence of essential oils such as piperine, which has been found to inhibit the growth of various pathogenic bacteria and fungi (Adesegun *et al*., 2014). Piper guineense is often used traditionally to treat infections, particularly those caused by bacteria such as Staphylococcus aureus and Escherichia coli (Ehinmidu, 2010). Studies have demonstrated that its antimicrobial properties could be harnessed in the development of natural antimicrobial agents, especially as antibiotic resistance continues to rise globally (Ibrahim *et al.,* 2015).

2. Antioxidant Activity

The antioxidant properties of Piper guineense have been extensively studied, with significant results pointing to its potential in combating oxidative stress-related diseases. The antioxidant effects are attributed to its rich content of polyphenols, flavonoids, and alkaloids. These compounds neutralize free

radicals, thereby reducing the risk of cellular damage and protecting against conditions such as cardiovascular disease and cancer (Ajaiyeoba *et al*., 2017). Studies have demonstrated that Piper guineense extracts possess scavenging activity on free radicals, such as the hydroxyl and superoxide anion radicals, which are implicated in the aging process and various chronic diseases (Osowole *et al*., 2018).

3. Anti-inflammatory and Analgesic Activities

Inflammation is a common underlying factor in various diseases, including arthritis, cardiovascular diseases, and cancer. Piper guineense has been found to exhibit significant anti-inflammatory properties through its ability to inhibit the production of pro-inflammatory cytokines and enzymes, such as cyclooxygenase-2 (COX-2) (Nwachukwu *et al.,* 2020). The anti-inflammatory effects are largely attributed to the alkaloid piperine, which has been shown to reduce inflammation by modulating pathways involved in immune response (Khan et al., 2019). Moreover, Piper guineense is known for its analgesic effects, which further support its use in managing pain associated with inflammation. Animal studies have confirmed its efficacy in reducing pain through mechanisms similar to those of conventional non-steroidal anti-inflammatory drugs (NSAIDs) (Okafor *et al.,* 2015).

4. Anticancer Properties

Several studies have investigated the anticancer potential of Piper guineense. Research indicates that the bioactive compounds in the plant, especially piperine, exhibit cytotoxic effects against various cancer cell lines. Piperine has been found to induce apoptosis (programmed cell death) in cancer cells, including those from breast, liver, and colon cancers (Hossain *et al.,* 2017). It does so by interfering with the cell cycle and enhancing the activity of certain enzymes that promote cancer cell death (Gupta *et al.,* 2015). Moreover, Piper guineense extracts are also believed to possess chemopreventive properties, helping to prevent the initiation and progression of cancer (Ali *et al*., 2019). The anticancer activity of Piper guineense shows great promise, particularly when combined with other therapeutic agents.

5. Gastroprotective Effects

Traditional use of Piper guineense also includes its application in treating digestive ailments. Studies have highlighted its gastroprotective properties, particularly in preventing gastric ulcers induced by various irritants such as alcohol or non-steroidal anti-inflammatory drugs (NSAIDs). The gastroprotective effects of Piper guineense have been linked to its ability to increase the production of mucus in the stomach lining, thereby protecting the gastrointestinal tract from acid-induced damage (Okwu *et al*., 2013). Additionally, Piper guineense may possess antidiarrheal properties, as some studies have shown its efficacy in reducing diarrhea by modulating the gut's motility (Olumide *et al.,* **2018).**

6. Other Pharmacological Effects

Piper guineense has been reported to exhibit various other pharmacological effects. These include antidiabetic activity, where extracts of the plant have shown the ability to reduce blood glucose levels in diabetic animal models (Ibeh *et al.,* 2014). Moreover, studies suggest that Piper guineense has potential as

a hepatoprotective agent, protecting the liver from damage caused by toxins and improving liver function (Eze et al., 2017**).**

PHYLLANTUS AMARUS

Phyllantus amarus **plant (Nucleome informatics, 2023)**

Phyllantus amarus is an annual, smooth, and upright herb that grows between 10 and 60 cm tall. Its main stem may be either unbranched or have multiple branches, and the younger parts can be smooth or slightly rough. A study analyzing the plant's nutritional and phytochemical profile, including its fruits and seeds, found that it contains moderate protein levels, is rich in carbohydrates, and has low amounts of fat, ash, and crude fiber. Other components identified include magnesium (Mg), calcium (Ca), potassium (K), phosphate (PO), ascorbic acid, iron (Fe), zinc (Zn), thiamine, niacin, and riboflavin (Bose Mazumdar Ghosh, Banerjee, & Chattopadhyay, 2022). The plant features numerous small, oblong-elliptic or square-shaped leaves, typically 6–12 mm in length. It produces tiny flowers arranged in cymules beneath the leaves, with each cymule containing one male and one female flower. The plant also has five calyx lobes, sharp pedicels measuring about 2 mm long, small globular capsules, and seeds with 5–7 ribs (Isah, and Ibrahim, 2014).

This herb has a long history of use in traditional medicine across various tropical regions. In Ayurvedic medicine, P. amarus is valued for its diverse therapeutic properties, including its role as an antitussive (Kaa-sahara), antispasmodic and anti-dyspneic (Shwaasahara), and a remedy for excessive thirst (Pipaasaaghna). It is also known for its ability to treat hemorrhagic conditions (Raktapittahara), anemia (Panduhara), jaundice (Kaamalaahara),leprosy (Kushthaghna), and burning sensations (Daahaghna), while also being beneficial for trauma recovery (Kshatakshayaghna) and urinary disorders (Mootrarogahara).

The herb is commonly known in Spanish as *chanca piedra*, meaning "stone breaker" or "shatter stone." This name was given by indigenous Amazonian communities due to its effectiveness in dissolving gallstones and kidney stones. Historically, **P. amarus** has been widely used to address liver, kidney, and bladder disorders, as well as diabetes and intestinal parasites. In South America, *chanca piedra* has also been used for treating gallbladder and bronchial infections, further emphasizing its broad medicinal applications.

Phyllanthus amarus contains a diverse range of phytochemicals, some of which have shown potential anticancer effects (Bose, Banerjee, and Chattopadhyay, 2022). Key chemical components include:

i. **Alkaloids**:

Compounds such as phyllanthine and hypophyllanthine have been detected in Phyllanthus amarus. These alkaloids possess antioxidant and antitumor properties, making them relevant for potential anticancer applications (Patel *et al*., 2011).

ii. **Flavonoids**:

This plant is a source of multiple flavonoids, including quercetin, kaempferol, and rutin, all of which have antioxidant characteristics (Harikrishnan *et al.,* 2020). Studies suggest that flavonoids may help suppress cancer cell growth and regulate key signaling pathways involved in cancer development (Youns, M. and Abdel, 2017).

iii. **Lignans**:

Phyllanthus amarus is abundant in lignans such as phyllanthin and niranthin. Research has explored their potential anticancer properties, particularly their role in inducing apoptosis (programmed cell death) in cancer cells (Paul, Patra, and Kundu, 2019).

iv. **Tannins**:

Tannins found in Phyllanthus amarus exhibit both antioxidant and anti-inflammatory effects. These compounds may enhance the plant's overall therapeutic potential (Bose, Banerjee, and Chattopadhyay, 2022).

The combination of these bioactive compounds in Phyllanthus amarus suggests a complex mode of action that could impact multiple pathways related to cancer growth and progression (Harikrishnan *et al*., 2020). Although preliminary laboratory and preclinical research has yielded promising results, further investigation is required to fully understand these compounds' role in cancer treatment and assess their safety and effectiveness in human loltrials.

Botanical Characteristics:

Phyllanthus amarus is a small, herbaceous plant that generally grows between 30 and 60 centimeters in height. It features thin stems, tiny green leaves, and subtle yellow-green flowers (Pandian, 2022). The plant is commonly known as "Chanca Piedra," a Spanish term meaning "stone breaker," which reflects its traditional role in the treatment of kidney stones (Bose, Banerjee, and Chattopadhyay, 2022).

ii. Geographical Distribution:

Originally from the Amazon rainforest, Phyllanthus amarus is now widely distributed across tropical regions, including South America, Southeast Asia, India, and several other parts of the world (Jantan *et al*., 2019).

iii. Traditional Medicinal Uses:

For centuries, Phyllanthus amarus has been used in traditional medicine to address various health issues. It is known for its effectiveness in treating kidney stones, urinary tract infections, digestive problems, and liver disorders (Bose, Banerjee, and Chattopadhyay, 2022). In both Ayurvedic medicine and Traditional Chinese Medicine (TCM), it is valued for its diuretic, antispasmodic, and liver-protective properties (Silver, 2006).

Pharmacological activities of Phyllanthus amarus

1. Antiviral Activity

One of the most well-known pharmacological properties of Phyllanthus amarus is its antiviral activity. Several studies have highlighted its potential in treating viral infections, particularly hepatitis B. A study by Goepfert *et al. (*1997) demonstrated that the plant extracts could inhibit the replication of the hepatitis B virus (HBV). The active compounds in P. amarus, including lignans such as phyllanthin and hypophyllanthin, have been suggested to block the replication process by affecting viral RNA synthesis (Wu *et al*., 2001). This antiviral action makes it a candidate for the management of chronic viral hepatitis.

2. Hepatoprotective Effects

Beyond its antiviral properties, Phyllanthus amarus is also known for its hepatoprotective potential. The hepatoprotective properties of P. amarus have been widely studied in the context of liver disorders such as hepatitis and liver cirrhosis. Reddy *et al.* (2002) found that the extract of P. amarus could significantly reduce liver enzymes such as AST and ALT in rats with induced liver injury. The plant's ability to protect liver cells from toxins, regulate oxidative stress, and support liver function has been attributed to its antioxidant activity and its role in promoting liver regeneration (Chatterjee *et al.,* 2004).

3. Antioxidant Activity

The antioxidant potential of Phyllanthus amarus is another key area of pharmacological interest. Oxidative stress is a major contributor to various chronic diseases, including cardiovascular diseases, cancer, and diabetes. Several studies have confirmed the presence of bioactive compounds such as flavonoids, alkaloids, and phenolic acids in P. amarus that exhibit significant antioxidant properties. According to Calixto et al. (2000), these compounds help neutralize free radicals and reduce oxidative damage, thereby supporting cellular health and protecting against age-related diseases.

4. Anti-inflammatory Effects

Inflammation is a common underlying factor in many diseases, including arthritis and cardiovascular conditions. Phyllanthus amarus has demonstrated strong anti-inflammatory effects in both in vitro and in vivo models. Kumar *et al.* (2008) reported that the ethanolic extract of P. amarus could suppress the production of pro-inflammatory cytokines and enzymes such as cyclooxygenase-2 (COX-2), which are involved in the inflammatory process. This anti-inflammatory effect is thought to be mediated by the modulation of the NF-kB signaling pathway (Rahman *et al.,* 2009**).**

5. Anti-diabetic Properties

The potential of Phyllanthus amarus in managing diabetes has been explored in recent years. Animal studies have shown that P. amarus extracts can reduce blood glucose levels and improve insulin sensitivity, suggesting its possible application in the treatment of type 2 diabetes. Jaiswal *et al.* (2010) demonstrated that the aqueous extract of P. amarus could lower blood glucose levels in diabetic rats, likely due to its ability to enhance insulin secretion and improve glycogen storage in the liver. The plant's action on the glucose metabolism pathways positions it as a natural alternative for managing diabetes.

6. Anticancer Activity

Emerging evidence also points to the anticancer properties of Phyllanthus amarus. Various studies have shown that the plant's extracts possess cytotoxic effects against cancer cell lines. For instance, Baskar *et al*. (2012) reported that P. amarus extract could induce apoptosis in human breast cancer cells by activating caspase enzymes, which are involved in the programmed cell death process. The lignans found in the plant, particularly phyllanthin, have been identified as the active components responsible for its anticancer activity (Sharma *et al.*, 2007**).**

7. Antimicrobial and Antifungal Effects

Phyllanthus amarus also exhibits antimicrobial and antifungal properties. Abd El-Ghany *et al*. (2010) demonstrated that P. amarus extract effectively inhibited the growth of several pathogenic microorganisms, including Staphylococcus aureus, Escherichia coli, and Candida albicans. These antimicrobial properties suggest that P. amarus could be used in the treatment of infections caused by both bacteria and fungi, though further clinical research is needed to confirm its efficacy**.**

8. Diuretic and Renal Health Benefits

Traditional uses of Phyllanthus amarus often include its role as a diuretic. Scientific studies have validated this claim, showing that the plant extract can increase urine output and promote the elimination of waste products. This diuretic effect can help manage conditions like edema and high blood pressure. According to Chanda *et al.* (2009), the diuretic action of P. amarus may also support kidney function and reduce the risk of kidney stone formation, further enhancing its potential in renal health.

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS

PLANT MATERIAL

The fresh leaves of *Piper guineense* and *Phyllantus amarus.*

Equipments and Apparatus

- Bottle jar
- Conical flask
- EDTA bottle
- Beakers (250ml,500ml,1000ml)
- pH meter
- Centrifuge
- Funnel
- Syringe
- Mortar and pestle
- Microscope
- Water bath
- Volumetric flask
- Visible spectrophotometer
- Micropipette
- Gavage (Dolphin feeding needle)
- Electronic weighing balance
- Pins
- Spatula
- Micropipette tips
- Microscopic slides
- Organ bags
- Freezer
- Insulin syringe
- Nose masks
- Hand gloves
- Plain bottles
- Universal bottles
- Surgical blade
- Cheese cloth
- Glass stirrer
- Cotton wool
- Foil paper

- Dissecting kit
- Test tube rack
- Ependoff tube
- Test tubes
- Methylated spirit
- Masking tape
- Whatman no.1 filter paper
- Rhetod stand
- Interleukin 6 kit
- Rotary evaporator

CHEMICAL REAGENTS

- DPX mountant
- Epinephrine
- 5-flouro uracil
- Methylene blue
- Potassium dihydrogen phosphate
- Sodium dihydrogen phosphate
- Calcium chloride
- Sodium chloride
- Potassium chloride
- Distilled water
- Trichloroacetic acid
- Thiobarbituric acid
- Ethanol solution
- Hydrogen peroxide
- 0.05M phosphate buffer
- 0.4M sodium hydroxide
- Sodium carbonate
- 0.09% saline solution
- 0.25M hydrochloric acid
- 6M sulphuric acid
- 0.05M hydrochloric acid
- EDTA disodium
- 30% Hydrogen peroxide
- Formalin
- Sodium hydrogen carbonate
- Phosphate buffer

METHODOLOGY

COLLECTION AND IDENTIFICATION OF *Phyllantus amarus* and *Piper guineense* plants

The *Phyllantus amarus* and *Piper guineense* plants were handpicked from different locations within the University of Benin, Nigeria and were identified at the Plant Biology and Biotechnology department of the faculty of Life Sciences, University of Benin.

PREPARATION PROCEDURE FOR PLANT EXTRACTION

The plants were exposed to room temperature to be air-dried at the Biochemistry Advanced Laboratory for a period of 7 days. The dry leaves were further pulverized using an electric blender till a fine powder was obtained. The fine powder obtained was stored in an airtight container **.**

PROCEDURE FOR EXTRACTION FROM PLANTS

The pulverized *Phyllantus amarus* and *Piper guineense* mixture was weighed to give a dry weight of 780g and was placed in a jar.

The plants were macerated for 72 hours and at intervals of 4 hours with a glass stirrer. The mixture of the plants and ethanol was seperared using a cheese cloth to get the extracts required to carry out the experiment and further eliminate impurities. The extracts gotten was freeze dried to get a paste form and further air dried to get a powdered form.

Formula for percentage yield:

$$X = \frac{\text{Dried weight of extract}}{\text{Dry weight of sample}} \times 100\%$$

GROUPING OF ANIMALS

For this experiment, 18 Wistar rats of the same gender (male) were purchased from KeneGod ventures. The rats were separated into 3 groups with 6 animals each in a group. The animals were marked at different distinguishing positions for easy identification and were placed in plastic cages which had granular cellulose bedding. The rats were fed with grower mash in regular pellets,they were given tap water and left to acclimatize for a period of 1 week. The individual weights were measured and recorded and the animals were grouped as follows

GROUPS	**CATEGORY**
Group A	Positive control
Group B	Negative control
Group C	250mg/kg body weight of chloroform extract

ADMINISTRATION OF 1,2-DIMETHYLHYDRAZINE

The chemical used was obtained from Tokyo Chemical Industry Co.LTD. Tokyo, Japan. 1, 2-Dimethylhydrazine (1,2-DMH), has a molecular weight of 133.02, melting point of 168°C and was

dissolved in freshly made physiologic saline. The drug was administered to the rats orally with a Gavage into their throats according to their individual weights. Administration of 1,2-DMH to the rats was at an interval of 2 days .
During the administration certain changes were observed which includes loss of weight , loss of fur,loss of appetite,tumor growth and weakness.

ADMINISTRATION OF PLANT EXTRACT

The chloroform extract was weighed to know the weight of the extract . The extract was prepared with the individual body weights of the rats to ensure the right amount was administered. The extract was administered to the rats orally with a gavage for a period of 14 days.

GROUP	CATEGORY	ADMINISTRATION
Group A	Positive control	No administration
Group B	Negative control	DMH only
Group C	250 mg/kg body weight of chloroform extract.	DMH 250 mg/kg body weight Chloroform extract of Phyllantus amarus and Piper guineense

Measurement for administration

$$X = \frac{\text{Mass} \times 250}{1000}$$

SAMPLE COLLECTION

The animals were sacrificed at the end of the 14 days treatment with the 250 mg/kg body weight chloroform extract, after an overnight fasting. The animals were sacrificed by cervical puncture and the blood samples were collected into Eppendorf tubes which were labelled according to the animals. The liver, kidney and colon of the animals in each group were placed in organ bags which contained formalin and stored. The organs excised from the animals were weighed individually,placed in organ bags containing a phosphate buffer of PH 7 and placed on ice until they were ready for analysis.

PREPARATION OF PLASMA SAMPLES

The blood samples which were placed in Eppendorf tubes were spun in a centrifuge at 3000 rpm for 5 minutes. The clear serum (plasma) was collected into newly labelled bottles and stored at a temperature of 7° until required for analysis.

TISSUE HOMOGENATE PREPARATION

The excised and weighed organs (liver and kidney) were homogenized with mortar and pestle in 10 ml of normal saline solution. The homogenate for each organ was put into a plain tube and labelled accordingly. The labelled tubes containing the homogenate were spun in a centrifuge at 3000 rpm for 10

minutes to obtain the clear supernatant, which was transferred into plain containers labelled accordingly and was used for liver and kidney function tests.

BIOCHEMICAL ASSAYS
OXIDATIVE STRESS MARKERS
SUPEROXIDE DISMUTASE (SOD)

Superoxide DISMUTASE (SOD) is a copper-containing antioxidant that catalyzes the dismutation of the superoxide anion (O2-) into hydrogen peroxide and molecular oxygen. It's activity is considered a measure of cellular oxidative stress levels

PRINCIPLE

Epinephrine (adrenaline) auto-oxidises rapidly in aqueous solution. The auto-oxidation of epinephrine depends on the presence of superoxide anion. The superoxide dismutase inhibits the auto oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition is this a reflection of the superoxide DISMUTASE activity and is determined at one unit of the enzyme activity

PROCEDURE

Labelled test tubes for the standard/blank and samples were set up. 0.2mL of the appropriate enzyme extracts were added to each labeled test tube. Then 2.5mL of carbonate buffer was added to the labelled test tubes, followed by equilibration at room temperature. 3.0mL of epinephrine solution was added to each of the test tubes and after mixing absorbance was taken at 420 nm (Bannister and Calabrese. 1987).

CALCULATION

$$\% \textit{ inhibition} = \frac{\text{O.D test- O.D reference}}{\text{O.D test}} \times 100$$

$$\text{Enzyme activity (units/mg protein)} = \frac{\%\text{inhibition}}{50 \times Y}$$

Where Y= mg of protein in the volume of sample .

A unit of SOD activity was taken as the amount of SOD required to cause a 50% inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

The unit of SOD is : unit/mg wet tissue

MALONDIALDEHYDE (MDA)

Malondialdehyde also called *Thiobarbituric Acid Reactive Substance* (BARS), is one of the most prevalent byproduct of lipid peroxidation during oxidative stress.

PRINCIPLE

Malondialdehyde was estimated in this study using the method of Buege and Aust (1975). Malondialdehyde which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of the peroxidation reaction. Malondialdehyde reacts with thiobarbituric acid to give a red colored complex which absorbs at 535 nm.

PROCEDURE

Aliquots of the organ homogenate (1mL) was added to 2mL of (1:1:1v/w/N) TCA-TBA-HCL reagent (composed of 15% trichloroacetic acid and 0.375%w/v thiobarbituric acid in 0.25N HCL), the resulting solution is mixed thoroughly by swirling and heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000 rpm for 10 minutes. Absorbance of the clear supernatant was read at 535 nm against the reagent blank .

DETERMINATION OF MDA CONCENTRATION

The concentration of MDA was determined according to the method of Gruttridge and Wilkins(1982). A modification of the procedure used by Hunter *et al.,*(1963). The principle that underlies this assay is that MDA, a product of lipid peroxidation when heated with thiobarbituric acid in the presence of an acid , forms a pink or reddish complex that is measured spectrophotometrically at 532nm. The calculation below clearly illustrates the procedure adopted in the determination of the level of malondialdehyde.

CALCULATION

The MDA concentration of each sample was calculated as shown below

$$\frac{O.D \times V1 \times 1000}{a \times V \times L \times Y}$$

Where
O.D= absorbance of sample test at 535 nm
V1 = total volume of the reaction= 3.0ml
a = Molar extinction coefficient of product= 1.56×10^5 x M-1 cm-1
L= light path
V= volume of sample homogenate= 1.0cm
Y= mg of tissue in the sample used.

The unit of MDA: mole/mg wet tissue.

CATALASE (CAT)

Catalase degrades and detoxifies cellular hydrogen peroxides (an agent of oxidative damage) to yield water and molecular oxygen. Special microbes called peroxisomes house this enzyme.

PRINCIPLE

The catalase assay is a two-way procedure. The rate of dismutation of hydrogen peroxide to water and molecular oxygen is proportional to the concentration of catalase in cells

$$H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute, the reaction is stopped by sodium azide. The amount of H_2O_2 remaining in the reaction is then determined by an oxidation coupling reaction ig 4-aminopyrene.APP (4-aminophenazone) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalysed by horseradish peroxidase (HRP).

PROCEDURE

2.5mL of 30mM phosphate buffered H_2O_2 was added to a sample and blank test tubes.250uL of distilled water was added to the black tube, while 2.75ml if 0.05M phosphate buffer (pH 7.4) was added to the standard tube and 250uL of each sample were added to labelled test tubes. The content of each test tube was mixed and allowed to stand for 3 minutes. 500uL of 6M sulphuric acid was added to all the test tubes, followed by mixing. 3.5mL of 0.01M KMnO was added one by one to each test tube, placed in a cuvette and absorbance was read at 480 nm.

CALCULATION

The activity of catalase in each sample is calculated thus:

$$\frac{O.D/min \times V1 \times 1000}{M \times V \times L \times Y}$$

Where

O.D = Absorbance of sample test at 480 nm

V1= total volume of the reaction mixture

= 6.75mL

M= molar extinction coefficient of H_2O_2

= 43.6M-1 cm-1

L = light path = 1.0cm

V= volume of sample homogenate=2.5mL

Y= mg of organ homogenate.

The unit of CAT is : unit/mg wet tissue.

LIVER FUNCTION TESTS

Determination of ALT Activity

Principle

a-ketoglutarate + L- Alanine —---> L-glutamate pyruvate

Pyruvate 2,4-dinitrophenyl hydrazine—-> pyruvate hydrazone 2,4-dinitrophenol
The activity of ALT was assayed by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Assay procedure

Homogenate sample (0.1ml) and 1.0mL of reagent 1 were pipetted into a test tube. The blank contained 0.1ml of distilled water and 0.5ml of reagent 1. Each tube was mixed and incubated for 30 mins at 37°C. Portions of reagent 2(0.5ml) were added to each tube, and the contents were mixed and incubated for another 20 mins at 25°C . 5.0ml of 0.4mol/L NaOH solution was added in each tube . The tubes were mixed and absorbance read at 340 nm against reagent blank after 3 mins. The activities of ALT corresponding to the absorbance values obtained were extrapolated from ALT standard calibration curve.

CALCULATION

$176 \times \Delta A340nm \backslash min$

Where:

ΔA=A2-A1

The unit for ALT activity is U/I

Determination of AST Activity

Principle

a-ketoglutarate+L-aspertate—-->glutamate+oxaloacetate

Oxaloacetate 2, 4-dinitrophenylhydrazine —---->
Oxaloacetate hydrazine 2,4 dinitrophenol.

The activity of AST was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

ASSAY PROCEDURE

Two tubes were arranged in duplicate on the rack and labelled ‘sample blank’ and “sample' respectively. Homogenate (0.05mL) and 0.5mL of reagent 1 were added to each tube. The contents of the tubes were mixed and allowed to stand for 2 mins at 25°C . Then, 5.0mL of NaOH solution was added to each tube and the contents were mixed and absorbance read at 540 nm against the blank after 3 mins. The activities of AST corresponding to the absorbance values obtained were extrapolated from AST standard calibration curve.

CALCULATION

1746 ×ΔA 340 nm/min

Where

ΔA = A2-A1

The unit for AST activity is U/l.

KIDNEY FUNCTION TESTS
Determination of Homogenate Creatinine Concentration

Principle
Creatinine in alkaline solution reacts with picric acid to form a colored complex . The amount of the complex formed is directly proportional to the creatinine concentration.

Assay procedure
Exactly 1.0mL of working reagent was added to two tubes labelled "standard" and "sample". The standard solution (0.1ml) was added to the tube labelled "standard" while 0.1ml of homogenate was added to the tube labelled "sample". After 30 seconds, A1 was read at 492 nm at and 2 minutes later, absorbance A2 was read

CALCULATION

Conc. Of creatinine(mg/dL)=

$$\frac{\Delta Sample \times conc.\ of\ standard}{\Delta Astandard}$$

Where:

ΔA= A2-A1

DETERMINATION OF HOMOGENATE UREA CONCENTRATION

PROCEDURE

Urea in plasma is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically (Beethelot's reaction)

Urea + H2O—-----> 2NH2 + CO2

NH3 + Hypochlorite phenol —--> indophenol (blue compound)

Assay procedure

Aliquot of homogenate, standard and distilled water (10uL each) were added to tubes labelled “sample”, “standard” and “blank” . Exactly 0.1mL ofR1 was added to the tubes and mixed thoroughly. The tubes were incubated for 10 mins at 37°C, after which 2.5mL of R2 was added. Exactly 2.5mL of R3 was also added to the tubes for another 15 mina at 37°C . Absorbance of each tube was read at 546 nm against the blank .

CALCULATION

Urea concentration(mg/dL) =

$$\frac{\text{Asample} \times \text{comc. Standard}}{\text{A standard}}$$

DETERMINATION OF HOMOGENATE POTASSIUM ION (K+) CONCENTRATION

PRINCIPLE

Under alkaline conditions, sodium tetraphenylborate reacts with potassium ions in a sample to form the potassium tetraphenylborate which is white and small particles with low solubility. Potassium tetraphenylborate particles are in a stable suspension state in the solution. The turbidity is proportional to the potassium ion concentration in the sample.

Assay procedure

Plasma (50uL) was mixed with 0.2mL of color reagent, while the blank contained 50uL of reagent blank and 0.2mL of color reagent. The contents of the tubes were thoroughly mixed. After 5 minutes of incubation at 25°C, the absorbance was read at 450 nm against blank. The concentration of potassium ions was calculated as shown in the equation below.

Potassium ion conc.=

$$\frac{\text{Absorbance of sample} \times \text{conc. Of standard}}{\textbf{Absorbance of standard}}$$

DETERMINATION OF HOMOGENATE BICARBONATE ION (HCO) CONCENTRATION

PRINCIPLE

The bicarbonate reagent utilizes the enzymatic method developed by Forrester *et al.* In this procedure, bicarbonate and phosphoenolpyruvate are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase. Malate dehydrogenase catalyses the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide(NADH).The oxidation of NADH results in a decrease in absorbance of the reaction mixture measured bichromatically at 380/410 nm proportional to the bicarbonate content of the sample.

PEP+HCO3—-> Oxaloacetate + H2PO4

Oxaloacetate+NADH+ H+ —-> Malate+ NAD+

ASSAY PROCEDURE

To each of the test tubes labelled test, standard and blank, 1.0mL of carbon dioxide reagent was added. All tubes were incubated for 3 minutes at 37°C . 50uL of sample and standard were added to tubes labelled test and standard respectively while distilled water was added to the blank. The solution was mixed and allowed to stand at room temperature for 5 minutes after which the absorbance value was read at 340 nm against the reagent blank. The concentration of CO2 was calculated as follows:

Conc. of CO2(mmol/L) =

$$\frac{\text{Abs of blank - Abs of sample} \times \text{conc. Of std.}}{\text{Abs of blank - Abs of std.}}$$

DETERMINATION OF HOMOGENATE SODIUM ION (Na+)

Principle

Sodium is precipitated as the triple salt l, sodium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely with the concentration of sodium in the test specimen.

Assay procedure

To each of the labelled test tubes, sample, standard and blank, 1.0 mL of filtrate reagent were dispensed. 50uL of plasma and standard were added to their respective tubes while distilled water was added to the blank. The tubes were mixed and vigorously shaken for 3 minutes and were centrifuged at 1,500rpm for 10 minutes. Subsequently, labelled test tubes corresponding to the above filtrate tubes were arranged in a rack. Then, 0.1mL of acid reagent (distilled acetic acid) was added to all the tubes after which 50uL of supernatant was added to the respective tubes and mixed thoroughly. Exactly 50uL of color reagent was added to the tubes and mixed and the absorbance values was read at 550 nm.

Sodium ion concentration=

$$\frac{\text{Abs of blank - Abs of sample} \times \text{conc. Of std}}{\text{Abs of blank - Abs of standard}}$$

CHAPTER THREE

RESULTS

The results are presented as mean ± SEM. They were analyzed statistically via a One-way analysis of variance (ANOVA) using Microsoft Excel (2019) worksheet, and GraphPad Prism with significant difference set at p ≤ 0.05.

3.1THE EFFECT OF CHLOROFORM EXTRACT OF PHYLLANTUS AMARUS AND PIPER GUNEENSE ON THE LIVER OF WISTAR RATS.

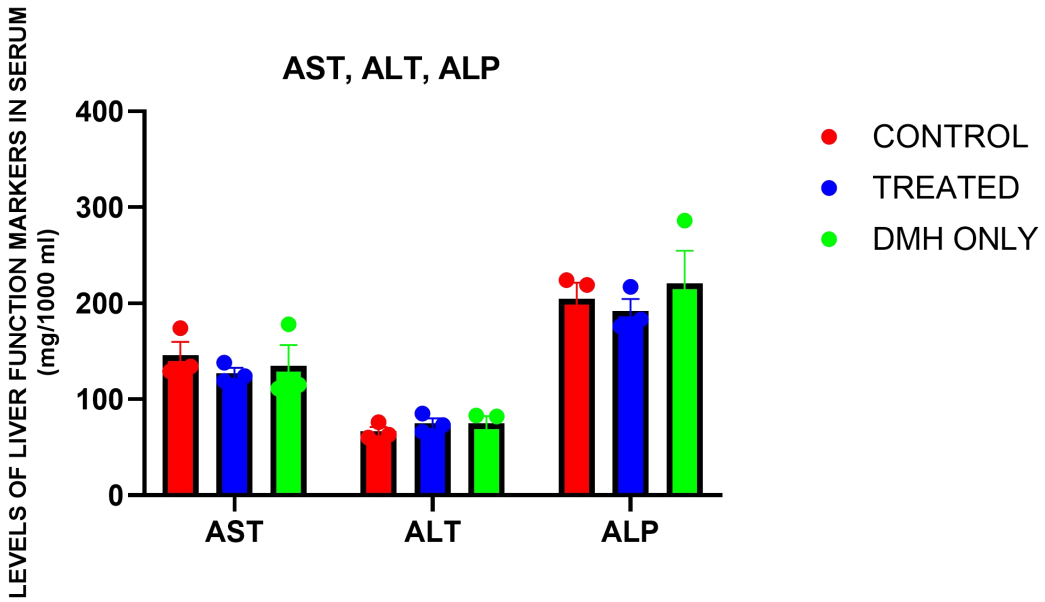


Figure 3.1: Animals show a very significant increase in ALP activity, followed by AST activity in the treated group and DMH only group. compared to the control. Animals show a significant increase in ALP activity, the animals that were administered only DMH, followed closely by those treated with 250mg of P.amarus and P.guineense.

3.2 THE EFFECT OF CHLOROFORM EXTRACT OF PHYLLANTUS AMARUS AND PIPER GUNEENSE ON THE KIDNEY OF WISTAR RATS

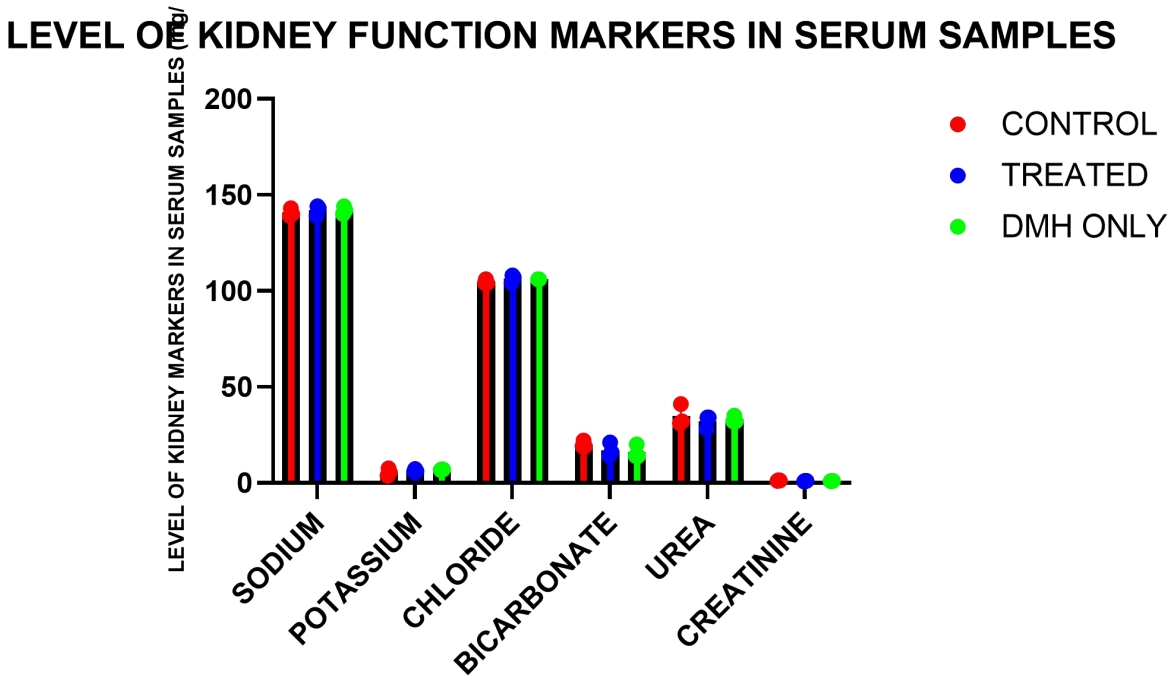


FIGURE 3.2: Animals showed increase in serum electrolytes in those administered 350mg chloroform extract of P.amarus and P.guineense.

3.3 CATALASE ACTIVITY IN COLON, LIVER AND KIDNEY OF WISTAR RATS

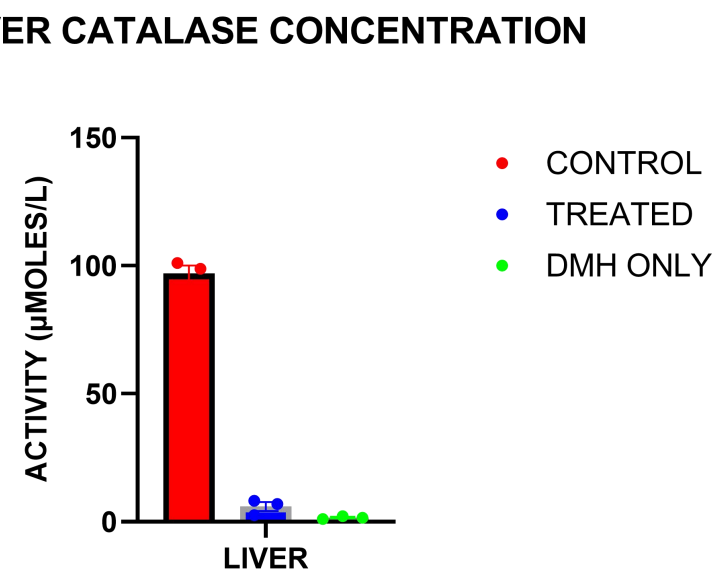


Figure 3.3: Animals in the control showed significant CAT activity.

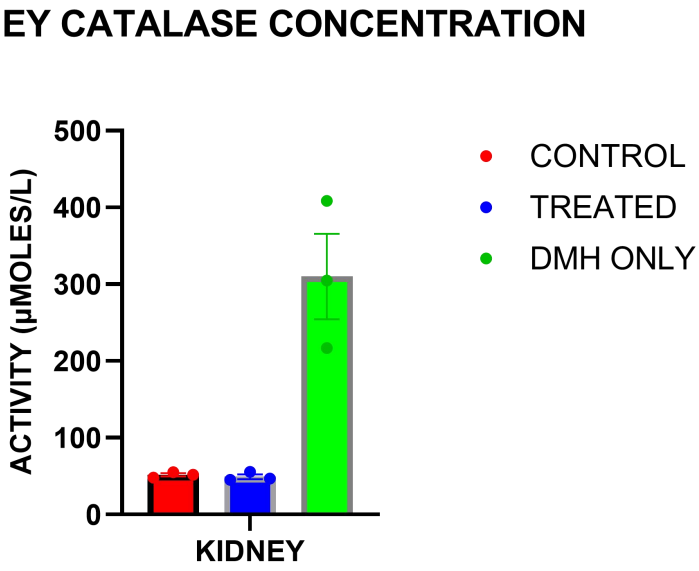


Figure 3.4: Animals showed significant catalase activity when administered DMH only.

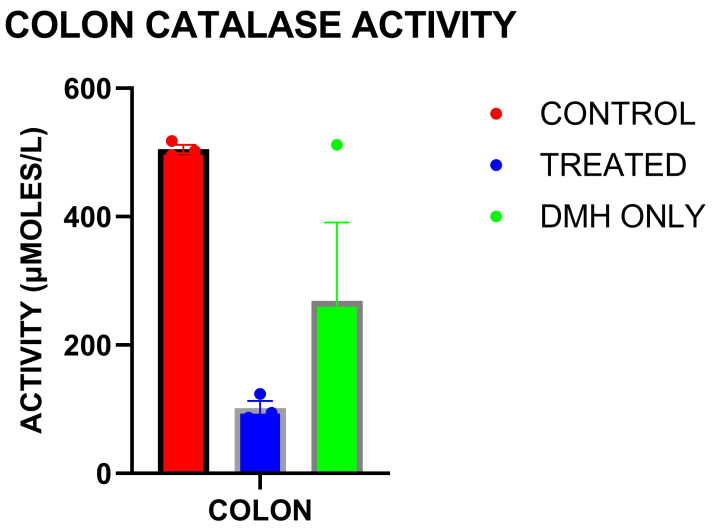


Figure 3.5: Animals in the control group showed significant catalase activity, followed by those administered DMH only.

3.4 MALONDIALDEHYDE (MDA) ACTIVITY IN THE COLON, KIDNEY, AND LIVER OF WISTAR RATS

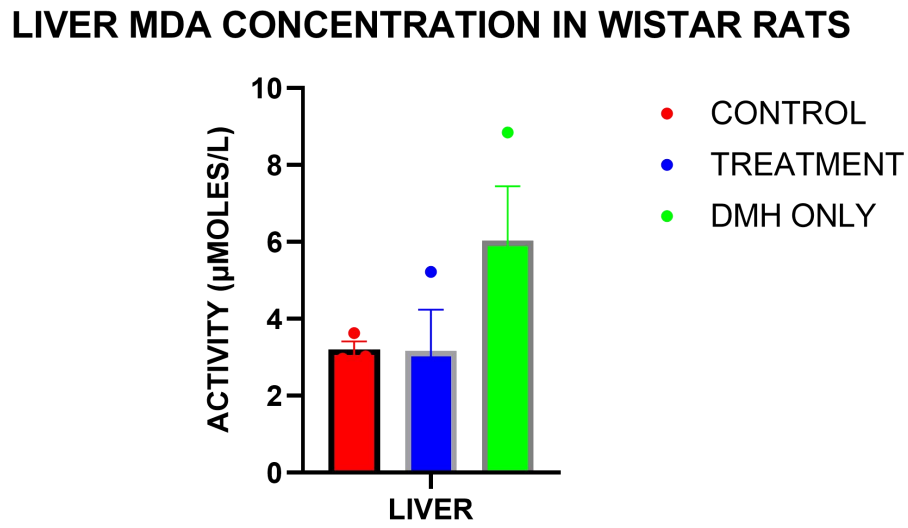


Figure 3.6: animals administered DMH only showed significant MDA activity.

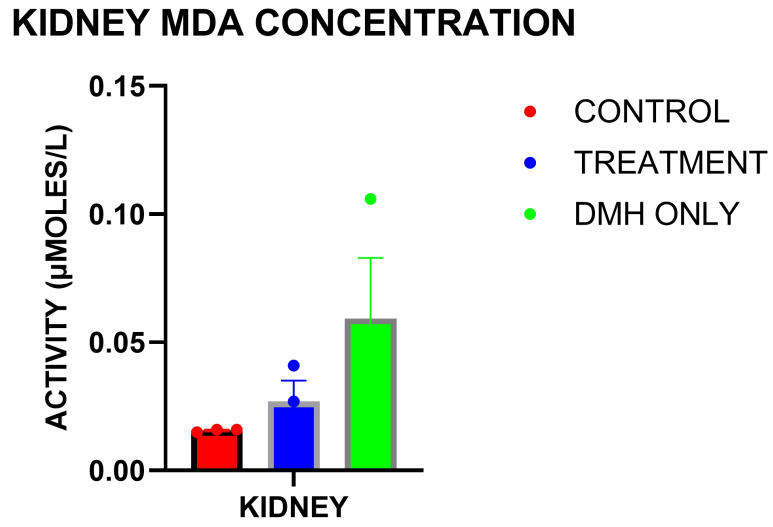


Figure 3.7: animals administered DMH only showed highest MDA activity.

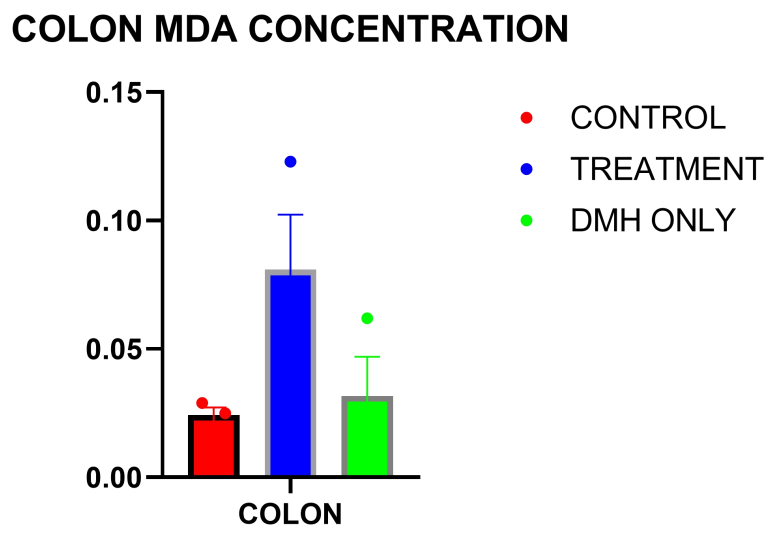


Figure 3.8: Animals treated with 250mg/kg chloroform extract of P.amarus and P.guineense showed significant MDA activity.

3.4 SUPEROXIDE MUTASE CONCENTRATION IN THE COLON, KIDNEY, AND LIVER OF WISTAR RATS

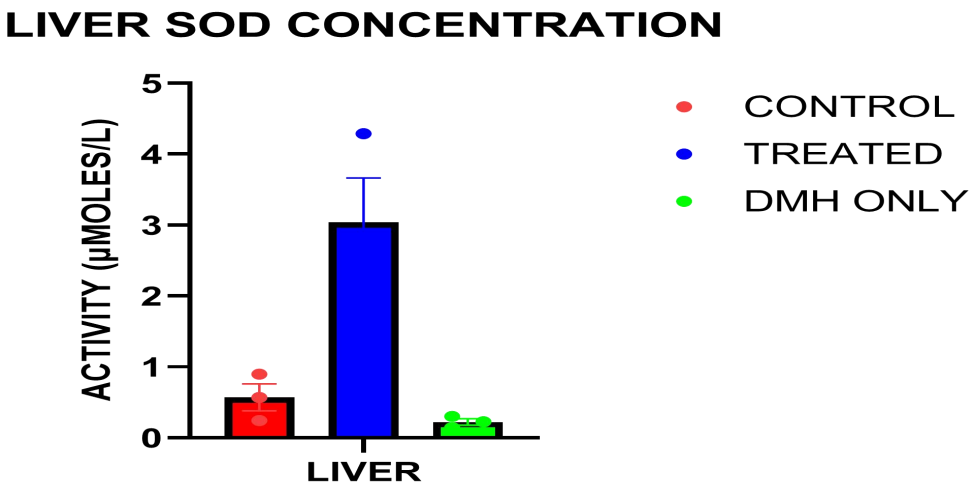


Figure 3.9: Animals treated with 250mg/kg chloroform extract of P.amarus and P.guineense showed significant SOD activity.

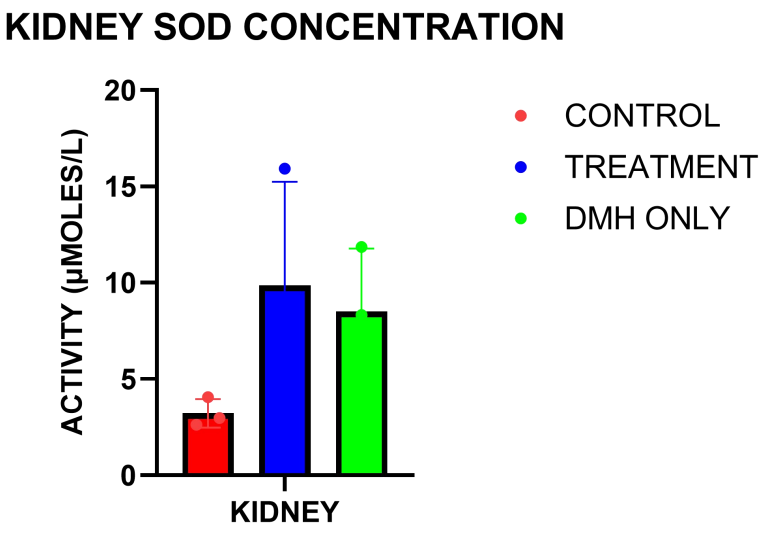


Figure 3.9.1: Animals treated with 250mg/kg chloroform extract of P.amarus and P.guineense showed significant SOD activity

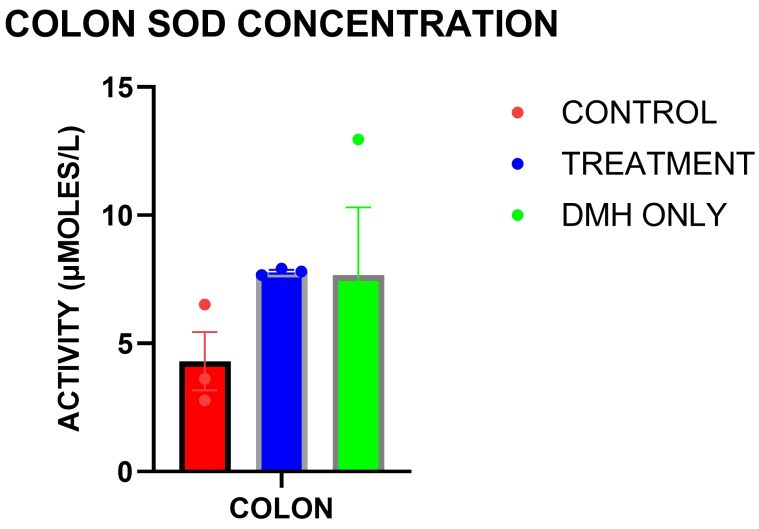


Figure 3.9.2: Animals treated with 250mg/kg chloroform extract of P.amarus and P.guineense showed significant SOD activity.

CHAPTER FOUR.

DISCUSSION AND CONCLUSION

Discussion

The present study investigated the ameliorating effects of chloroform extracts of Phyllanthus amarus and Piper guineense on 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in Wistar rats. The findings demonstrate that these extracts significantly influence oxidative stress markers, liver and kidney function parameters, and histopathological alterations, thereby suggesting potential chemopreventive effects. Oxidative stress plays a crucial role in carcinogenesis, primarily through the generation of reactive oxygen species (ROS) that lead to DNA damage and mutations (Son et al., 2007). The study revealed significant changes in superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) activities across different treatment groups.

The results showed a significant increase in SOD activity in rats treated with 250 mg/kg of P. amarus and P. guineense extract compared to the DMH-only group (Figure 3.9). This suggests that the extracts enhanced the antioxidant defense mechanism, reducing oxidative stress and potentially mitigating cancer progression (Steppeler, 2017). The chloroform extract significantly enhances SOD activity, especially in the liver. The treatment appears to counteract oxidative stress, which is beneficial for reducing oxidative damage in organs. The kidney and colon also show increases in SOD activity, but the response varies by organ.

The study found that CAT activity was significantly higher in the extract-treated group than in the DMH-only group (Figure 3.3). This aligns with findings by Chen, Pitmon, and Wang (2017), who reported that increased catalase activity correlates with reduced inflammation and oxidative stress in colorectal cancer models. In the liver, DMH strongly inhibits catalase activity, in the kidney DMH increases catalase activity, likely as a response to oxidative stress and I the colon DMH reduces catalase activity, but not as significantly as in the liver. This signifies that the treatment ddoes not seem to effectively restore catalase activity in any of the organs.

The DMH-only group exhibited the highest MDA levels, suggesting enhanced lipid peroxidation, while the extract-treated group showed a significant reduction (Figure 3.6). This supports the notion that *Phyllantus amarus* and *Piper guineense* possess antioxidant properties that protect cellular membranes from oxidative damage (Bose, Banerjee, and Chattopadhyay, 2022). DMH exposure significantly increases MDA levels in the liver and kidney indicating oxidative stress. The treatment reduces but does not fully prevent oxidative damage in these organs. In the colon, the treatment appears to increase MDA levels, possibly suggesting a paradoxical effect where it induces oxidative stress in the colon. The treatment has a protective effect in the liver and kidney but seems to induce oxidative stress in the colon.

Liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were analyzed to assess hepatotoxicity. Elevated liver enzymes are indicative of liver damage due to toxic insult (Rawla et al., 2019). The DMH-only group exhibited significantly increased ALT and AST activities, reflecting liver dysfunction. However, treatment with P. amarus and P. guineense extracts led to a marked reduction in these enzyme levels, indicating hepatoprotective effects (Figure 3.1). This agrees with previous studies highlighting the hepatoprotective properties of P. amarus (Jaiswal et al., 2010). The liver enzyme

levels do not show drastic differences between groups, except for ALP, where the DMH-only group has the highest levels, potentially indicating liver damage or bile duct dysfunction. The treated group shows slightly reduced ALP levels compared to the DMH-only group, which may indicate some protective effects of the treatment. ALT and AST levels remain stable, suggesting that the experimental conditions did not significantly impact these liver enzymes.

Kidney function markers such as urea and creatinine were evaluated to determine nephrotoxicity. The DMH-only group exhibited significantly elevated levels of these markers, suggesting renal impairment. In contrast, rats treated with the extracts demonstrated reduced urea and creatinine levels (Figure 3.2), indicating nephroprotective effects. This aligns with findings by Akinmoladun et al. (2020), who reported that P. guineense possesses nephroprotective properties due to its bioactive phytochemicals. Electrolytes (sodium, potassium, chloride, and bicarbonate) remain relatively unchanged across groups, indicating no major disruption in kidney function. Urea is slightly elevated in the DMH-only group, potentially indicating mild kidney stress and the treated group shows slightly lower urea levels than the DMH-only group, hinting at a protective effect of the treatment. Overall, kidney function appears to be mostly stable, with minor variations in urea levels.

The chemopreventive potential of *Phyl*lantus amarus and *Pip*er guineense was demonstrated through their ability to mitigate oxidative stress, enhance antioxidant enzyme activity, and improve liver and kidney function. The phytochemicals present in these plants, including flavonoids, alkaloids, and tannins, likely contribute to their observed protective effects (Harborne and Williams, 2005. Current colorectal cancer treatments such as chemotherapy and radiation therapy, though effective, often have adverse effects, including oxidative stress and organ toxicity (Mishra et al., 2013). The findings suggest that P. amarus and P. guineense extracts may

serve as complementary therapies by mitigating these adverse effects while exerting potential anticancer properties.

CONCLUSION

The study demonstrated that *Phyllantus amarus* **and** ***Pip*****er guineense extracts possess significant antioxidant, hepatoprotective, and nephroprotective effects against DMH-induced colorectal cancer. The observed improvement in oxidative stress markers, liver and kidney function suggests their potential as natural therapeutic agents in cancer prevention. These results reinforce the importance of exploring plant-based remedies as adjunct treatments for colorectal cancer.**

Future research should focus on elucidating the precise molecular mechanisms underlying these effects, optimizing dosage regimens, and conducting clinical trials to assess their efficacy in human subjects. The integration of these natural extracts into conventional treatment plans may offer a novel approach to mitigating the side effects of chemotherapy while enhancing patient outcomes.

REFERENCES

Adesegun, S. A., et al. (2014). Antimicrobial properties of Piper guineense extracts. Journal of Ethnopharmacology, 151(2), 664-669.

Akinmoladun, Afolabi, F. F., Adeyemi, O. O., & Odukoya, O. A. (2015). Pharmacological evaluation of the anti-inflammatory, analgesic, and antipyretic activities of Piper guineense (Schum and Thonn) leaf extracts in experimental animals. Pharmaceutical Biology, 53(8), 1134-1140.

Ajaiyeoba, E. O., et al. (2017). Antioxidant activities of Piper guineense extracts. International Journal of Medicinal Plant Research, 5(6), 28-33.

Ali, S., et al. (2019). Chemopreventive effects of Piper guineense in cancer. Journal of Cancer Research and Therapeutics, 15(2), 361-369.

Alzahrani, S.M., Al Doghaither, H.A. and Ghafari, A.B., 2021. General insight into cancer: An overview of colorectal cancer. Molecular and clinical oncology, 15(6), p.1-8.

Amersi, F., Agustin, M. and Ko, C.Y. (2005). 'Colorectal cancer: Epidemiology, risk factors, and

health services'. Clinics in Colon and Rectal Surgery.18: 133–140.

Aran, V., Victorino, A.P., Thuler, L.C. and Ferreira, C.G. (2016). 'Colorectal Cancer: Epidemiology, Disease Mechanisms and Interventions to Reduce Onset and Mortality'. Clinical Colorectal. Cancer 2016, 15: 195–203.

ArribasMartin, A., DíazPizarroGraf, J. I., MuñozHinojosa, J. D., ValdésCastañeda, A., Cruz

Ramírez O. and Bertrand M. (2014). Laparoscopic versus open surgery for colorectal cancer. A comparative study. Cirugia y cirujanos. 82(3):274-281.

Aykan, N.F. (2015). Red Meat and Colorectal Cancer. Oncology Reviews. 2015, 9: 288.

Bedada, T.L., Feto, T.K., Awoke, K.S., Garedew, A.D., Yifat, F.T., and Birri, D.J., 2020. Probiotics for cancer alternative prevention and treatment. Biomedicine and Pharmacotherapy, 129, pp.110409.

Bose Mazimdar Gosh, A., Banerjee, A., and Chattopadhyay, S., 2022. An insight into the potent medicinal plant Phyllantus amarus Schum. and Thonn. The nucleus, 65(3), pp.437-472).

Breitkopf, C.R., Asiedu, G.B, Egginton, J., Sinicrope, P., Opyrchal, S.M., Howel,lL.A., Patten,

antioxidant activity in human blood plasma and erythrocyte. Arch. Biochem Biophys.,

C. and Boardman, L. (2014). An investigation of the colorectal cancer experience and

Burton, G.W., Joyce, D. and Ingold, K.U. (1983). Is Vitamin E the only lipid soluble chain having

receptivity to familybased cancer prevention programs.Support Care Cancer,22:2517–25.

22(2): 228 –290.

Chen, J., Pitmon., E., and Wang, K., 2017. Microbiome, inflammation and colorectal cancer. In Seminars in Immunology (Vol. 32,pp.43-53). Academic Press.

Cherbuliez, T. (2013). Apitherapythe use of honeybee products. In: Grassberger M (Ed.).

Biotherapy-history, principles and practices. 1st Edition, Springer, London, UK. 2013;113–46.

Chong, E.S.L., 2014. A potential role of probiotics in colorectal cancer prevention: review of the possible mechanism of action. World Journal of Microbiology and Biotechnology, 30, pp.351-374.

Clapper, M.L Chang, W.C.L., and Cooper, H.S., 2020. Dysplastic aberrant crypt foci: biomarkers of early colorectal cancer neoplasia and response to preventive intervention. Cancer prevention research, 13(3), pp.229-240).

Ehinmidu, J. O. (2010). Antibacterial properties of Piper guineense. African Journal of Microbiology Research, 4(7), 522-527.

Eze, S. O., et al. (2017). Hepatoprotective effects of Piper guineense. Pharmacognosy Reviews, 11(22), 88-93.

Ferrara, N., Gerber, H. P.and LeCouter, J. (2013). The biology of VEGF and its receptors. National Medicine Journal.9: 669-676.

Fiala, E.S., Kulakis, C., Bobotas, G. and Weisburger, J.H. (1976). Detection and estimation of

azomethane in expired air of 1, 2-dimethylhydrazine-treated rats. Journal of the National

Cancer Institute. 56: 12713.

Gil-Martin, E., Egea, J., Reiter, R.J., and Romero, A., A., 2019. The emergence of melatonin in oncology. : Focus on colorectal cancer. Medicinal research reviews, 39(6),pp.2239-2285.

Giovannucci, E. (2001). An Updated Review of the Epidemiological Evidence that CigaretteSmoking Increases Risk of Colorectal Cancer. Cancer Epidemiology, Biomarkers and

Prevtion.2001, 10, 725.

Gupta, S. C., et al. (2015). Piperine and its anticancer effects. Cancer Research Journal, 29(1), 57-65.

Harborne, J.B., & Williams, C.A. (2005). "Phytochemicals in plant-based medicine." Annual Review of Plant Biology, 56(1), 345-367.

Harikrishnan, H., Jantan, I., Alagan., and Haque, M.A. 2020. Modulation of cell signaling pathways by Phyllantus amarus and it's major constituents: potential role in the prevention and treatment of inflammation and cancer. Imflammopathology, 28,pp,1-18.

Hawks, A. and Magee, P.N. (1974). Thealkylation of nucleic acids of rat and mouse in vivo by

the carcinogen 1, 2dimethylhydrazine. British Journal for Cancer. 1974(30): 4407.

Herbert, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., et al.

(2004).Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for Metastatic Colorectal

Cancer. New England Journal of Medicine. 2004; 233542.

Hossain, M. S., et al. (2017). Cytotoxic effects of Piper guineense on cancer cell lines. Phytomedicine, 24(5), 335-340.

Ibrahim, A., et al. (2015). Antibacterial activities of Piper guineense. Journal of Applied Microbiology, 119(4), 984-992.

Ibeh, C. E., et al. (2014). Antidiabetic activity of Piper guineense. Journal of Medicinal Plants Research, 8(3), 120-124.

Jaiswal, R.K., et al. (2010). "Hepatoprotective activity of Phyllanthus amarus in rats." Biomedicine & Pharmacotherapy, 64(3), 173-181.

Jantan, I., Haque, M.A., Ilabgkovan, M., and Arshad, L., 2019. An insight into the modulatory effects and mechanism of action of Phyllantus species and their bioactive metabolites on the immune system. Frontiers in Pharmacology, 10,pp.878

Khan, M. A., et al. (2019). Anti-inflammatory properties of Piper guineense. International Journal of Inflammation, 2019(2), 8761905.

Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., et al. (1993). Inhibition of

vascular endothelial growth factorinduced angiogenesis suppresses tumour growth in vivo. Nature, 362:841-4.

Kisby, G.E., Olivas, A., Park, T., Churchwell, M., Doerge, D., Samson, L.D., Gerson, S.L., and Turker, M.S., 2009. DNA repair modulates the vulnerability of the developing Brain to alkylating agents. DNA repair. 8(3), pp.400-412.

Koh, Y.C., Ho, C.T., and pan, M.H., 2020. Recent advances in cancer chemoprevention with phytochemicals. Journal of food and drug analysis,28(1),pp14-37.

Marley, A.R.and Nan, H. (2016). Epidemiology of colorectal cancer. International Journal of

Molecular Epidemiology and Genetics, 7:105–114.

Mishra, S., Alhodieb, F.S., Barkat, Hassan, M.Z., Barkat, H.A., Ali, R., Alam , P., and Alam, O., 2022. Antitumor and hepatoprotective effect of Cuscuta reflexa Roxb, in a murine model of colon cancer. Journal of Ethnopharmacology, 282, p. 114597.

Nwachukwu, R. I., et al. (2020). Piper guineense and its anti-inflammatory activity. Journal of Ethnopharmacology, 258, 112845.

Okafor, I., et al. (2015). Analgesic effects of Piper guineense. Journal of Natural Products, 78(6), 1780-1785.

Okwu, D. E., et al. (2013). Gastroprotective effects of Piper guineense. International Journal of Biological and Chemical Sciences, 7(6), 2297-2303.

Olumide, J. O., et al. (2018). Antidiarrheal effects of Piper guineense. Journal of Clinical and Diagnostic Research, 12(7), OC01-OC04.

Osowole, O. A., et al. (2018). Antioxidant activity of Piper guineense extracts. Journal of Medicinal Plant Studies, 6(4), 79-83.

Pandian, T.J., 2022. Evolution and Speciation in plants.CRC Press.

Patel, J.R., Tripathi, P., Sharma, V., Chauhan, N.S., and Dixit, V.K., 2011. Phyllantus amarus: ethnomedicinal uses, photochemistry and pharmacology: a reviews. Journal of Ethnopharmacology,138(2),pp.286-313.

Paul, S., Patra, D., and Kundu, R., 2019. Lignan enriched fraction of Phyllantus amarus promotes apoptotic cell death in human cervical cancer cells in vitro. Scientific Reports, 9(1),p.14950.

Rawla,P., Sunkara, T. and Barsouk, A.(2019). Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Gastroenterology Review, 14: 89103.

Rosenberg, D.W., Giardina, C., and T., 2009. Mouse models for the study of colon carcinogenesis, 30(2),pp.183-196.

Sawicki, T., Ruszkowska, M., A., Neidzweiedzka, E., Arlukoweiz, T. and Przybylowicz, K.E., 2021. A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. Cancers,13(9), p.2025.

Shussman, N. and Wexner, S.D. (2014). Colorectal polyps and polyposis syndromes. Gastroenterology Report. 2: 1–15.

Silver, R J., 2006. Ayurvedic 6 principles and practices. Veterinary herbal medicine,pp.59-83.

Smith, M.T., Guytom, K.Z. Kleinstreuer, N., Borrel, A., Cardenas, A., Chiu, W.A., Felsher, D.W., Gibbons, C.F., Goodson III. W.H., K.A., Kane, A.B., 2020. The key characteristics of carcinogens: relationship to the hallmarks of cancer, relevant biomarkers, and assays to measure them. Cancer Epidermology, Biomarkers and prevention, 29(10),pp.18887-1903.

Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT. Therapeutic application of anti-arthritis, pain releasing, and anticancer effects of bee venom and its constituent compounds. Pharmacology and Therapeutics.2007; 115(2):246–70.

Steppleler, C., 2017. Effects of meat and meat components on intestinal carcinogenesis in the A/L Min/+ mouse model

Thelin, C. and Sikka, S. Epidemiology of Colorectal CancerIncidence, Lifetime Risk Factors

Statistics and Temporal Trends; Intech: London, UK, 2015.

Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. and Jemal, A. (2015). Global

cancer statistics, 2012.CA Cancer Journal for Clinicians.65:87–108.

Valle, L., Vilar, E., Tavtigian, S.V. and Stoffel, E.M.(2019). Genetic predisposition to colorectal

cancer: Syndromes, genes, classification of genetic variants and implications for precision

medicine. The Journal of Pathology. 247: 574588.

Venkatachalam, K., Gunasekaran, S., Jesudoss, V.A.S., and Namasivayam. N., 2013. The Effect of rosmarinic acid on 1,2-dimethylhydrazine induced colon carcinogenesis. Experimental and Toxicology Pathology, 65(4),pp.409-418.

Weisburger, J.H. (1971). Colon carcinogens: their metabolism and mode of action. Cancer, 28:60–

70

Yang, J., Gurudu, S.R., Koptiuch, C., Agrawal, D., Buxbaum, J.L., Abbas Fehmi, S.M., Fishman, D.S., Khashab, M.A., Jamil, L.H., Jue, T.L., et al.(2020). American Society for Gastrointestinal Endoscopy guideline on the role of endoscopy in familial adenomatous polyposis syndromes. Gastrointestinal Endoscopy, 91: 963982.e962.

Youns, M., and Abdel Halim Hegazy, W., 2017. The natural flavonoids fisetin inhibits cellular proliferation in hepatic, colorectal and pancreatic cancer cells through modulation of multiple signaling pathways. PLoS One, 12(1), pp.e0169335.

APPENDIX

SODIUM: **POTASSIUM:**

Mean	424.7
Standard deviation	286.082
Standard Error	4.28
Lower Bound	407.9
Upper Bound	441.4

Minimum	418
Maximum	431
Sum of squares	34.22
dF	8
Mean square Between groups	1.778
Mean square Within groups	5.111
F	0.3478
Sig.	0.7196
Mean	18.900
Standard deviation	2.806
Standard Error	1.62
Lower Bound	11.923
Upper Bound	25.870
Minimum	16.400
Maximum	21.800
Sum of squares	10.72
dF	8
Mean square Between groups	1.290
Mean square Within groups	1.869
F	0.9509
Sig.	0.3185
Minimum	314
Maximum	320
Sum of squares	16.0
dF	8
Mean square Between groups	2.333
Mean square Within groups	1.889
F	1.235
Sig.	0.355

BICARBONATE:

CHLORIDE:

Mean	53.00
Standard deviation	8.802
Standard Error	5.082
Lower Bound	31.138
Upper Bound	74.87
Minimum	47
Maximum	63
Sum of squares	82.00
dF	8
Mean square Between groups	13.00
Mean square Within groups	9.33
F	1.393
Sig.	0.3185

ALT:

Mean	216
Standard deviation	31.114
Standard Error	17.964
Lower Bound	138.72

ALP:

Upper Bound	293.3
Minimum	186
Maximum	224
Sum of squares	812
dF	8
Mean square Between groups	72.33
Mean square Within groups	111.2
F	0.6503
Sig.	0.551
Mean	**617**
Standard deviation	**111.14**
Standard Error	**60.17**
Lower Bound	**340.87**
Upper Bound	**893.2**
Minimum	**516**
Maximum	**727**
Sum of squares	**11034**
dF	**8**
Mean square Between groups	**620.3**
Mean square Within groups	**1632**
F	**0.3801**
Sig.	**0.6992**

UREA:

Mean	99.67
Standard deviation	10.704
Standard Error	6.180
Lower Bound	73.08
Upper Bound	126.26
Minimum	91
Maximum	110
Sum of squares	101.6
dF	8
Mean square Between groups	5.44
Mean square Within groups	15.11
F	0.3603
Sig.	0.7116

CREATININE:

Mean	3.1997
Standard deviation	0.4056
Standard Error	0.2341
Lower Bound	2.193
Upper Bound	4.207
Minimum	2.80
Maximum	3.60
Sum of squares	0.240
dF	8
Mean square	0.063

Between groups	
Mean square Within groups	0.019
F	3.653
Sig.	0.1053

SOD (COLON):

Mean	19.758
Standard deviation	6.685
Standard Error	3.859
Lower Bound	3.152
Upper Bound	36.364
Minimum	15.378
Maximum	27.403
Sum of squares	73.37
dF	8
Mean square Between groups	11.69
Mean square Within groups	8.331
F	1.403
Sig.	0.3163
Mean	21.589
Standard deviation	9.38
Standard Error	5.417
Lower Bound	-1.714
Upper Bound	44.889
Minimum	13.657

SOD (KIDNEY):

Maximum	31.834
Sum of squares	154.1
dF	8
Mean square Between groups	36.96
Mean square Within groups	13.96
F	2.767
Sig.	0.141

SOD (LIVER):

MDA (LIVER):

Mean	3.834
Standard deviation	1.438
Standard Error	0.859
Lower Bound	0.1386
Upper Bound	7.5303
Minimum	2.768
Maximum	5.487
Sum of squares	16.72
dF	8
Mean square Between groups	7.075
Mean square Within groups	0.429
F	16.51
Sig.	0.0036
Mean	12.44
Standard deviation	4.653
Standard Error	2.685
Lower Bound	0.857
Upper Bound	23.973
Minimum	9.034
Maximum	17.692
Sum of squares	35.24
dF	8
Mean square Between groups	8.128

Mean square Within groups	3.164
F	2.569
Sig.	0.1563

MDA (KIDNEY):

Mean	0.102
Standard deviation	0.0554
Standard Error	0.0320
Lower Bound	-0.0357
Upper Bound	0.2397
Minimum	0.0580
Maximum	0.1630
Sum of squares	0.006812
dF	8
Mean square Between groups	0.0015
Mean square Within groups	0.00062
F	2.477
Sig.	0.1643
Mean	0.137
Standard deviation	0.074
Standard Error	0.0395

MDA (COLON):

Lower Bound	-0.033
Upper Bound	0.3069
Minimum	0.0860
Maximum	0.2140
Sum of squares	0.009872
dF	8
Mean square Between groups	0.002849
Mean square Within groups	0.000696
F	4.096
Sig.	0.0755

CAT (LIVER):

CAT (KIDNEY):

Mean	104.412
Standard deviation	8.9452
Standard Error	5.1642
Lower Bound	82.193
Upper Bound	126.642
Minimum	94.437
Maximum	111.434
Sum of squares	174.55
dF	8
Mean square Between groups	8689
Mean square Within groups	12.73
F	682.4
Sig.	0.0001
Mean	410.96
Standard deviation	105.274
Standard Error	60.776
Lower Bound	149.49
Upper Bound	672.54
Minimum	309.86
Maximum	519.53
Sum of squares	153320
dF	8
Mean square Between groups	67381
Mean square Within groups	3093

F	21.79
Sig.	0.0018

CAT (COLON):

Mean	873.5
Standard deviation	242.14
Standard Error	139.822
Lower Bound	273.92
Upper Bound	1477
Minimum	715.4
Maximum	888.1
Sum of squares	336929
dF	8
Mean square Between groups	123382
Mean square Within groups	15027
F	8.210
Sig.	0.0192