SUBACUTE TOXICITY STUDY ON LIVER FUNCTION INDICES OF DICHLOROMEHTANE FRACTION OF *Detarium microcarpium* IN SWISS ALBINO MICE

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CERTIFICATION

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DEDICATION

This report is dedicated to Almighty God for his infinite mercy and for giving me the strength and grace to successfully carry out my project.

ACKNOWLEDGEMENT

Special appreciation to the Almighty God, who gave me the gift of life, and has made all things beautiful in His time.

I wish to express my profound gratitude to my wonderful family, most especially my Parents, Br. Justin Okonkwo and Mrs Christiana Okonkwo for their unending support towards the success of my studies. To my siblings, I also wish to say thank you for your moral support and encouragement, thank you for standing strong with me during this time.

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ABSTRACT

In tropical Africa, *Detarium microcarpium* serves as both vital industrial source and medicinal herb. An overview of *D. microcarpium* verified pharmacological qualities, including its effect on certain organ enzyme activity, acute and subacute toxicity, was the goal of this research. The leave of *Detarium microcarpium* are fit for several commercial and industrial uses, as was discovered after a comprehensive literature review. Traditionally, *D. microcarpium* has been used to cure or alleviate the symptoms different illnesses and conditions in humans. In herbal therapy, this species is used to treat a wide range of conditions, from chest discomfort to cough to infertility to liver issues to menstrual pain to STDs. Antibacterial, antifungal, antihyperglycemic, antimalarial, antioxidant, and iron absorption are only some of the many pharmacological actions associated with *D. microcarpium*, as shown by pharmacological research. The fact that plants are natural does not guarantee their health benefit and safety. Some of these natural plants have been reported to cause renal and hepatic toxicity by deactivating and reducing the activity of both hepatic and renal enzymes. It is therefore expedient that these popularly known herbal medicines are widely studied with regards to their pharmacological and toxicological aspects in order to understand their safe doses and adverse effects. The chemical, nutritional, and toxicological qualities of *Detarium microcarpium* are interesting enough to warrant in-depth scientific examination. Animal experiments, randomized clinical trials, and subacute toxicity tests on specific organs are all necessary parts of a detailed research of *Detarium microcarpium* and its derivatives.

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CHAPTER ONE

1.1 BACKGROUND OF STUDY

Phytomedicine, the ancient practice of preventing and treating various human health issues using plant parts such as leaves, stem-bark, seeds, and roots, has a rich history. Records dating back to 2838–2698 B.C. reveal that Chinese Emperor Shen Nung documented 365 medicinal herbs, while the Egyptian manuscript "Ebers Papyrus" from 1500 B.C. recorded 811 prescriptions and 700 drugs. Remarkably, plants like ginseng (Panax spp.), Ephedra spp., Cassia spp., and Rheum palmatum L., mentioned in these ancient texts, are still utilized by the pharmaceutical industry. Additionally, indigenous tribes have integrated medicinal plants into their rituals and disease treatments (Firmo et al., 2011).

The Ministry of Health in Brazil reported a remarkable 161% increase in patients seeking treatment through medicinal plants and phytopharmaceuticals between 2013 and 2015. This surge in popularity is likely attributed to the effectiveness, accessibility, and cost efficiency of herbal remedies. The World Health Organization (WHO) noted in 2014 that 70% to 95% of the global population relies on herbal medicines in primary healthcare. Consequently, WHO recommends that countries develop national policies and regulations for traditional medicines with proven effectiveness (Goncalves et al., 2013).

It's essential to recognize that, despite being natural, not all plants guarantee health benefits and safety. Some natural plants have been associated with renal and hepatic toxicity. Therefore, it is crucial to extensively study these well-known herbal medicines, examining their pharmacological and toxicological aspects to establish safe dosages and understand potential adverse effects (Araújo-Filho et al., 2016). order to understand their safe doses and adverse effects (Araújo-Filho *et al.,* 2016).

1.2 JUSTIFICATION FOR THIS STUDY

Most research on the subacute effect of *Detarium microcarpium* on albino rats in recent times have been on the subchronic effects of the aqueous, hexane and ethanol extracts of the leaves of *Detarium microcarpium*. In essence, there is very little data available on the subchronic effects of the methanol extract of stem-bark of *Detarium microcarpium*. This study is focused at drawing attention to the possible subchronic toxic effects of the methanol extract of stem bark of *Detarium microcarpium* on male Swiss albino mice

1.3 AIM AND OBJECTIVES

This study is aimed at ascertaining the acute and subacute oral toxicity studies of fractions of *Detarium microcarpium* in swiss albino male wistar mice.

The following were the objectives of the study includes assessment of Liver Function Enzymes such as:

1. AST (Aspartate transaminase) levels

2. ALT (Alanine transaminase) levels

3. ALP (Alkaline phosphatase) levels

4. Albumin levels

1.4 LITERATURE REVIEW

1.4.1 PLANT PROFILE

In recent times, with the proliferation of infectious diseases and the emergence of drug-resistant organisms, the pursuit of innovative treatments has surged. Throughout history, humans have consistently drawn inspiration from the unique healing properties of plants. Traditional African medicine places significant reliance on medicinal plants for addressing a wide array of ailments. Indigenous communities have harnessed the power of these plants for centuries to both cure and prevent diseases, while also utilizing them as sources of valuable pharmaceuticals and health aids. Given the escalating challenges posed by infectious diseases and resistant pathogens, the search for new medications has gained even more momentum.

Within plants, numerous chemical compounds with physiological functions exist, and they can be harnessed for therapeutic or preventive purposes. These compounds include flavonoids, tannins, saponins, alkaloids, glycosides, and phenolics. Plants house a diverse array of physiologically active molecules that hold promise in disease treatment and prevention.

One such remarkable plant is Detarium microcarpum, a native African plant thriving in the wild across several African nations, particularly in savannah regions. Every part of the D. microcarpum plant possesses therapeutic properties, earning it the distinction of being a "miracle plant" in traditional herbalism. Folk medicine places a heavy emphasis on the leaves and fruits of this plant.

The demand for novel drugs derived from various medicinal plant species continues to grow in contemporary times. The objective of this study is to consolidate scattered information regarding the biological effects of D. microcarpum, presenting a comprehensive understanding of the plant and providing clearer guidance on how to harness its components effectively (Chen et al., 2013).

1.4.2 BOTANICAL CLASSIFICATION OF *DETARIUM MICROCARPUM*

Kingdom: *Plantae*

Subkingdom: Tracheophytes

Division: Angiosperms

Class: Eudicots

Subclass: Rosids

Order: Fabales

Family: Fabaceae

Genus: *Detarium*

Species: *D. microcarpum*

Binomial name: *Detarium microcarpum*

Plate 1.1: *D**etarium microcarpum*** plant(Chen *et al.*, 2013).

Plate 1.2: Stem bark of *Detarium microcarpum*

Plate 1.3: Fruit of *Detarium microcarpum*

1.4.3 PLANT DESCRIPTION

Detarium microcarpum, an African tree belonging to the Fabaceae family, which is commonly referred to as legumes, displays a wide range of growth characteristics. While typically classified as a small tree or shrub, it has the capacity to attain heights of up to 15 meters, with some specimens even reaching as tall as 25 meters in areas with ample moisture. In terms of its growth rate, the shoots originating from the trunk can ascend to heights of 1.5 to 2 meters within a span of just 1 to 2 years, showcasing notably robust growth compared to seedlings. On average,

seedlings tend to reach heights of approximately 0.6 meters after 3 years, with the potential to achieve 1.5 meters in height within a 4-year period.

This tree enters its flowering phase during the rainy season, which typically occurs from July through September, sometimes extending into November. It's worth noting, however, that the primary flowering period is relatively brief, lasting no more than 8 days. The fruit-bearing season for Detarium microcarpum spans from September to January, occasionally extending into May. Furthermore, in the month of November, the tree undergoes a shedding of its leaves, subsequently sprouting new foliage in March.

1.4.4 Geographical distribution of *Detarium microcarpum*

Detarium microcarpum is naturally distributed throughout the arid regions of West and Central Africa, encompassing countries such as Benin, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sudan, and Togo. It distinguishes itself from other species within its family as Detarium microcarpum thrives in dry savanna environments, while Detarium senegalense prefers dry forests, and Detarium macrocarpum thrives in humid forests. This species goes by various local names, including "sweet dattock" or "tallow tree" in English, "dankh" or "petit détar" in French, and "Abu-laili" in Sudan or "Tamba Dala" in Mali.

Propagation of Detarium microcarpum can occur through vegetative means or by using seeds. It demonstrates vegetative propagation through coppice regeneration and the formation of suckers from stumps or roots. Additionally, propagation is feasible through rooted cuttings and grafting,

using scions sourced from mature trees. This species primarily inhabits shallow, stony, and lateritic soils, often on hilly terrain, and is commonly found in areas with an annual rainfall ranging from 600 to 1000 mm. Its most prevalent habitat consists of wooded savannahs, semi-cleared dry forest regions, and fallow lands, where it thrives in sandy or hard soils characterized by a high iron content.

1.4.5 Traditional Uses of *Detarium microcarpum*

Ethnomedicinal plants serve as a prominent source for pioneering medication development. The traditional usage of various parts of Detarium microcarpum, such as its roots, leaves, and bark, along with its valued high-quality firewood, has influenced a diversity of regional approaches to its management. This African plant holds a significant place in traditional medicine, with a history of being employed to address numerous ailments, including diarrhea, bronchitis, fever, meningitis, convulsions, malaria, diabetes, bacterial, and fungal infections. Known by several names, including "sweet dattock" or "tallow tree" in English and "Abu Laila" in western Sudan, it carries cultural and medicinal significance. In Senegal, it goes by "dank," and in Mali, it is referred to as "tamba dala."

For treating wounds and averting infections, fresh bark or leaves are applied, while a boiled bark powder serves as a painkiller. The bark infusion is reported to possess anti-inflammatory, anti-parasitic, and diuretic properties, whereas the fruits and leaves are employed in managing diarrhea and syphilis. The roots, stems, bark, leaves, and fruits of this plant have proven effective against a wide spectrum of conditions, including diarrhea, tuberculosis, and meningitis. In Mali, the bark is used for measles, while the roots and leaves help alleviate cramps and diarrhea in

humans and cattle, respectively. In Burkina Faso, the fruit pulp is used for skin ailments, and in Niger Republic and Togo, it's utilized for dizziness. All parts of the plant, including the fruit, leaves, and seeds, are edible.

This versatile plant has additional applications; its infusion is believed to possess antiparasitic, diuretic, and anti-inflammatory properties, and the fruits and leaves are valuable in addressing diarrhea and syphilis. Certain African tribes incorporate the fruit and leaves into their cuisine for added flavor. The bark infusion is employed against diarrhea and syphilis, while the fruits and leaves are used for dysentery and syphilis, respectively.

Detarium microcarpum's medicinal qualities make it a popular folk remedy for conditions like diarrhea, meningitis, tuberculosis, and hemorrhoids. Its leaves are consumed as a vegetable, used as an enema for diarrhea, an eye wash for conjunctivitis, and a traditional wash for itch. Additionally, the bark is employed for treating anemia and expelling retained placenta. In Senegal, it's used to manage bronchitis, pneumonia, and other respiratory conditions through palm wine maceration. In Nigeria, it's even used in cancer treatment. In Sudan, the aromatic heated roots are used as perfume, and in Chad Republic, they serve as a mosquito repellent. Its excellent ignition properties, especially in the presence of moisture, also make it valuable as firewood and charcoal.

This study confirms the traditional therapeutic effectiveness of Detarium microcarpum, underscoring its potential as a valuable source of medicine (Chen et al., 2013). Notably, all reviewed studies highlight the plant's favorable activity against tested parasites. It's intriguing to

observe that the diverse antiparasitic effects of these extracts can be attributed to both their chemical composition and the nature of the targeted promastigote species. Furthermore, the mechanism of action of these extracts appears to involve cell membrane disruption and the induction of cell death in specific cellular targets. In this context, contact with the mitochondrial membrane is suggested as an additional method to trigger parasite apoptosis (Kim et al., 2013).

1.4.6 Antidiabetic Activity of *Detarium microcarpum*

Diabetes mellitus is a prevalent and widespread condition affecting populations across underdeveloped, developed, and developing nations. It is projected to impact approximately 25 percent of the global population. Diabetes is characterized by an abnormal glucose metabolism linked to low blood insulin levels. The ongoing quest for innovative treatments persists, driven by the growing demand.

In experiments with alloxan-induced diabetic rats, the root extract significantly reduced blood sugar levels at a significance level of $p < 0.05$. Similarly, the seed extract demonstrated significant reductions in both blood sugar and cholesterol levels. Notably, the extract exhibited no impact on hematological or blood chemical markers, indicating its safety profile. Moreover, the methanolic seed extract lowered alpha-amylase and glucosidase levels to 69.3 and 31.1 mg/mL, respectively. In human trials, the seed extract contributed to reduced postprandial blood glucose and insulin levels. These reductions in incremental blood glucose and postprandial glucose levels were statistically significant at $p < 0.05$, with the area-under-curve of glucose registering at 62%.

Consequently, Detarium microcarpum has undergone extensive in vitro and in vivo research as a potential source of antidiabetic medication. The trunk bark of the plant was found to contain myo-inositol (4), L-quino-1,5-lactone (1), D-pinitol (3), D-(-)-bornesitol (2), sucrose, D-glucose, and D-fructose. Notably, D-pinitol and its derivatives are renowned for their beneficial effects in cases of insulin resistance. The hypoglycemic effects of D. microcarpum's crude extract and compounds are likely attributed to mechanisms such as hindering glucose absorption in the small intestine, stimulating insulin secretion in the pancreas, suppressing glucose production in the liver, or enhancing glucose uptake in peripheral tissues via glucose transporters (Lobo et al., 2010).

1.4.7 Toxicity Evaluation of *Detarium microcarpum*

The utilization of medicinal plants is a widespread practice worldwide, particularly in developing nations, owing to their affordability and local availability. However, there exists a common belief among consumers across the globe that herbal medicines are inherently safe due to their natural origins. Contrary to this perception, accumulating evidence suggests otherwise. If not appropriately selected and prepared, these herbal remedies can possess a high degree of toxicity. Therefore, it becomes imperative to assess the safety of plant extracts.

Numerous studies have revealed that medicinal plants house a diverse array of bioactive compounds with beneficial effects. Toxicological tests have determined that methanolic leaf extracts at a concentration of 5000 mg/kg are safe. Additionally, dietary inclusion of seeds, even at a significance level of $p > 0.05$, exhibited no discernible impact on hematological or

biochemical parameters. In summary, tallow seed meal can be integrated into broiler chicken feed up to 20% without affecting organ weight, hematological indices, or biochemical parameters.

Nevertheless, long-term administration of the methanolic stem bark extract for treating various medical conditions has been linked to adverse effects on vital organs. Findings indicate that the fruit of D. microcarpum may have detrimental effects on rats and possibly contains antinutrients that interfere with digestion and absorption, resulting in observed growth retardation. The LC50 (lethal concentration for 50% of subjects) of the methanolic stem bark extract for brine shrimp larvae was determined to be 158.49 g/mL. Based on these findings, D. microcarpum is deemed toxic and potentially unsafe, especially when administered in large doses without careful monitoring and treatment. The extract led to the mortality of mice at doses of 2900 mg/kg and 1600 mg/kg body weight, with an LD50 (lethal dose for 50% of subjects) of 3,807.89 mg/kg.

Considering these conclusions, excessive consumption of any part of D. microcarpum extract may carry toxicological risks. Therefore, caution is advised, and only modest doses should be employed. Additionally, individual compounds should undergo toxicity assessments, serving as the foundational step for drug development or herbal formulation (Swedko et al., 2003).

1.4.7.1 Acute Toxicity of *Detarium microcarpum*

In an acute toxicity assessment, the oral administration of a single 2000 mg/kg dose of the aqueous bark extract from D. microcarpum led to a minor decrease of 1.56% in rat body weight by day 14 compared to their initial weight. It's worth noting that according to Subramanion et al., a weight loss exceeding 10% typically signifies adverse effects induced by a drug or chemical. In

this context, the results indicate that administering the bark extract of D. microcarpum orally did not have a significant impact on the growth of the rats. Throughout the 14-day experimental period, there were no notable alterations in rat behavior, and no fatalities occurred.

Consequently, it can be inferred that the extract is well-tolerated by rats, and the LD50 (lethal dose for 50% of subjects) would likely be higher than the administered 2000 mg/kg dose. In fact, substances or compounds with LD50 values greater than 1000 mg/kg are typically considered safe and only mildly toxic. This suggests that the administration of D. microcarpum extract at a single dose of 2000 mg/kg body weight is either non-toxic or exhibits only minimal toxicity. To validate these findings, further investigations were conducted, examining hematological and biochemical parameters following the medium-term administration of the D. microcarpum extract (Bouyahya et al., 2017).

1.4.7.2 Sub acute Toxicity of *Detarium microcarpum*

In a research study involving Detarium microcarpum, a sub-chronic toxicity investigation was conducted over a 14-day period. During this duration, a daily dosage of 1000 mg/kg body weight of D. microcarpum extract was administered to rats. Careful observations for behavioral changes and any occurrences of rat fatalities revealed that the extract did not result in any deaths or alterations in the rats' habits. These outcomes align with those obtained in the acute toxicity study, indicating that the extract is either weakly toxic or non-toxic.

Changes in body and organ weights serve as indicators of a substance's potential toxicity. Throughout the study, assessments were made regarding the body weight of the animals and the

relative weights of organs such as the heart, liver, kidneys, brain, and lungs. Between the initial measurement (day 0) and day 14, there was no significant variation in body weight among rats in the test group compared to the control group. However, by day 28, it became evident that the weight variation in the test group (+1.19%) was notably lower than that in the control group (+19.48%). This weight change, while indicating some degree of weight loss, is noteworthy for the test group as the final weight variation (+1.19%) was considerably higher than the initial weight (0%). This suggests that the extract contributes to impaired growth in rats.

On the other hand, the relative weights of organs such as the liver, heart, and kidneys showed no significant differences in the test group receiving D. microcarpum bark extract compared to the control group. This weight loss appears to be associated with the extract's ability to prevent the accumulation of fat or its potential to negatively affect fat digestion and absorption. However, scientific studies have established that weight loss can be a result of the absence of fat buildup and the physiological adaptive response to plant extracts (El Menyiy et al., 2021).

1.4.8 *Detarium microcarpum* as an Insecticide

Sustained use of liquid and gaseous insecticides plays a vital role in managing insect populations within stored products. However, their frequent application over many years has disrupted natural biological control mechanisms, resulting in insect pest outbreaks, the widespread development of resistance, adverse effects on non-target organisms, and concerns for both the environment and human health. Given the abundance of bioactive compounds found in plants, they offer a promising alternative to conventional pesticides.

These plants contain various active ingredients, notably including compounds like 3,13E-clerodien-15-oic acid, 4 (18)-clerodien-15-oic acid, 18-oxo-3,13E-clerodien-15-oic acid, and 2-oxo-3,13E-clerodien-15-oic acid. With the exception of the last compound, these chemicals have not been previously linked to D. microcarpum. Remarkably, all four of these substances exhibited significant antifeedant properties at a concentration as low as 1%. Further research is warranted to validate the effectiveness of plant extracts in controlling pests (Kagambega et al., 2020).

1.4.9 Anticancer Activity of *Detarium microcarpum*

Cancer is a disease characterized by uncontrolled and aberrant cell division. In the year 2012, there were approximately 14 million new cancer cases reported, resulting in 8.2 million cancer-related deaths on a global scale. Cancer stands as the second leading cause of mortality worldwide, trailing only cardiovascular disease, and it poses a substantial and pressing public health challenge. The incidence of cancer and associated fatalities are on the rise across the globe. Delving deeper into the biological properties of medicinal plants with potential anticancer attributes holds promise for the treatment and management of this condition.

The IC50 values, denoting the concentration at which methanol and aqueous extracts from three different plants inhibit the growth of MCF7 cells, ranged from 78 to >500 µg/mL. Notably, the stem bark extracts exhibited the most robust antioxidant and antiproliferative characteristics, suggesting that anti-breast cancer compounds might be present in the stem bark of these plants. Additionally, dAgNps (silver nanoparticles) displayed an inhibitory effect on the proliferation of

HeLa cells, with IC50 values of 31.5 µg/mL. Among the extracts, the chloroform and ethyl acetate variants demonstrated the highest effectiveness in restraining osteosarcoma cells. The ethyl acetate extract proved to be lethal to all osteosarcoma cells across all tested concentrations, while the chloroform extract exhibited cell-killing effects at concentrations of 250 and 500 µg/mL.

Furthermore, plant extracts and chemical compounds were observed to inhibit apoptosis while enhancing survival signaling pathways and disrupting proapoptotic intermediates. These bioactive substances hold the potential to impact the angiogenesis pathway, which involves the formation of blood vessels within tumors and represents a critical step in the process of metastasis (Khouchlaa et al., 2021).

1.4.10 Phytochemistry and pharmacology

Numerous investigations into D. microcarpum in West Africa have underscored its medicinal significance, prompting a range of phytochemical and pharmacological examinations across its various components. In particular, the trunk bark yielded a diverse array of compounds, including L-quino-1,5-lactone (1), D-(-)-bornesitol (2), D-pinitol (3), myo-inositol (4), sucrose, D-glucose, and D-fructose. Notably, D-pinitol and its derivatives have garnered recognition for their advantageous effects in conditions associated with insulin resistance, such as diabetes and its associated complications such as obesity, hyperlipidemia, atherosclerosis, and hypertension. Furthermore, it has exhibited anthelmintic and larvicidal properties against Aedes aegypti and Culex quinquefasciatus, along with anti-inflammatory attributes in rat models, particularly in cases of acute and subacute inflammation.

Regarding myo-inositol, it serves as a pivotal compound with critical roles in various biological processes, both in humans and animals. Notably, it plays an indispensable role in the growth of rodents. In humans, a portion of this compound is endogenously produced. Remarkably, myo-inositol has been associated with therapeutic benefits, including its positive impact on conditions such as depression, panic attacks, and obsessive-compulsive disorders.

1.4.10.1 Phenolic compounds

From the trunk bark, coumarin (5) and melilotoside (6) were successfully extracted, while fruits yielded methyl gallate (7). Coumarin is noted for its anti-edematous properties, finding application in cases of lymphedema of the upper limb following radiosurgical breast cancer treatment. However, due to an association with cases of hepatitis, the corresponding drug was withdrawn from the market in France in 1996, subsequently in other European countries. Notably, within the realm of coumarin derivatives, certain compounds exhibit pharmacological activities, primarily functioning as anticoagulants. Dicoumarol and esculoside, well-recognized among them, serve as both venotonic and vasculoprotective agents.

Melilotoside (6) has exhibited antimicrobial activities against Entamoeba histolytica and Giardia lamblia in in vitro studies.

1.4.10.2 Flavonoids

Catechin (8), epicatechin (9), catechin-7-O-galloylester (10), and epicatechin-3-O-galloylester (11) were obtained from the trunk bark, while kaempferol-3-O-β-glucopyranoside (12) was sourced from the leaves, and luteolin (13) along with epicatechin were isolated from the fruits.

Catechin, a fundamental component within the flavan-3-ol family, is ubiquitously present in numerous plant species and serves as the foundational structure for catechic tannins. Much like most flavonoids, it exhibits potent antioxidant properties.

The anti-HIV-1 activity of compounds (8) through (11) was assessed using a cell line infected with the HIV-1IIIB strain, with compound (11) demonstrating notable toxicity.

1.4.10.3 Steroids

β-sitosterol (14), campesterol (15), stigmasterol (16), and sitosterol-3-β-O-[6'-O-palmitoyl-2',3',4'-O-triacetyl-β-D-glucopyranoside] were derived from the trunk bark of D. microcarpum, while β-sitosterol from 3-O-β-D-glucopyranoside was sourced from the fruits. Among these compounds, β-sitosterol, campesterol, and stigmasterol stand out as the three most commonly encountered sterols within the unsaponifiable fraction of oils.

1.4.10.4 Terpenoids

Compound (19) a diterpene of the clerodane type and compound (20) a diastereomer of this molecule were isolated from the trunk bark. Compounds (19) 2-oxo-kolavenic acid, (21) kolavenic acid, (22) 2-pentenoic acid and (23) ent-4(18), 13E-clerodien-15-oic acid are four clerodaney type diterpenes isolated from leaves.

1.4.11 THE LIVER

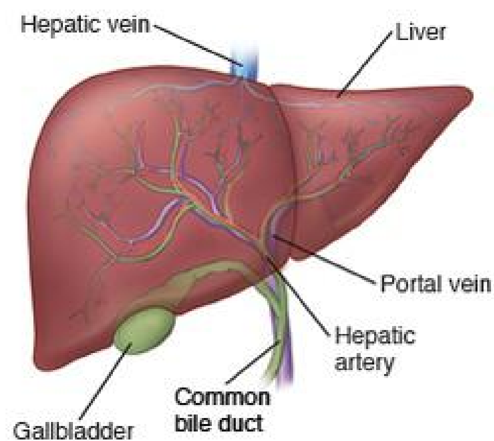

Figure 1.3: A labelled Image of the liver(Teschke, 2018)

Situated in the upper right section of the abdominal cavity, positioned below the diaphragm and resting atop the stomach, right kidney, and intestines, the liver assumes the form of a conical, dark reddish-brown organ with a weight of approximately 3 pounds. It receives its blood supply from two separate sources, namely:

1. Oxygenated blood flows in from the hepatic artery
2. Nutrient-rich blood flows in from the hepatic portal vein

At any given time, the liver retains approximately one pint, equivalent to 13%, of the body's total blood supply. The liver's structure comprises two primary lobes, each composed of eight segments housing a total of 1,000 lobules, which are small units. These lobules are interconnected by small ducts, ultimately merging into larger ducts to create the common hepatic duct. This common hepatic duct serves as the conduit for liver cell-produced bile to reach the gallbladder and the duodenum (the initial section of the small intestine) through the common bile duct (Smuckler, 2015)..

Functions of the Liver

The liver plays a pivotal role in overseeing the majority of chemical levels within the bloodstream and expelling a substance known as bile, which aids in the removal of waste products from the liver. Every drop of blood departing from the stomach and intestines traverses through the liver. Here, the liver meticulously processes this blood, performing tasks such as breakdown, equilibrium maintenance, and the synthesis of essential nutrients. Additionally, it metabolizes drugs into forms that are more easily utilized by the body or rendered nontoxic. Remarkably, the liver is associated with over 500 critical functions. Among the more recognizable ones are::

1. Production of bile, which helps carry away waste and break down fats in the small intestine during digestion
2. Production of certain proteins for blood plasma
3. Production of cholesterol and special proteins to help carry fats through the body

4. Conversion of excess glucose into glycogen for storage (glycogen can later be converted back to glucose for energy) and to balance and make glucose as needed
5. Regulation of blood levels of amino acids, which form the building blocks of proteins
6. Processing of hemoglobin for use of its iron content (the liver stores iron)
7. Conversion of poisonous ammonia to urea (urea is an end product of protein metabolism and is excreted in the urine)
8. Clearing the blood of drugs and other poisonous substances
9. Regulating blood clotting
10. Resisting infections by making immune factors and removing bacteria from the bloodstream
11. Clearance of bilirubin, also from red blood cells. If there is an accumulation of bilirubin, the skin and eyes turn yellow(Washington *et al.*, 2015).

After the liver has metabolized detrimental substances, the resulting by-products are either discharged into the bile or the bloodstream. Those within the bile proceed into the intestine and are eventually eliminated from the body through feces. Conversely, blood-borne by-products are sifted out by the kidneys, exiting the body in the form of urine (Weber et al., 2010).

1.4.12 BIOCHEMICAL INDICES

1.4.12.1 LIVER FUNCTION INDICES

From a scientific perspective, the examination of liver pathology plays a crucial role in identifying and characterizing liver damage, helping to detect clinical or biochemical alterations. During hepatotoxicity, liver injury can manifest in various patterns, including hepatitis, cholestasis, zonal necrosis, and more. As explained by Singh et al. (2011), hepatotoxins can

induce a wide range of histopathological changes and clinical symptoms related to liver damage. To diagnose liver injury, various biomarkers like alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, and others are utilized (Singh et al., 2011).

It's worth noting that elevated enzyme activity levels in the bloodstream serve as critical indicators of liver toxicity, while an increase in total bilirubin levels assesses overall liver function. Consequently, when transaminase levels rise above the normal range alongside a bilirubin level exceeding double its upper normal limit, it signifies a concerning sign of hepatotoxicity. However, it's important to recognize that although liver enzymes increase in acute hepatotoxicity, they tend to decrease with prolonged exposure due to liver damage (Obi et al., 2004).

Singh et al. (2011) also revealed that the histopathological findings, in conjunction with additional clinical or biochemical parameters, contribute to the confirmation of hepatotoxicity. These tests prove invaluable for assessing and managing individuals with liver dysfunction. Nevertheless, relying solely on a single laboratory liver test may not always provide sufficient evidence to conclude liver disease, as some serious liver conditions or infections may be associated with normal enzyme levels, and abnormal levels may also occur in asymptomatic healthy individuals.

1.4.12.2 Alanine Aminotransferase

Alanine aminotransferase (ALT), formerly known as serum glutamic pyruvic transferase (SGPT), is primarily abundant in the liver, surpassing its presence in other parts of the body such as

muscle and the heart. ALT plays a key role in catalyzing transamination, a biochemical reaction that involves the conversion of α-oxoglutarate and L-alanine into L-glutamate and pyruvate.

The normal ALT range typically falls within 7–56 U/L, and levels exceeding this range may suggest injury to liver cells. Elevations in ALT levels, up to approximately 300 U/L, are generally nonspecific. However, notable increases in ALT values, surpassing 500 U/L, are most frequently observed in patients with hepatic conditions like ischemic liver injury (commonly known as shock liver), viral hepatitis, and liver damage induced by toxins. In essence, ALT serves as a crucial biomarker for identifying hepatocellular injury. It's important to note that while significantly elevated ALT levels are associated with hepatocellular diseases, the absolute peak of ALT elevation does not necessarily correlate with the extent of liver cell damage (Gowda et al., 2009).

1.4.12.3 Aspartate Aminotransferase

Aspartate Aminotransferase (AST), formerly known as serum glutamic oxaloacetic transaminase (SGOT), plays a pivotal role in catalyzing transamination reactions. AST typically occurs in two genetically distinct isoenzyme forms, the cytoplasmic and mitochondrial variants. Notably, it is more prevalent in the heart compared to other body tissues such as skeletal muscle, kidney, and liver.

Normal serum levels of AST typically fall within the range of 0 to 35 U/L. Elevated levels of mitochondrial AST are observed in cases of severe tissue necrosis, such as during myocardial infarction, and in chronic liver conditions characterized by tissue necrosis and degeneration. It's

worth noting that after significant liver damage, AST values may increase to levels 10 to 20 times higher than the norm, whereas ALT can rise to approximately 50 times its normal value. AST, however, lacks specificity for liver disease. Therefore, the AST to ALT ratio (AST/ALT) becomes crucial in distinguishing between liver damage and damage to other organs (Huang et al., 2006).

1.4.12.4 Alkaline Phosphatase

Alkaline Phosphatase (ALP) is distributed in various body tissues, including the placenta, the epithelial mucosa of the small intestine, the liver, the proximal convoluted tubule of the kidney, and bone. Its functions encompass contributing to bone calcification and aiding in lipid transport within the intestine. The majority of ALP activity in the bloodstream arises from the liver and bone.

Normal serum ALP levels typically range from 41 to 133 U/L. In cases of acute viral hepatitis, ALP levels often remain within the normal range or show moderate elevation. However, when ALP levels are elevated alongside persistent itching, it may be associated with Hepatitis A and indicate the presence of cholestasis.

It's important to note that ALP is not highly specific for assessing liver damage. To distinguish between liver disorders and bone-related conditions, the level of gamma glutamyl transferase is evaluated, as it tends to increase specifically in cholestatic disorders and not in bone diseases (Gowda et al., 2009).

1.4.12.5 Albumin

Albumin, primarily synthesized in the liver, stands as the predominant protein within the blood serum. Albumin assumes a multifaceted role in the body, including the transportation of various substances like drugs, hormones, and vitamins throughout the body. Additionally, it plays a critical role in preventing the leakage of fluids from blood vessels and in nourishing tissues.

A decrease in albumin levels within the bloodstream may indicate potential issues such as kidney damage, shock, liver impairment, severe inflammation, or malnutrition. Consequently, albumin values serve as a valuable tool for clinicians to assess and screen for conditions related to kidney or liver health, which may be attributed to factors like lupus and others (Walker et al., 1990).

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 REAGENTS/CHEMICALS

1. Alanine aminotransferase test kits- Randox laboratories Ltd, United Kingdom
2. Aspartate aminotransferase test kits- Randox laboratories Ltd, United Kingdom
3. Albumin test kits- Randox laboratories Ltd, United Kingdom
4. Creatinine test kits- Randox laboratories Ltd, United Kingdom
5. Total protein test kits- Randox laboratories Ltd, United Kingdom
6. Urea test kits- Randox laboratories Ltd, United Kingdom
7. Alkaline phosphatase test kits- Randox laboratories Ltd, United Kingdom
8. Malondialdehyde test kits- Fortress diagnostics Ltd, United Kingdom
9. Bilirubin test kits- Randox laboratories Ltd, United Kingdom
10. Methanol
11. Ethylacetate
12. Dichloromethane
13. Chloroform
14. Normal saline

2.1.2 EQUIPMENT

1. Water bath
2. Analytical Weighing balance

3. Centrifuge
4. Spectrophotometer
5. Masking tape
6. Cotton wool
7. Gloves
8. Separation funnel
9. Rotary evaporator
10. Beakers
11. Stirring rod
12. Magnetic stirrer
13. Electric oven
14. Test tubes
15. Bowls
16. Eppendoff tubes
17. EDTA bottles
18. Ice block
19. 2ml syringe

20. Foil paper

21. Masking tape

22. Gloves

23. Cotton wool

2.2 METHODS

2.2.1 PLANT MATERIALS(SAMPLES)

Detarium microcarpium stem bark was bought from a local seller at Badagary, Lagos State, Nigeria. The stem barks was allowed to air dried, ground and fluid extracted from the grounded samples each using analytical methanol for soaking.

2.2.2 **Preparation of Plant Extracts**

About 2.5 kg of powdered stem bark of ***Detarium microcarpium*** was soaked with 4.5 liters of methanol. Extraction was by maceration over a 72 hour period. After the 72 hours, filtration was carried out using a Muslin cloth. The filtrate was concentrated using a rotary evaporator, after which it was dried. The dried sample (2.0 g) was dissolved in 100 ml of distilled water to obtain the stock solution.

2.2.3 EXPERIMENTAL ANIMALS

Adult male albino mice used in this experiment were procured from a local breeder in Benin City, Edo State. They were acclimatized for two weeks at the Animal Unit of the Department of

Biochemistry, Faculty of Life Sciences, University of Benin. They were given growers mash and water *ad libitum* throughout the duration of the experiment.

2.2.4 EXPERIMENTAL DESIGN

Twenty-five male albino rats weighing 25- 33 g were divided into six groups of five rats each. They were given growers mash and water *ad libitum*. Groups one (control), two, three, four and five were administered 0, 250, 500, 250, 500mg/kg body weight of the methanol stem bark extract respectively for a period of 14 days. Measurement of body weights were carried out on the 15th day. After 14 days, the rats were subjected to overnight fast, they were sacrificed and blood samples collected into sterile containers with anticoagulant.

2.2.5 PREPARATION OF ANIMAL SAMPLES

10 % liver homogenates were used for liver assay respectively. This was achieved by grinding 0.001 kg of the kidney and liver tissue with a homogenizer and normal saline. The mixture was centrifuged at a speed of 10,000 x g for 10 minutes. The supernatant was cautiously decanted and stored in refrigerator for use in biochemical assay.

2.2.6 SUB-ACUTE TOXICITIES OF FRACTIONS OF *Detarium microcarpium*

The sub-acute toxicities of the fractions of *Detarium microcarpium* will be determined using the Organization for Economic Co-operation and Development Protocol (OECD, 2001) with modifications. The appropriate fraction dose for each animal will be arrived at with the formula given below and dissolved in an appropriate vehicle (Carboxyl methyl cellulose – CMC) before administering to the animals at their various doses.

Amount of Extract = Dose/1000 × mean body weight (g)

= X mg.

Principle

The Organization for Economic Co-operation and Development Protocol (OECD, 2001) method of sub-acute toxicity involves the use of a certain number of animals per group (in this case, six). The test will allow the establishment of the short term effects of the plant fractions in-vivo by assaying for some biochemical parameters.

Procedure

Twenty five (25) mice of weights 25 to 33g will be divided into 5 groups as shown below. Group 1 will be the control group which will be receiving a fraction dose level of 0mg/kg body weight plant fraction (i.e., only CMC vehicle). Groups 2 and 3 will be respectively administered 250mg/kg and 500mg/kg of fractions of *Spondias mombin*. Also, groups 4 and 5 will be administered 250mg/kg and 500mg/kg respectively of fractions of *Detarium microcarpium.* The administration will be done once daily for a period of 14days after which the animals will be fasted overnight and sacrificed on day 15.

Table 1: Experimental groupings of Sub-acute toxicity Study

S/N	Groups	Administered Substance
Group 1	Normal control	0.2 mL Carboxyl methylcellulose (CMC) daily
Group 2	S.M 250mg/kg body weight	S. M 250mg/kg body weight daily
Group 3	S.M 500mg/kg body weight	S. M 500mg/kg body weight daily
Group 4	D. M 250mg/kg body weight	D. M 250mg/kg body weight daily
Group 5	D.M 500mg/kg body weight	D M 500mg/kg body weight daily

2.2.7 Sample Collection

The animals will be fasted overnight prior to their sacrifice after which they will be sacrificed by cervical dislocation. Blood samples will be collected from the aorta and heart with a syringe into plain tubes (for serum) and EDTA tubes (for hematological analysis). The collected blood

samples in the plain tubes will be allowed to clot for some time and thereafter centrifuged at 1500g for 15minutes so as to obtain serum.

2.2.8 Preparation of Tissue Homogenates

After sacrificing the mice, the organs of interest (liver) was harvested and kept in plain tubes (universal bottles) with 10 mL of normal saline and stored in ice (4^0C). One gram (1g) of each tissue will be homogenized in 10ml of 0.9% ice-cold phosphate buffered saline. Thereafter, the homogenates will be centrifuged at 5000 rpm for 10 min with the separated supernatant being stored for later use.

2.2.9 Assessment of Liver Function Enzymes

The following liver functions will be assessed using standard procedures: Alanine Aminotransferase (ALT) Activity (using the Reitman-Frankel colorimetric method (1957)), Aspartate Aminotransferase (AST) Activity (using the Reitman-Frankel colorimetric method (1957)), Alkaline Phosphatase activity (using the Reitman-Frankel colorimetric method (1957) and Albumin.

2.3 TISSUE STATUS AND ENZYME ACTIVITIES

2.3.1 LIVER FUNCTION INDICES

2.3.1.1 Determination of Alanine Aminotransferase (ALT) Activity

This was determined by the method of Reitman and Frankel (1957).

Principle

Alanine aminotransferase is measured by observing the amount of pyruvate hydrazone produced with 2,4-dinitrophenylhydrazine.

Procedure

Exactly 0.1 mL of the sample was mixed with 0.5 mL of ALT R1 solution in the sample test tube while for the blank 0.1 ml of distilled water was mixed with 0.5 mL of ALT R1 solution. The mixtures were incubated for 30 minutes at 37°C. After incubation, 0.5 mL of ALT solution R2 was added across all test tubes. The mixture was allowed to stand for 20 minutes at 20°C. Finally, 5.0 mL of sodium hydroxide was added across all test tubes. The absorbance was read at 546 nm after 5 minutes.

Calculation for ALT activity

Absorbance	U/I	Absorbance	U/I
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72

0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

2.3.1.1 Determination of Aspartate Aminotransferase (AST) Activity

Reitman and Frankel (1957) method was used to determine AST.

Principle

α- Oxoglutarate + L-Aspartate $\xrightarrow{AST}$ L-Glutamate + Oxaloacetate

Aspartate aminotransferase is measured by observing the amount of oxaloacetate hydrazone produced with 2,4-dinitrophenylhydrazine.

Procedure

Exactly 0.1 mL of the sample was mixed with 0.5 mL of AST R1 solution in the sample test tube while for the blank 0.1 ml of distilled water was mixed with 0.5 mL of AST R1 solution. The mixtures were incubated for 30 minutes at 37°C. After incubation, 0.5 mL of AST solution R2 was added across all test tubes. The mixture was allowed to stand for 20 minutes at 20°C. Finally, 5.0 mL of sodium hydroxide was added across all test tubes. After 5minutes the absorbance was read at 546 nm.

Calculation for AST activity

Absorbance	U/I	Absorbance	U/I
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

2.4.3 Determination of Alkaline Phosphatase (ALP) Activity

The method developed by Tietz, (1976) was used in the evaluation of serum alkaline phosphatase activity.

Principle

p-Nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p-nitrophenol

Procedure

Exactly 0.5 mL of alkaline phosphatase substrate was dispensed into blank, standard and sample test tubes and it was left to equilibrate to 37°C for three minutes. Then, 0.05 ml of distilled water, standard reagent and sample were introduced into their respective test tubes, and then mixed thoroughly. The mixture was incubated for 10 minutes at 37°C. Alkaline phosphatase colour developer (2.5 ml) was added to all test tubes and mixed. Absorbance of samples was read against blank at 590 nm.

Calculation for ALP activity

U/L = 2760 × ÆA 405 nm/min

2.4.4 DETERMINATION OF ALBUMIN

This was determined by the method of Doumas *et al.* (1971).

Principle

The estimation of albumin concentration in the serum is on the basis of its quantitative binding to 3, 3', 5, 5'- tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG) (indicator). At 578 nm, the albumin-BCG-complex is known to absorb at a maximum capacity and the concentration of albumin is directly proportional to the absorbance read.

Procedure

Exactly 0.01 mL of distilled water was mixed with 3.00 mL of BCG reagent (blank). The standard solution (0.01 ml) was also mixed with 3 ml of BCG reagent (standard). Serum (0.01 ml) was mixed with 3.00 mL of BCG reagent (R1). The separate mixtures were incubated for 5

minutes at 20 - 25°C. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) was estimated against the reagent blank at 630 nm.

Manual calculation for albumin

Albumin conc (g/l)

$$\frac{A_{sample}}{A_{standard}} \times \text{Concentration of standard}$$

2.4.6 DATA ANALYSIS`

All data were represented as mean ± standard error of mean (SEM). Values obtained were examined using analyses of variance (ANOVA), and the least significance difference (LSD) analysis, to evaluate the differences among groups and for multiple comparisons among different groups. $p < 0.05$ was the level of significance used. EXCEL/PC software was used in all calculations.

CHAPTER THREE

RESULTS

3.1. *IN VIVO* SUBACUTE TOXICITY OF DICHLOROMETHANE EXTRACT OF *DETARIUM MICROCARPIUN* FRACTIONS ON ALT, AST, ALP AND ALBUMIN ACTIVITY.

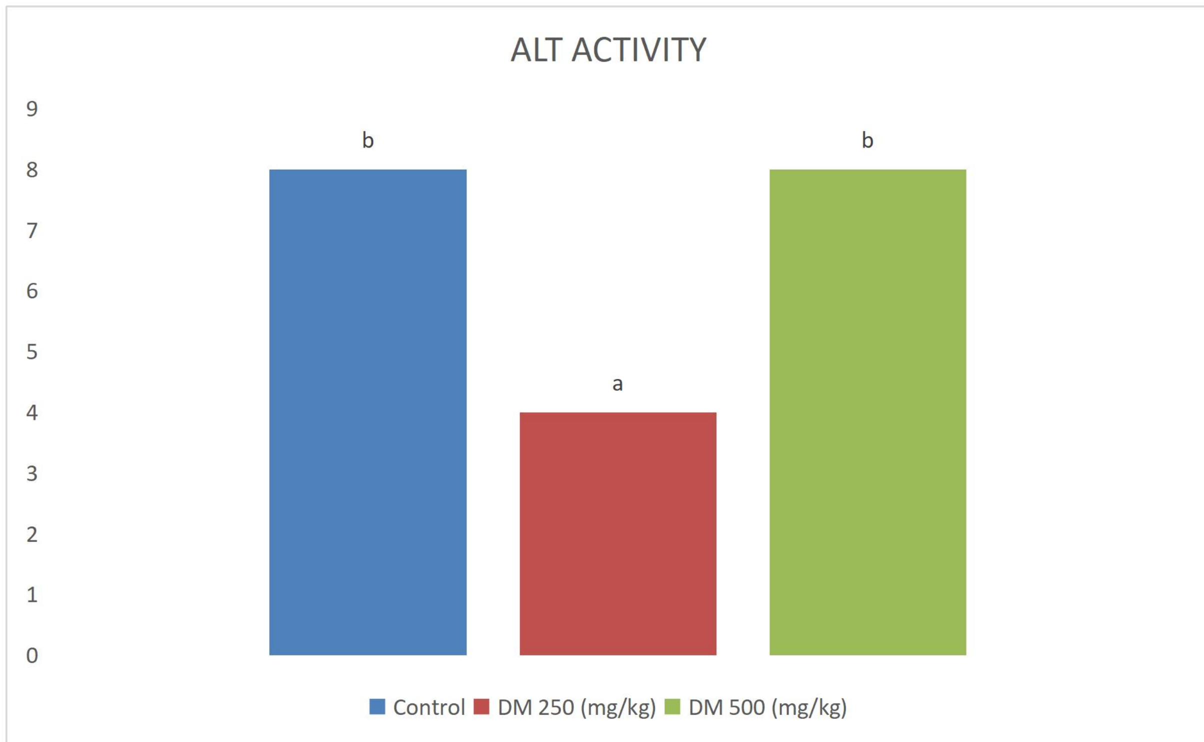

Figure 1: ALT activity of dichloromethane fraction of *Detarium microcarpiun* leaves in comparison to the control. (Data expressed as mean ± SEM, n=3). Different lowercase letters represent significant difference between mean at $p \leq 0.05$.

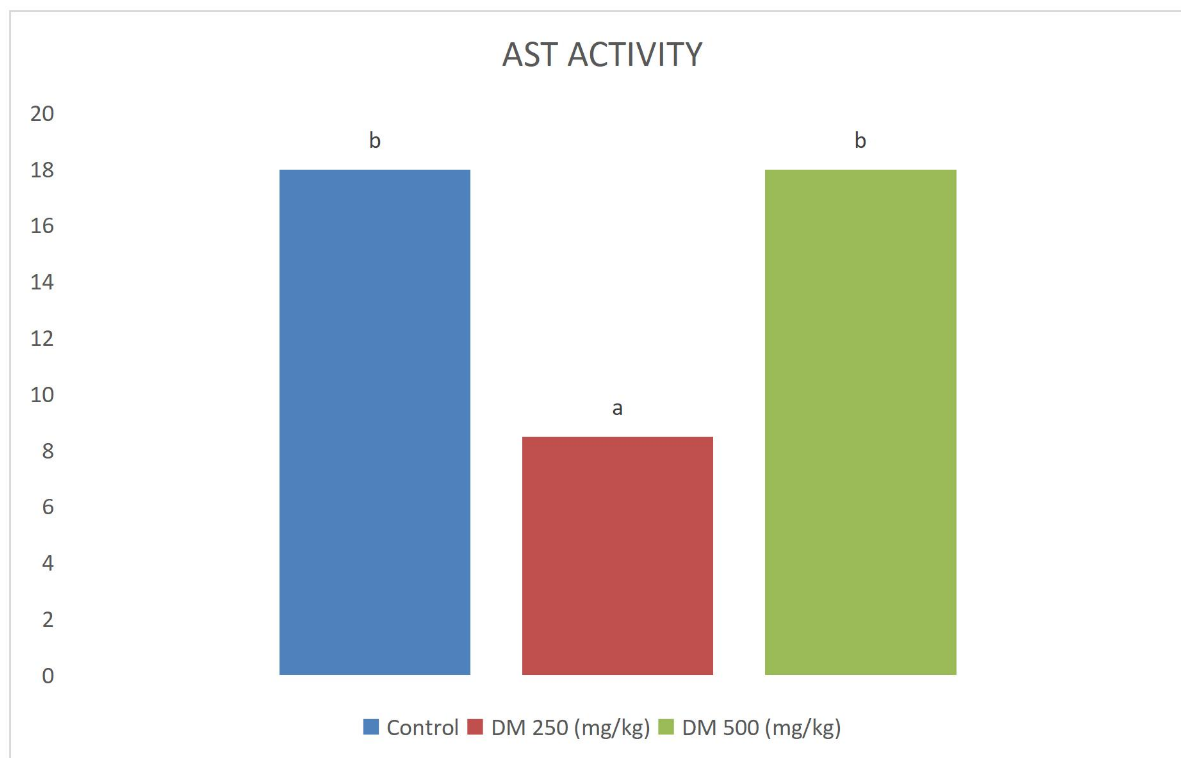

Figure 2: AST activity of dichloromethane fraction of *Detarium microcarpiun* leaves in comparison to the control. (Data expressed as mean ± SEM, n=3). Different lowercase letters represent significant difference between mean at $p \leq 0.05$.

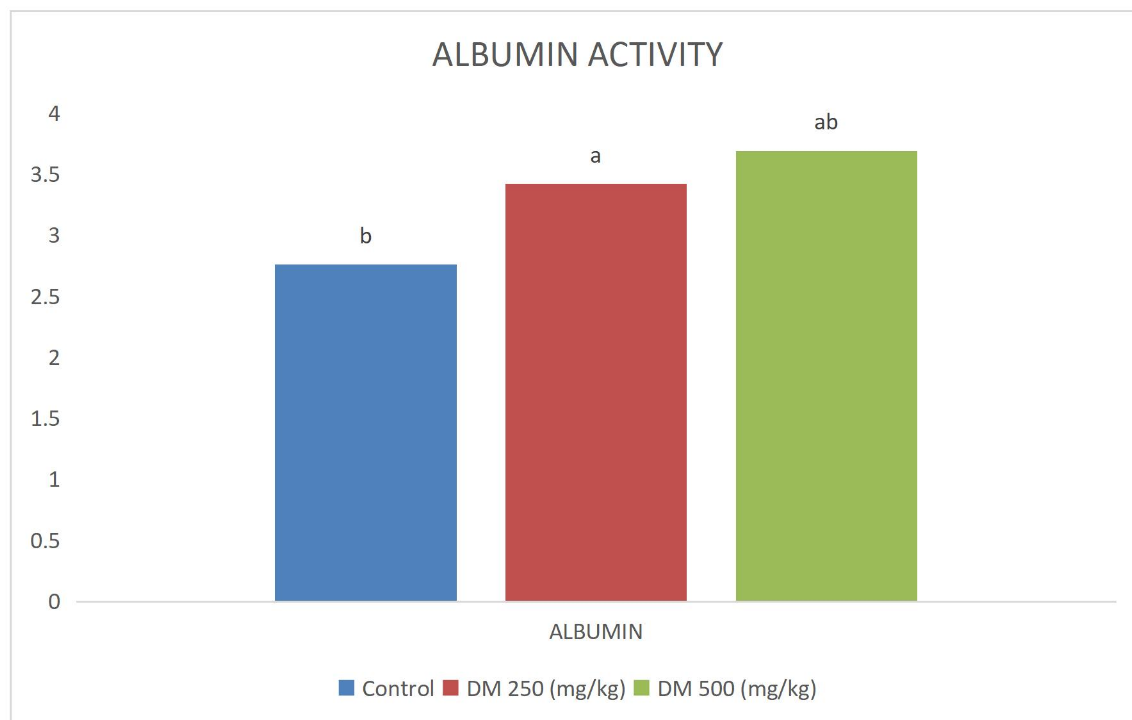

Figure 3: ALBUMIN activity of dichloromethane fraction of *Detarium microcarpiun* leaves in comparison to the control. (Data expressed as mean ± SEM, n=3). Different lowercase letters represent significant difference between mean at $p \leq 0.05$.

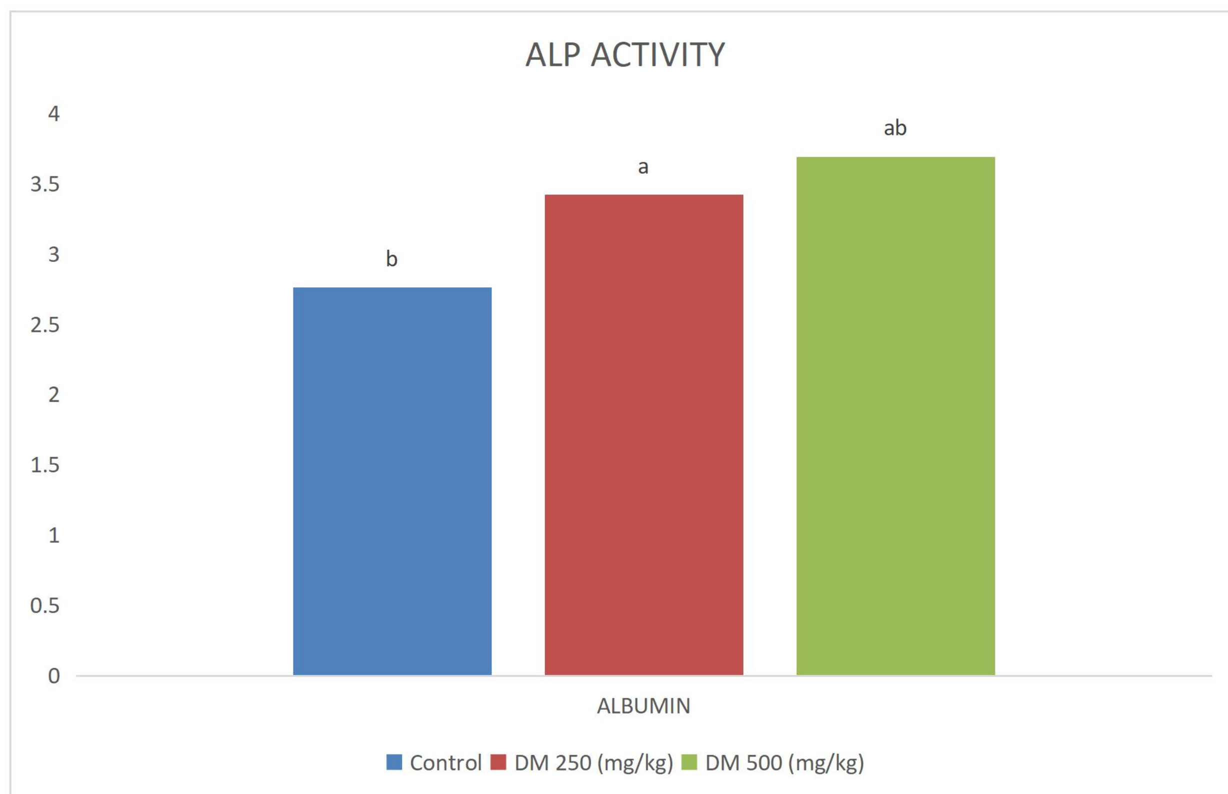

Figure 4: ALP activity of dichloromethane fraction of *Detarium microcarpiun* leaves in comparison to the control. (Data expressed as mean ± SEM, n=3). Different lowercase letters represent significant difference between mean at $p \leq 0.05$.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The utilization of medicinal plants has always been a fundamental aspect of human culture and is extensively prevalent in Africa. Throughout recent decades, herbs and spices have been employed in culinary and traditional therapeutic practices for the management of various ailments. The nutritional and medicinal characteristics of the plant may be interconnected through both nutrient and non-nutrient phytochemicals. Among the plant species that has been ethnobotanically documented to have diverse medicinal applications but not without acute and subacute toxic effect is the *Detarium microcarpium* (Lawal *et al.,* 2012).

Measurement of the activity of an enzyme is an indispensable tool in the assessment of cellular toxicity caused by chemical compounds including plant extracts (Malomo. 2000; Yakubu *et al*., 2003). The aminotransferases (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al*., 2006). The increase in the liver AST and ALT support the suggested hepatotoxicity. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974) and is used to assess the integrity of the plasma membrane (Akanji et al., 1993). The significant reduction in the activity of alkaline phosphatase in the liver is an indication that the enzyme have leaked from the organ through the disrupted plasma membrane into the serum. This is suggestive of permeability changes due to the chemical components in the extract. The loss in ALP activity in the liver of the animals may adversely affect the transfer of

metabolites or required ions across the cell membrane and consequently insufficient ions and metabolites to these organs (Akanji et al., 1993). A previous report had shown that the plant extract contains saponins (Ijeh and Ukweni, 2007). Saponins are known for their wide range of biological activities which include disruption of biological membranes resulting in escape of large quantities of metabolites including enzymes and generation of free radicals (Francis et al., 2002; Nadi et al., 2004; Sparge et al., 2004). Therefore, the reduction in ALP activity may suggest saponin-induced toxicity. This observation however has not been reported in previous toxicological studies of the plant leaf extract.

4.2 CONCLUSION

In conclusion, dichloromethane leaf extract of *Detarium microcarpium* has selective adverse effects on some parameters of liver function of the animals. The changes in levels of some enzymes and parameters were not reflected in the histology of the liver. This shows that further studies are required, which would be long-term, and multiple organs should be involved to examine the effect of dose, duration and toxicity of consumption of dichloromethane leaf extract of *Detarium microcarpium* on the biological system.

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APPENDIX

1.1 WEIGHT OF ANIMALS USED

GP	S/N	wt. on day1 (in g)	wt. on day15 (in g)	wt. of Liver (in g)
GP 1	1T	23.04	21.06	1.40
	1B	25.33	26.36	1.26
	1RL	23.17	22.79	1.70
	1H	24.58	23.10	1.13
GP2	2RL	25.33	26.89	1.21
	2LL	30.08	21.21	1.49
GP3	3RL	31.15	28.47	1.18

	3T	28.85	27.35	1.31
	3H	31.53	28.23	1.73
GP4	4T	29.17	26.03	1.49
	4RL	27.18	23.10	1.58
	4LL	29.67	29.33	1.16
	4B	28.53	18.89	1.04
	4H	30.31	27.02	1.53
	4A	29.84	26.50	1.60
GP5	5LL	24.22	16.24	1.49
	5B	24.22	24.00	1.15
	5T	27.36	25.36	1.38
	5H	32.12	30.02	1.40
	5A	31.48	29.98	1.56
	5RL	27.84	28.54	1.49

1.2 ABSORBANCE VALUES

GP	AST @546nm	ALT @546nm	ALP @405nm(0 min)	ALP @405nm(1 min)	ALP @405nm(2 min)	ALP @405nm(3 min)	ALBUMIN @578nm
GP 1	0.031	0.032	0.148	0.204	0.418	0.777	0.063
	0.067	0.039	0.147	0.225	0.467	0.879	0.068
GP 2	0.086	0.125	0.078	0.168	0.379	0.663	0.016
	0.043	0.048	0.013	0.074	0.264	0.548	0.064
GP 3	0.02	0.002	0.035	0.11	0.29	0.592	0.04
	0.017	0.018	0.032	0.093	0.271	0.546	0.059
GP 4	0.021	0.022	0.07	0.129	0.241	0.436	0.081
	0.012	0.024	0.061	0.111	0.225	0.414	0.094
GP 5	0.062	0.017	0.068	0.142	0.317	0.605	0.048
	0.031	0.072	0.064	0.144	0.347	0.685	0.03

1.3 CONCENTRATION VALUES

GROUP	LFT AST(U/L)	LFT ALT(U/L)	LFT ALP(U/L)	LFT ALBUMIN (g/dl)
1	13	8	154.56	2.66
	23	8	215.28	2.87
	16	8	184.92	2.76
2	31	21	248.4	0.67
	16	8	168.4	2.70
	23	17	207	2.02
3	7	4	207	0.36
	7	4	168.36	1.68
	7	4	187.68	4.17
4	10	4	162.87	3.41
	7	4	138	3.96
	7	4	150.42	3.69
5	23	4	204.24	2.02

13	12	220.8	1.26
16	8	212.52	1.65

1.4 AST

One-way ANOVA: Control, SM 250mg/kg, SM 500mg/kg, DM 250mg/kg, DM 500mg/kg

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	Levels	Values
Factor	5	Control, SM 250mg/kg, SM 500mg/kg, DM 250mg/kg, DM 500mg/kg

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	577.6	144.40	6.45	0.008
Error	10	224.0	22.40		
Total	14	801.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.73286	72.06%	60.88%	37.13%

Means

Factor	N	Mean	StDev	95% CI
Control	3	17.33	5.13	(11.24, 23.42)
SM 250mg/kg	3	23.33	7.51	(17.24, 29.42)
SM 500mg/kg	3	7.000	0.000	(0.912, 13.088)
DM 250mg/kg	3	8.00	1.73	(1.91, 14.09)
DM 500mg/kg	3	17.33	5.13	(11.24, 23.42)

Pooled StDev = 4.73286

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
Control	3	17.33	A
SM 250mg/kg	3	23.33	A
SM 500mg/kg	3	7.000	B
DM 250mg/kg	3	8.000	B
DM 500mg/kg	3	17.33	A

Factor	N	Mean	Grouping
SM 250mg/kg	3	23.33	A
DM 500mg/kg	3	17.33	A
Control	3	17.33	A
DM 250mg/kg	3	8.00	B
SM 500mg/kg	3	7.000	B

Means that do not share a letter are significantly different.

Fisher Individual 95% CIs

ALT

One-way ANOVA: Control_1, SM 250mg/kg_1, SM 500mg/kg_1, DM 250mg/kg_1, DM 500mg/kg_1

Method

Null hypothesis All means are equal

Alternative hypothesis At least one mean is different

Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	Control_1, SM 250mg/kg_1, SM 500mg/kg_1, DM 250mg/kg_1, DM 500mg/kg_1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	257.1	64.27	5.33	0.015
Error	10	120.7	12.07		
Total	14	377.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.47371	68.06%	55.28%	28.12%

Means

Factor N Mean StDev 95% CI

Control_1 3 8.000 0.000 (3.531, 12.469)

SM 250mg/kg_1 3 15.

Factor	N	Mean	Grouping
Control	3	8.000	
SM 250mg/kg	3		
SM 500mg/kg	3		
DM 250mg/kg	3		
DM 250mg/kg	3		

33 6.66 (10.86, 19.80)

SM 500mg/kg_1 3 4.000 0.000 (-0.469, 8.469)

DM 250mg/kg_1 3 4.000 0.000 (-0.469, 8.469)

DM 500mg/kg_1 3 8.00 4.00 (3.53, 12.47)

Pooled StDev = 3.4737

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
Control	3	8.000	B

SM 250mg/kg	3	15.33	A
SM 500mg/kg	3	4.000	B
DM 250mg/kg	3	4.000	B
DM 500mg/kg	3	8.000	B

Factor N Mean Grouping

SM 250mg/kg_1 3 15.33 A

DM 500mg/kg_1 3 8.00 B

Control_1 3 8.000 B

DM 250mg/kg_1 3 4.000 B

SM 500mg/kg_1 3 4.000 B

Means that do not share a letter are significantly different.

Fisher Individual 95% CIs

ALP

One-way ANOVA: Control_2, SM 250mg/kg_2, SM 500mg/kg_2, DM 250mg/kg_2, DM 500mg/kg_2

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	Control_2, SM 250mg/kg_2, SM 500mg/kg_2, DM 250mg/kg_2, DM 500mg/kg_2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	7252	1813.1	2.91	0.078
Error	10	6240	624.0		
Total	14	13493			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
24.9802	53.75%	35.25%	0.00%

Factor	N	Mean	StDev	95% CI
Control	3	184.9	30.4	(152.8, 217.1)
SM 250mg/kg	3	207.9	40.0	(175.8, 240.1)
SM 500mg/kg	3	187.7	19.3	(155.5, 219.8)
DM 250mg/kg	3	150.42	12.42	(118.29, 182.55)
DM 500mg/kg	3	212.52	8.28	(180.39, 244.65)

Pooled StDev = 24.9802

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
Control	3	184.9	AB
SM 250mg/kg	3	207.9	A
SM 500mg/kg	3	187.7	AB
DM 250mg/kg	3	150.42	B
DM 500mg/kg	3	212.52	A

Factor N Mean Grouping

DM 500mg/kg_2 3 212.52 A

SM 250mg/kg_2 3 207.9 A

SM 500mg/kg_2 3 187.7 A B

Control_2 3 184.9 A B

DM 250mg/kg_2 3 150.42 B

Means that do not share a letter are significantly different.

Fisher Individual 95% CIs

ALBUMIN

ONEe-way ANOVA: Control_3, SM 250mg/kg_3, SM 500mg/kg_3, DM 250mg/kg_3, DM 500mg/kg_3

Method

Null hypothesis All means are equal

Alternative hypothesis At least one mean is different

Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	Control_3, SM 250mg/kg_3, SM 500mg/kg_3, DM 250mg/kg_3, DM 500mg/kg_3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	8.498	2.124	2.11	0.155
Error	10	10.084	1.008		
Total	14	18.582			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.00417	45.73%	24.03%	0.00%

Means

Factor	N	Mean	StDev	95% CI
Control_3	3	2.7633	0.1050	(1.4715, 4.0551)
SM 250mg/kg_3	3	1.797	1.033	(0.505, 3.088)
SM 500mg/kg_3	3	2.07	1.93	(0.78, 3.36)
DM 250mg/kg_3	3	3.687	0.275	(2.395, 4.978)
DM 500mg/kg_3	3	1.643	0.380	(0.352, 2.935)

Pooled StDev = 1.00417

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping
Control	3	2.7633	AB
SM 250mg/kg	3	1.797	B
SM 500mg/kg	3	2.07	AB
DM 250mg/kg	3	3.687	A
DM 500mg/kg	3	1.643	B

Factor N Mean Grouping

DM 250mg/kg_3 3 3.687 A

Control_3 3 2.7633 A B

SM 500mg/kg_3 3 2.07 A B

SM 250mg/kg_3 3 1.797 B

DM 500mg/kg_3 3 1.643 B

Means that do not share a letter are significantly different.

Fisher Individual 95% CIs