THE EFFECTS OF MIRACLE SEED ULTIMA® ON LIVER AND LIPID PROFILE OF MALE WISTAR RATS

BY

PRECIOUS IZEHIESE OSAIGBOVO

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CERTIFICATION

This is to certify that this project work was carried out by PRECIOUS IZEHIESE OSAIGBOVO with matriculation number BMS2201869, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfillment of the requirements for the award of Bachelor of science (B.sc) degree in Medical Biochemistry.

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DR. J.C ANIONYE Date

(Project Supervisor)

_____ _____

DR. N. B AGUEBOR OGIE Date

(A .g,Head of Department)

_____ _____

EXTERNAL EXAMINER

DEDICATION

I dedicate this work to God Almighty,my source of strength, inspiration,wisdom and understanding and to my lecturers who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development

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ABSTRACT

Miracle Seed Ultima® (MSU) is widely used as a natural supplement, yet scientific evidence on its safety profile remains limited. This study therefore evaluated the sub-acute toxicity effects of MSU on liver function and lipid metabolism in male Wistar rats. Twenty rats were randomly assigned to four groups and administered 0 mg/kg (control), 100 mg/kg, 300 mg/kg, and 1000 mg/kg of MSU orally for 28 days. Liver biomarkers including ALT, AST, ALP, total protein, albumin, total bilirubin, and direct bilirubin were analysed, while lipid profile parameters (total cholesterol, triglycerides, HDL, LDL, and VLDL) were assessed.The results showed no statistically significant differences ($P > 0.05$) in AST, ALP, total bilirubin, direct bilirubin, or albumin levels across all doses. However, ALT increased significantly ($P < 0.05$) in the 300 mg/kg and 1000 mg/kg groups compared with the control, with mean values of 147.35 ± 12.9^{a} (control), 135.00 ± 8.7^{abd} (300 mg/kg), and 200.56 ± 22.2^{ace} (1000 mg/kg), indicating a dose-related biochemical change. Total protein decreased significantly in the 100 mg/kg group 4.06 ± 0.1^{a} (control) and 3.39 ± 0.1^{bc}(100mg/kg), although values remained within physiological ranges. Lipid parameters showed no statistically significant alterations, indicating that the observed slight increase in total cholesterol in Group 3 and reduced triglycerides in Group 2 were not biologically meaningful since they were not statistically supported.Overall, the findings indicate that MSU did not produce broad hepatic or lipid toxicity within the 28-day period. Although ALT increased at higher doses, a single enzyme elevation without corresponding changes in other hepatic markers does not conclusively indicate liver damage. Nevertheless, the dose-dependent rise suggests a potential early biochemical response that warrants further attention. The study highlights the need for additional investigations involving histopathology, oxidative stress markers, and long-term exposure to more conclusively establish the safety profile of MSU.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Herbal dietary supplements and “natural” seed-based remedies enjoy widespread use Worldwide, and especially in West Africa, where traditional medicines and commercial herbal blends are regularly consumed for general health, fertility, and “blood tonic” claims, herbal dietary supplements and “natural” seed-based remedies are widely used (Miracle Seed Ultima, 2025; Konga, 2025). There is an evidentiary gap between public use and scientific understanding of toxicity and organ-specific effects since many commercial seed formulations are poorly defined and lack rigorous preclinical safety data, despite their widespread use.This gap is significant because concentrated phytochemicals (alkaloids, glucosinolates, fatty acids, saponins, etc.) found in seeds and seed-derived oils can have positive pharmacologic effects at low doses but cause organ damage, particularly hepatic damage, or metabolic disorders at higher or longer exposures (Huang *et al*., 2023).

“Miracle seed ultima” is advertised as a multi-seed/fruit combination that is meant to strengthen the immune system, improve fertility, and act as a “blood tonic.” The product’s website and retail listings provide the composition as advertised (a blend of several seeds and fruits in a liquid preparation) and the commercial claims; however, the publicly accessible materials lack peer-reviewed toxicological or pharmacokinetic data to support safety claims

(Miracle Seed Ultima, 2025; Konga, 2025). Pharmacodynamic interactions and cumulative toxicity in organs involved in metabolism and clearance—primarily the liver—can occur when a product's composition includes a complex mixture of botanicals and seeds.Therefore, before assuming safety for human usage, preclinical investigations that assess organ function and biochemical indicators following repeated dosage are crucial (Huang *et al.,* 2023).

The primary organ for the metabolism of xenobiotics is the liver, which is also frequently the focus of toxicity from seed oils and herbal medicines. Hepatic biomarkers, such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin, are often measured in subacute (repeated-dose, usually 14–28 day) toxicity studies in addition to histopathology and organ weights to identify early hepatotoxic signals that might not manifest after a single dose (acute testing).Assessing serum lipid profiles (total cholesterol, LDL, HDL, triglycerides) along with liver function provides a more comprehensive picture of metabolic and hepatic safety after repeated exposure because many seeds and seed oils have an impact on lipid metabolism, either positively (such as lipid-lowering phytosterols, unsaturated fatty acids) or negatively (such as hepatosteatosis from altered lipid handling). This integrative method is recommended by OECD guidelines and a number of published subacute studies for screening plant extracts and seed oils for potential harmful effects (OECD, 2008; Aragie *et al*., 2025; Iweala *et al.,* 2024).

The necessity of product-specific evaluation is demonstrated by a number of recent preclinical studies of distinct seed types. For instance, experimental subacute investigations of seed oils or seed meals, such as the seed fractions of Persea americana (avocado) and Lepidium sativum (garden cress), have revealed alterations in clinical chemistry, bodyweight and organ histology in Wistar or albino rats following repeated dosage; results are dependent on dose, preparation (fixed oil, ethanol extract, defatted meal), and length of research (Aragie *et al*., 2025; Iweala *et al.,* 2024). Other seed studies, on the other hand, show minimal or no

negative effects at moderate dosages, highlighting the fact that formulation and exposure schedule have a significant impact on toxicity. A focused subacute investigation is warranted since Miracle Seed Ultima is a multi-seed blend (composition not completely reported in peer-reviewed literature), making it impossible to properly predict its toxicological profile from individual-seed data (Huang *et al*., 2023). Wistar rats are a popular rodent model for subacute oral toxicity assessment because they are sensitive and repeatable. Male and/or female Wistar rats are used in numerous peer-reviewed research and the OECD Test Guideline 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents) to identify biochemical, hematological, and histological alterations after repeated oral dose (OECD, 2008). Clinical observations, body and organ weights, liver enzymes, full lipid panel, and microscopic histopathology of the liver and other target organs (kidney, heart) are typical measured endpoints; using male Wistar rats for an initial subacute study can reduce inter-sex variability and is acceptable for initial safety screening. When combined, these endpoints provide a sensitive indicator for lipid metabolic disorders and hepatic damage that may result from long-term usage of seed-based supplements (OECD, 2008: Aragie *et al.,*2025*).*

1.2 Problem statement

Numerous herbal products on the market are used without a sufficient scientific assessment of their safety. The majority of these products have not been evaluated to ascertain their impact on key organs, despite the claims made by producers. Although Miracle Seed Ultima is a well-known herbal seed mixture, there are no published toxicological studies to confirm its safety for frequent use. It is crucial to look into if long-term consumption of Miracle Seed Ultima could affect lipid levels or liver function because comparable seed-based extracts have demonstrated both positive and negative effects on the liver and metabolism in animal tests. Users could unintentionally suffer organ toxicity in the absence of such evidence.

1.3 Justification of study

Toxicological examination is required due to the growing usage of herbal and seed-based supplements. It is crucial to evaluate Miracle Seed Ultima's safety in order to safeguard customers and offer trustworthy scientific data. Any product taken orally on a regular basis has the potential to damage the liver or alter lipid metabolism because the liver is the primary location for detoxification. The purpose of this study is to ascertain whether long-term usage of Miracle Seed Ultima presents any health hazards. Additionally, it will provide important information for regulatory evaluation, public health monitoring, and the safe use of herbal remedies.

1.4 Research questions/Hypothesis

What is the effect of Miracle Seed Ultima on liver enzyme levels such as bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

What is the Evaluation in the impact of Miracle Seed Ultima on lipid profile markers including high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and total cholesterol.

1.5 Aim of study

The main aim of this study is to evaluate the sub-acute toxicity effects of Miracle Seed Ultima on liver function and lipid profile in male Wistar rats.

1.6 Objectives of study

The specific objectives are to:

Ascertain the effect of Miracle Seed Ultima on liver enzyme levels such as bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Evaluate the impact of Miracle Seed Ultima on lipid profile markers including high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and total cholesterol.

1.7 Scope and limitations

This study used male Wistar rats to examine the sub-acute (28-day) oral toxicity of Miracle Seed Ultima. The examination examines the liver and lipid profile from a biochemical, physiological, and histological perspective. Genotoxicity, reproductive, and chronic tests are not included. To avoid hormonal differences that could impact the results, only male rats are employed. The precise composition and concentration of Miracle Seed Ultima's

constituents may not be entirely known because it is a commercial multi-herbal product, which could restrict the ability to interpret results in great details

CHAPTER TWO

LITERATURE REVIEW

2.1. sub-acute toxicity studies in experimental animals

2.1.1Description and purpose of sub-acute

Sub-acute toxicity studies aim to assess the effects of administering a test substance repeatedly over a period of 14 to 28 days, usually in rodents. Before severe toxicity manifests, these investigations aid in identifying possible target organs, establishing dose-response relationships, and identifying early toxic effects. (Gandhare *et al.,*2013), they are essential for forecasting human safety in situations involving recurrent exposure. Sub-acute toxicity studies are a crucial stage in pre-clinical safety review, acting as a link between acute single-dose studies and longer-term sub-chronic or chronic investigations, according to the Organization for Economic Co-operation and Development (OECD TG 407, 2008).No-Observed-Adverse-Effect Levels (NOAELs), reversibility of hazardous effects, and possible biochemical or histological alterations are all provided by the generated data (OECD, 2008).

2.1.2 common parameters measured

a. Clinical Signs and Behavior

Every day, physical and behavioral changes in animals, including salivation, convulsions, tremors, pilo-erection, posture, fur condition, and movement, are monitored. Changes in these symptoms may be early warning indications of neurotoxicity or systemic toxicity (Gandhare *et al*., 2013).

b. Body Weight and Food/Water Consumption

One sensitive measure of overall health is body weight. Throughout the experiment, regular monitoring aids in determining whether the test drug has an impact on metabolism or growth rate. A notable decrease in body weight gain could be a symptom of toxicity, metabolic imbalance, or inadequate nutrition absorption (Yimam *et al.,* 2020). Data on food and water intake can be used to assess if changes in weight are due to metabolic changes or decreased consumption.

c. Organ weights (Absolute and Relative)

Major organs such the liver, kidney, heart, spleen, and lungs are removed, weighed, and compared between groups at the conclusion of the 28-day research. Relative organweights, or organ-to-body weight ratios, can reveal potential hypertrophy, atrophy, or congestion. For example, hepatocellular hypertrophy or fatty infiltration, which are prevalent under hepatic stress, may be indicated by increased liver weight (Tennekoon *et al.,* 1991).

d. Hematological Indicies

Red blood cell (RBC), white blood cell (WBC), hemoglobin concentration, hematocrit (PCV), mean corpuscular volume (MCV), and platelet count are examples of hematological parameters that are crucial markers of how chemicals affect blood-forming tissues (hematopoietic system). Changes in these numbers may indicate inflammatory reactions, immunological suppression, or anemia (Yimam *et al.*, 2020).

e. Clinical biochemistry

Liver enzymes (ALT, AST, ALP), bilirubin, renal markers (urea, creatinine), proteins (albumin, total protein), lipids (cholesterol, triglycerides, HDL, LDL), glucose, and electrolytes are examples of important markers. Changes indicate organ dysfunction. For

instance, male Wistar rats' blood AST and ALT activity altered after 21 days in a study including Terminalia schimperiana root extract.

f. Histopathology

Cellular and tissue damage, including necrosis, fatty degeneration, and inflammation, can be seen confirmed by histological examination of organs such the liver, kidney, spleen, and heart. It confirms biochemical results and shows whether effects are progressive or reversible (Gandhare *et al.*, 2013).

2.1.3 Animal model : Male Wistar rat

Because of their calm disposition, well-defined physiology, and consistent reactions to poisons, male Wistar rats are frequently employed in sub-acute toxicity investigations. They are perfect laboratory models because of their affordability and controllable size (Tennekoon *et al*., 1991). Because hormonal changes in females during the estrous cycle can affect metabolic and biochemical outcomes and complicate data interpretation, male rats are preferable. Furthermore, the data's extrapolative usefulness is strengthened by the similarities between human and rat hepatic metabolism (Gandhare *et al.*, 2013).

2.1.4 Interpretation of findings

Analyzing dose-response relationships, biological relevance, and consistency across several parameters are all important aspects of interpreting sub-acute toxicity outcomes. When biochemical changes are dose-dependent, outside of normal physiological ranges, and corroborated by histopathology results, it is considered a real hazardous impact (OECD, 2008). Hepatocellular damage, for instance, is suggested by elevated blood ALT and AST values in conjunction with liver histological abnormalities including fatty degeneration or

necrosis (Ozer *et al*., 2008). Conversely, reversible alterations or minor deviations within normal ranges are

frequently regarded as adaptive physiological reactions. The No-Observed-Adverse-Effect Level (NOAEL) is ultimately determined by integrating behavioral, biochemical, and histological data, which also offers a scientific foundation for risk assessment (Gandhare *et al.,* 2013).

2.2 The liver and its role in metabolism and detoxification

2.2.1 Anatomy and physiology of the liver

As the body's largest internal organ, the liver is essential to preserving homeostasis. It is separated anatomically into the left and right lobes, which are made up of lobules—structural and functional units. Hepatocytes are distributed in plates that radiate from the central vein in each lobule, and sinusoids facilitate the exchange of liver cells with blood. Most of the liver's metabolic and detoxifying functions are carried out by these hepatocytes.(Guyton and Hall,2021) The hepatic artery provides oxygenated blood to the liver, whereas the portal vein provides nutrient-rich blood. Substances absorbed from the gastrointestinal tract are efficiently processed before entering the systemic circulation thanks to this special circulation. Additionally, it facilitates the liver's filtration, detoxification, and biotransformation processes, which make it an essential organ in toxicological research.(Trefts *et al*.,2017)

2.2.2 Functions of the liver in metabolism and detoxification

The body's metabolic powerhouse is the liver. By sustaining blood glucose through glycogenesis, glycogenolysis, and gluconeogenesis, it contributes to the metabolism of carbohydrates. The liver produces phospholipids, lipoproteins, and cholesterol as part of lipid

metabolism. It also transforms extra carbs into fatty acids. Additionally, it is essential for protein metabolism, which

includes the production of plasma proteins including albumin and clotting factors (Zhao *et al.,* 2021). Detoxification, or the biotransformation of xenobiotics (foreign chemicals including medications, poisons, and herbal compounds) into less harmful or more water-soluble metabolites that can be eliminated by bile or urine, is an essential liver function.

Two enzymatic phases are usually involved in this process: Phase I (oxidation, reduction, hydrolysis), which is mostly catalyzed by cytochrome P450 enzymes, and Phase II (conjugation processes), which includes acetylation, sulfation, and glucuronidation (Sharma *et al*., 2020). The liver is extremely vulnerable to damage from chemicals due to its high metabolic activity. Reactive metabolite-producing substances can raise biochemical indicators of liver injury by causing oxidative stress, lipid peroxidation, or hepatocyte necrosis.

2.2.3 Common Biochemical markers of liver functions

In toxicity studies, biochemical markers are frequently assessed to evaluate liver function and identify hepatocellular or cholestatic injury. Among the most prevalent markers are:

1.Aminotransferase Alanine (ALT)

Hepatocyte cytoplasm contains the majority of the enzyme ALT. Because hepatocyte membrane damage releases the enzyme into circulation, elevated blood ALT levels are thought to be a sensitive and specific sign of hepatocellular injury (Ozer *et al.*, 2008).

2. Aminotransferase of Aspartate (AST)

Hepatocytes' cytoplasm and mitochondria contain AST, which is also found in cardiac and skeletal muscle to a lesser degree. Elevated AST levels may be a sign of muscle damage as

well as liver impairment. As a result, hepatic and extra-hepatic causes can be distinguished using the AST/ALT ratio (Dufour *et al.,* 2000).

3. Alkaline phosphatase (ALP)

The sinusoidal membranes and biliary canaliculi contain ALP. Instead of direct hepatocellular damage, its rise usually indicates cholestatic liver disorders or restriction of bile flow. Elevated ALP in subacute toxicity may be a sign of poor excretion of bile components or injury to the bile duct (Li *et al.,* 2019).

4. Bilirubin

A byproduct of hemoglobin breakdown is bilirubin. There are two types of it: conjugated (direct) and unconjugated (indirect). For excretion, the liver combines bilirubin with glucuronic acid. Increased total or direct bilirubin levels may indicate bile duct blockage or compromised hepatic excretory function (Yuan *et al*., 2020).

5. Total protein and albumin

The majority of plasma proteins, such as albumin and globulins, are produced by the liver. Reduced albumin or serum total protein is a sign of compromised hepatic synthetic function, which is frequently seen in severe sub-acute or chronic toxicity where protein synthesis is interfered with (Ahmed *et al.*, 2022).

6. Lipid Profile Parameters

Toxicity studies also evaluate biochemical indicators including total cholesterol, triglycerides, HDL, and LDL as the liver controls lipid metabolism. Changes in lipid transport brought on by exposure to toxicants or hepatic metabolic stress may be reflected in disturbances in these parameters (Zhao *et al.*, 2021). When taken as a whole, these biochemical indicators offer a

thorough picture of the physiological condition of the liver and are essential diagnostic instruments in sub-acute toxicity assessment.

2.2.4 Effects of toxins or herbal compounds on liver structure and function

The liver may undergo a series of biochemical and structural alterations as a result of exposure to xenobiotics or unproven herbal remedies. Alkaloids, saponins, and flavonoids are examples of bioactive substances found in many herbal extracts. Although these substances are beneficial at low concentrations, prolonged exposure to them might result in the production of harmful metabolites (Teschke and Eickhoff, 2015). Hepatocyte degeneration, necrosis, fibrosis, or fatty infiltration are histopathological manifestations of toxic liver injury. Biochemically, these changes are associated with higher bilirubin, elevated serum transaminases, and altered lipid profiles. For example, rats given Moringa oleifera seed extract subacutely showed minor hepatic vacuolation and elevated ALT and AST activity, suggesting dose-related hepatotoxic effects (Adedapo *et al*., 2009).

2.3 Lipid profile and it's physiological significance

2.3.1 Overview of lipid metabolism

Lipids are essential for cellular structure, signaling pathways, and energy storage. In order to maintain homeostasis, the liver is essential for the synthesis, storage, and export of lipids (Nguyen *et al.*, 2008). Endogenous lipids are produced in the liver and delivered into the bloodstream as very low-density lipoproteins (VLDL), whereas dietary fats are absorbed as chylomicrons. Low-density lipoproteins (LDL) and high-density lipoproteins (HDL), each with specific physiological functions, are gradually produced from them (Choi *et al*., 2020). Dyslipidemia, or disruptions in lipid metabolism, can be brought on by xenobiotic exposure, endocrine imbalance, or hepatic damage. According to (Sinha *et al.* (2013), toxic chemicals,

such as some herbal medicines, can affect lipid synthesis or clearance, resulting in altered blood lipid levels that are indicative of liver dysfunction.

2.3.2 Components of Lipid profile

1. Total cholesterol

In addition to being a precursor of bile acids, steroid hormones, and vitamin D, cholesterol is a crucial structural element of cell membranes. On the other hand, high total cholesterol can be a sign of increased synthesis brought on by metabolic stress or poor hepatic cholesterol clearance (Tiniakos *et al*., 2010). Increased serum cholesterol in toxicity studies may indicate lipid dysregulation brought on by oxidative stress, biliary obstruction, or hepatic dysfunction (Reddy *et al.*, 2012).

2. Triglycerides

The primary source of stored energy in adipose tissue is triglycerides. Triglyceride production and packing into VLDL particles are controlled by the liver. Triglyceride buildup in hepatocytes (hepatic steatosis) or increased plasma triglyceride levels might result from toxicant exposure that interferes with β-oxidation or lipoprotein secretion (Friedman *et al.,* 2018). As a result, in sub-acute research, triglyceride estimation is a sensitive indicator of disruptions in lipid metabolism.

3. High Density Lipoprotein (HDL)

Reverse cholesterol transport, which involves removing extra cholesterol from peripheral tissues and transporting it to the liver for excretion, is carried out by HDL. Hepatic damage, inflammation, and oxidative stress are linked to low HDL levels (Tziomalos and Athyros, 2015). During sub-acute toxicity investigations, a substantial decrease in HDL could be a symptom of increased lipid peroxidation or compromised hepatic synthetic activity.

4. low-density lipoprotein(LDL)

LDL carries cholesterol to peripheral tissues from the liver. Overdosing on LDL increases oxidative stress and the risk of atherosclerosis by encouraging the buildup of cholesterol in tissues and blood vessels. Elevated LDL concentrations can result from toxicants that disrupt hepatic receptor activation or lipid metabolism (Wang *et al*., 2017).

2.3.3 Relationship between lipid metabolism and liver function

Lipid homeostasis is immediately affected by hepatic injury since the liver is the center of lipid synthesis and transport. Lipid profile levels may change as a result of an imbalance between lipid synthesis and utilization caused by liver disease. Hepatic damage caused by xenobiotics or herbal substances frequently results in elevated total cholesterol and triglycerides while lowering HDL, indicating decreased lipid clearance and increased oxidative stress(Nduka *et al.,* 2016) Furthermore, lipid peroxidation, or the oxidative breakdown of lipids, is a major factor in liver damage. Hepatocytes are further harmed by free radicals' attacks on polyunsaturated fatty acids in cell membranes, which result in the production of malondialdehyde (MDA) and other byproducts. This mechanism causes hepatotoxicity and metabolic syndrome by interfering with lipid metabolism (Ayala *et al*.,2014)

2.3.4 Effects of toxic substances or herbal formulations on lipid metabolism

Since untested herbal formulations contain phytochemicals that disrupt hepatic enzymes or mitochondrial function, exposure to them may change lipid metabolism. For instance, it has been demonstrated that long-term use of Cassia occidentalis seed extract causes hepatic steatosis and hypercholesterolemia in rats (Singh *et al.*, 2011). Similarly, depending on dosage and length of exposure, extracts from Moringa oleifera and Nigella sativa have been shown to either increase or decrease lipid levels (Mehta *et al*., 2009). These differences

highlight how crucial it is to carry out controlled sub-acute toxicity studies to assess the lipid-modulating effects of herbal remedies like Miracle Seed Ultima, making sure their safety and possible therapeutic benefits are verified by scientists

2.4 Herbal medicine and safety evaluation

2.4.1. Overview of Herbal medicine use

Herbal medicines (HM) are used as complementary or alternative treatments all throughout the world, particularly in developing nations. Because they are "natural," many people believe they are intrinsically safe. Nonetheless, the extensive use of herbal products has surpassed thorough scientific assessments of their safety, standardization, and regulatory supervision (Mahasiswa and Levita, 2023). Herbal remedies are frequently taken in Nigeria and other African nations with little understanding of their composition, dose, potential interactions, or toxicological hazards (Okoro *et al*., 2018).

2.4.2. Perception of herbal products as "safe" alternatives

Given that herbal remedies are made from plants, the general population believes they must be harmless, which frequently results in self-medication without expert advice. A significant

percentage of herbal medicine consumers are ignorant about possible side effects or interactions with conventional medications (Afolabi *et al.,*2022). Because many herbal products contain bioactive substances, unidentified combinations, or unreported additions that may induce organ toxicity or metabolic disruption, this perception may be problematic.

2.4.3 Needs for scientific validation and toxicity testing

Applying scientific techniques for toxicity testing, quality control, and standardization of herbal products is crucial given the rise in the usage of herbal therapies. Strict evaluation of herbal medicinal products is advised by regulatory recommendations (such as those from the European Medicines Agency (EMA) and other regulatory organizations). These guidelines address botanical identity, manufacturing quality, safety data, and post-market surveillance (EMA, 2016). However, dossiers submitted to regulatory bodies frequently reveal significant shortcomings in toxicological evidence, and many herbal medications on the market lack adequate safety data (Kassimu *et al*., 2023).

2.4.4. Cases of herbal-induced liver injury (HILI) and metabolic disturbances

HILI, or herbal-induced liver injury, has become a serious safety concern. Hundreds of cases of HILI have been documented worldwide, frequently involving multi-ingredient products or undisclosed adulterants; some of these cases required transplantation or were fatal (Lee *et al*., 2023; Huang *et al.*, 2024). Misidentification of plant species, contamination/adulteration, unknown synergistic interactions of numerous herbs, and low consumer understanding are factors that raise danger (Zhang *et al*., 2021). Many herbal products may also create metabolic disorders that are not usually immediately noticeable since they also have an impact on lipid metabolism or organ systems involved in detoxification.

2.4.5 Safety Evaluation frameworks and regulatory considerations

Numerous frameworks and regulatory models have been put out to guarantee the safe use of herbal goods. These include specialized criteria for herbal medicinal products (e.g., EMA herbal monographs) and tiered safety evaluation (beginning with in silico/in vitro screening, followed by in vivo repeated-dose trials, chronic exposure, and post-market surveillance). Crucial components consist of:

Verification of the portion utilized and botanical identity quantifying the active ingredients and standardizing the extract.

Quality control (looking for adulterants, heavy metals, and pollutants) Subacute, subchronic, and chronic investigations are examples of toxicological testing.

Adverse-event reporting and post-market pharmacovigilance.

However, a lot of herbal drugs avoid these thorough evaluations, which makes it difficult to completely estimate benefit-risk profiles (Kassimu *et al*., 2023).

2.4.6. Implications for the present study

Using a toxicity-screening approach is essential since the product under investigation—a multi-seed herbal formulation—falls within the category of herbal medicinal-type items. Sub-acute toxicity evaluation (as designed in this project) becomes an essential first step because such formulations lack reliable safety data. Given the known occurrences of herbal medicines producing hepatic and metabolic abnormalities, monitoring liver and lipid endpoints is particularly important. The findings will help close the evidence gap and provide information for consumer

protection, regulatory monitoring, and safe use guidelines.

2.5 Miracle Seed Ultima and Its Claimed Benefits

2.5.1Description and Composition

Miracle Seed Ultima is labeled as “100% organic” and is said to include “multi dynamic curative seeds and fruits” that promote wellbeing. The substance is marketed as having a variety of health benefits, such as immune support, blood-tonic effects, improved fertility,

and general detoxification, according to the manufacturer's website. (Miracle Seed Natural Care Ltd, 2025) .

Nevertheless, there is no peer-reviewed, publicly available record of the precise components of fruit and seed, their ratios, or standardized extract properties. The product is marketed in Nigeria in 500 ml bottles with organic certification claims; however the active phytochemicals and types of constituent seeds are not made clear.

Figure1. Image of miracle seed ultima®

2.5.2 Claimed Pharmacological and Health Benefits

promotional material the benefits claimed include:

Improved immune system functioning (e.g., "boost immune system")

Supporting fertility in men and women ("boost fertility in men and women")

Serving as a blood tonic (suggesting improved hematological status)

Helping to alleviate a variety of ailments including ulcers, diabetes, low sperm count, open wounds, as per the manufacturer's blog and marketing copy

Because of these claims, the product places itself within the category of general health-support supplements rather than a targeted pharmaceutical formulation.

2.5.3 Lack of Scientific Data on Its Toxicological Profile

Profile of Toxicology Despite the widespread marketing and consumption of Miracle Seed Ultima, there is a significant lack of publicly accessible, peer-reviewed toxicological research evaluating its safety in people or animal models. As far as I'm aware, the branded formulation has not been the subject of any published sub-acute or chronic toxicity studies. This indicates that this product has no established No-Observed-Adverse-Effect Level (NOAEL) and no recorded information on changes in lipid profiles, liver enzyme response, or histopathology results after repeated administration. The absence of official safety data is a major research gap given the wide claims of impact and multi-seed composition.

2.5.4 Possible Bioactive Compounds and Mechanistic Considerations

Mechanistic Aspects Multi-seed herbal products frequently contain bioactive phytochemicals such polyphenols, flavonoids, saponins, alkaloids, and essential fatty acids, even though the exact seed

blend is unknown. These substances may have positive effects (anti-inflammatory, antioxidant), but if ingested in large quantities or in complicated mixes, they may also cause metabolic or organ-specific toxicity. For instance, high-fatty acid seed oils may have an impact on lipid metabolism; flavonoids may modify the liver's cytochrome P450 enzymes, changing the metabolism of xenobiotics. These mechanistic options offer a justification for examining the product's effects on lipid profile and liver function in an experimental model.

2.5.5 Relevance to the Present Study

Miracle Seed Ultima's testing in a sub-acute toxicity model (male Wistar rats) with objectives of liver enzymes, lipid profile, organ weights, and histology is warranted because it is often used by customers and lacks a thorough safety review. Both metabolic (lipid) and organ (liver) endpoints may be impacted by the combination of purported effects (fertility, blood-tonic, detox) and unknown composition. Evidence-based recommendations for this kind of product will benefit from the establishment of baseline safety data.

2.6 Review of Related Studies

2.6.1 Studies Showing Hepatoprotective/Beneficial Effects of Seed Extracts

Certain seed extracts have been found in numerous trials to enhance lipid metabolism and preserve the liver. For example, following 28 days of dosing in rats, Nigella sativa seed extract decreased blood ALT and AST levels and preserved liver histology, suggesting hepatoprotective potential (Dollah *et al.,* 2013). In experimental models of chemical liver injury, Moringa oleifera seed and leaf extracts also enhanced lipid balance and liver enzyme profiles (Adeyemi *et al.*, 2014; Omodanisi *et al.,* 2017). According to these investigations, certain bioactive substances obtained from seeds may have lipid-regulating and antioxidant qualities that support liver function.

2.6.2 Studies Reporting Hepatotoxic or Dose-Dependent Adverse Effects

According to other studies, long-term or high-dose consumption of some seed extracts may cause toxicity or liver damage. For instance, in sub-acute toxicity studies on Wistar rats, Lepidium sativum seed oil resulted in dose-dependent increases in liver enzymes and histological alterations (Aragie *et al*., 2025). Similarly, modest to moderate changes in liver structure and enzyme activity were caused by Raphia hookeri seed extract, suggesting

possible hepatotoxicity at larger doses (Mbaka *et al.,* 2014). These results show that, depending on the dosage, solvent, and length of exposure, some seeds can be toxic while others are beneficial.

2.6.3 Comparative Pattern Across Studies

The effects of seed extracts exhibit predictable patterns, according to comparative assessments. Higher doses typically cause adverse hepatic reactions, whereas smaller doses are frequently advantageous or harmless (Dollah *et al.,* 2013; Aragie *et al*., 2025). The type of extract is particularly important because the phytochemical composition of ethanolic and aqueous forms can differ in terms of toxicity (Silva *et al.,* 2024). Furthermore, some "protective" experiments were carried out in models that first caused liver damage, so the findings cannot be directly compared to safety in animals in good condition (Krishnan *et al*., 2012; Ebuehi *et al*., 2020).

2.6.4 Study Design Features Important For Interpretation

The OECD Test Guideline 407, that requires for a 28-day oral exposure with assessments of body and organ weights, hematological, biochemistry, and histology, is followed by the majority of reliable toxicity studies (OECD, 2008; Sanpinit *et al.,* 2023). Because of their constant reaction patterns and metabolic similarities to humans, male Wistar rats are frequently used (Aragie *et al.,* 2025). Data comparability and interpretation may be impacted by differences in reporting requirements, dose spacing, and administration methods (Mbaka *et al.,* 2014).

2.6.5 Research Gaps Identified in Literature

The commercial formulation "Miracle Seed Ultima" has no published toxicity data, despite the large number of trials on individual seeds. Inconsistencies in dosage estimations and

extraction techniques are also evident in existing data, making cross-study comparisons more difficult. Furthermore, despite the liver's critical role in lipid control, few studies have thoroughly examined the lipid profile (TC, TG, HDL, and LDL) in toxicity situations (Adeyemi *et al*.,2014).

2.6.6 How These Studies Inform the Present Research

According to the reviewed literature, depending on dose, extraction, and experimental design, seed extracts can have either positive or negative effects on the lipid and liver. Therefore, to determine Miracle Seed Ultima's safety and possible dose limitations, 28-days sub-acute research in male Wistar rats is required, monitoring body and organ weights, liver enzymes, lipid parameters, and histology (Dollah *et al.,* 2013; Aragie *et al.,* 2025).

CHAPTER THREE

MATERIALS AND METHODS

3.1 METHODS

3.1.1 APPARATUS

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

1. Animal Cages

2. Scissors[Tecmel Tecmel,USA]

3. Plastic rubber

4. Binding wire

5. Hand Gloves[Fantastik, England]

6. Nose mask [Fantastik, England]

7. Detergent

8. Dettol

9. Cardboard papers

10. Cotton wool

11. Dissecting set[Tecmel Tecmel, USA]

12. EDTA container[Fantastik, England]

13. Feed [Edo, Nigeria]

14. Paper tape

15. Measuring cylinder[Pyrex, England]

16. Micro pipettes(1,10 and 25ml)[Pyrex, England]

18. Oro-gastric Gavage

19. Syringes(1ml,2ml,5ml and 10ml)[Pyrex, England]

20. Universal bottles[Fantastik, England]

21. Filter paper [Whatman, England]

22. Cuvettes [Pyrex, England]

23. Measuring scale

24. Conical flasks [Pyrex, England]

25. Container (food/water)

26. Pins

27. Rubber gloves

28. Beaker (50,150 and 250ml)[Pyrex, England]

29. Retort Stand

30. Test tube racks and test tubes

31.. Wistar rats

3.1.2 EQUIPMENT

Major equipment used include:

1. Refrigerator [Citizens PRC4246]
2. Water bath [B.Bran SC.Inst. England]

3. Spectrometer [PG Instruments Ltd.,UK]
4. Centrifuge [B. Bran SC. Inst. England]
5. Microplate reader [PG Instruments Ltd.,UK]

3.1.3 REAGENTS

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

1. Chloroform, hydrochloric acid (HCL) [May and Bayer, England]

2. Distilled water [Trigas, UNIBEN]

3. Buffered formalin

4. Set of kits for lipid profile parameters (Randox lab kit, UK)

5. Set of kits for assessment of liver function status parameters(Randox lab kit ,UK)

6. Methylated spirit

7. Gentian Violet

3.2 METHODS

3.21 EXPERIMENTAL ANIMALS

20 Male wistar rats weighing an average of 120g were obtained from the Department of Anatomy, Faculty of School of Basic Medical Science, University Of Benin, Benin City, Edo State, Nigeria. The animals were maintained under controlled conditions and 12-hour light-dark cycles.

The animals were acclimatized for 7 Days (a week) before the commencement of the study. The were housed in the cages and given free access to food and water. The rats were fed with pelleted grower mash.

The animals were divided into 4 groups. Four of these groups had five rats each. The animals were then stained using gentian Violet in various body parts for purpose of identification. The stained parts include head, back, left leg, left hand, right leg, right hand, abdomen and tail. The cages. their surroundings, receptacle tray below it's bedding were clean and disinfected daily

3.2 EXPERIMENTAL DIETS

Table 3.1: Composition of the basal diet (g/1000g) based on the standard pelleted grower mash Of Jerrison Agro Allied service, Benin City. Nigeria

INGREDIENTS	BASAL DIETS (g)
Maize	**500.0**
Wheat offal	**60.0**
Palm Kernel Cake	**50.0**
Soyabean Meal	**200.0**
Groundnut Cake	**100.0**
Lysine	**1.0**
Bone meal	**32.0**
Limestone	**28.0**
Methionine	**1.0**
Starter premix	**2.0**
Salt	**26.0**
Total	**1000.0**

3.3 TEST SUBSTANCE/SAMPLE PREPARATION

The multi-herbal seed-based supplement Miracle Seed Ultima was purchased from a reputable local distributor in Benin City, Nigeria. Natural seed extracts such Allium Sativum, Zingiber Officinale, Aplaceae, Hibiscus Sabdariffa, Cucumis Sativus, and Thespesia Carekeana were mentioned on the product label. Two weeks dosage was made by dissolving

in distilled water and kept in a refrigerator .to keep it fresh in aseptic conditions (Mbaka *et al.,* 2014; Dollah *et al.,* 2013).

3.4 EXPERIMENTAL DESIGN

The 20 Male wistar rats were arranged into four groups with the weight of those in a group being representative of the weight range of all the rats, such that average weight of all the groups at the onset of the experimental period was 135g to 180g. The groups are ;

- Group 1: This is the normal control; only distilled water was given
- Group 2: Low dose of miracle seed ultima (100mg/kg)
- Group 3: Medium dose of miracle seed ultima (300mg/kg)
- Group 4: High dose of miracle seed ultima (1000mg/kg)

For the animals in group 2,3 and 4 , miracle seed ultima was orally administered once daily via gavage for 4 weeks (28 days).

3.5 OBSERVATION AND DATA COLLECTION

Rats were monitored every day for indications of toxicity, including changes in posture, fur condition, feeding behavior, locomotor activity, and death, for the course of the experiment. Body weights were taken at the beginning and then every week to track any patterns in weight reduction or increase. In order to evaluate hunger and metabolic changes, feed and water consumption were also assessed (Sanpinit *et al.,* 2023). Standard OECD observation checklists, which include symptoms including tremors, piloerection, lethargy, and diarrhea, were used to record all behavioral and physiological changes (OECD, 2008). Any fatality or serious adverse response was noted, and the afflicted animal was put to death in a compassionate manner.

3.6 SAMPLE COLLECTION AND PREPARATION

Rats were anesthetized with mild chloroform vapor after an overnight fast at the conclusion of the 28-day therapy. Organs such the liver, kidneys, heart,spleen and lungs were removed after dissection and weighed to establish the absolute and relative organ weights. The liver was then kept in formalin for histological investigations (Dollah *et al*., 2013; Mbaka *et al*., 2014).

CHAPTER FOUR

RESULT

The experimental findings from the 28-day sub-acute toxicity study carried out to evaluate the effects of Miracle Seed Ultima® on liver function and lipid profile in male *Wistar* rats. The animals' biochemical reactions to various extract dosages are described in detail in this chapter, with special attention paid to the levels of albumin, total protein, bilirubin (direct and total), and liver enzymes (ALT, AST, and ALP). In order to identify any changes in lipid metabolism, it also looks at lipid markers such total cholesterol, triglycerides , High Density Lipoprotein(HDL) cholesterol, and Low Density Lipoprotein(LDL) cholesterol. Together with statistical analyses demonstrating significant or non-significant differences, the results are displayed in tabular form. To show the pattern of physiological response to the extract, dose-dependent trends are examined.

These combined results offer a comprehensive assessment of the hepatic safety profile of Miracle Seed Ultima®, forming the basis for the interpretation and conclusions drawn in subsequent chapters.

Non-significant difference ($P > 0.05$) in Serum Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), Albumin, Total bilirubin and Direct bilirubin were observed across all groups, while the serum Alanine Amino Transferase (ALT) experienced statistical difference ($P < 0.05$) in group three and four (300mg/kg and 1000mg/kg MSU) when compared with group one (control). Statistical difference ($P < 0.05$) in Total protein was also observed in group two (100mg/kg MSU) when compared with group one (control) (Table 1).

There was no statistical difference ($P > 0.05$) in all the Serum lipid profile parameters across all groups investigated in this study (Table 2)

Table 4.1: Effect of Miracle Seed Ultima® on Liver function parameters

Groups	ALT (U/L)	ALP (UI)	AST (U/L)	PROTEIN (g/dl)	Albumin (g/dL)	Total Bilirubin (mg/dl)	Direct Bilirubin (Mg/dl)
1	147.35±12.9^{a}	34.67±0.6^{a}	133.56±9.0^{a}	4.06±0.1^{a}	1.63±0.0^{a}	0.48±0.0^{a}	0.37±0.0^{a}
2	129.54±2.5^{ab}	32.49±0.3^{ab}	125.88±3.6^{ab}	3.39±0.1^{bc}	1.54±0.0^{ab}	0.57±0.0^{ab}	0.48±0.0^{ab}
3	135.00±8.7^{abd}	33.71±1.9^{abc}	136.37±11.3^{abc}	3.63±0.2^{acd}	1.57±0.0^{abc}	0.60±0.0^{abc}	0.48±0.0^{abc}
4	200.56±22.2^{ace}	33.23±0.8^{abc}	175.08±19.4^{abc}	3.92±0.2^{acd}	1.55±0.0^{abc}	0.58±0.0^{abc}	0.49±0.0^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly (P< 0.05) from each other.

Table 4.2: Effects of Miracle Seed Ultima® on Lipid profile parameters

Groups	TOTAL CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	LOW DENSITY LIPOPROTEIN (mg/dl)	HIGH DENSITY LIPOPROTEIN (mg/dl)	VERY LOW DENSITY LIPOPROTEIN (mg/dl)
1	179.62±3.9^{a}	134.33±14.7^{a}	122.01±5.7^{a}	30.74±0.8^{a}	26.87±2.9^{a}
2	174.67±3.3^{ab}	115.08±10.5^{ab}	122.00±2.8^{ab}	29.65±0.9^{ab}	23.02±2.1^{ab}
3	186.92±1.9^{abc}	134.33±13.9^{abc}	129.63±4.4^{abc}	30.43±2.2^{abc}	26.87±2.8^{abc}
4	179.37±4.2^{abc}	135.19±18.4^{abc}	123.24±4.2^{abc}	29.11±0.6^{abc}	27.04±3.7^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly (P< 0.05) from each other.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigated the sub-acute toxicity effects of Miracle Seed Ultima (MSU) on liver function and lipid profile in male Wistar rats over a 28-day period. The goal of the study was to present scientific proof of the safety of this widely used botanical substance. The investigation was motivated by the growing worry that, despite the fact that many herbal formulations are promoted as safe and useful, numerous seed-based treatments have shown dose-dependent hepatotoxic or metabolic abnormalities in earlier mouse studies (Aragie et al., 2025; Mbaka *et al.,* 2014).

Therefore, by assessing important biochemical markers frequently used to identify early changes in hepatic or lipid metabolism, this study addressed the issue of a lack of toxicological evidence for MSU. The results of this study add to the larger body of research demonstrating that, depending on dosage, extract type, and frequency of exposure, some botanicals may cause major physiological changes while others have hepatoprotective or neutral effects.

(Dollah et al., 2013; Adeyemi *et al.,* 2017).

Understanding that markers like Alanine amino transferase (ALT), aspartate amino transferase(AST) an alkaline phosphatase(ALP) give extremely sensitive measures of hepatocellular integrity is essential to the assessment of liver function parameters in toxicology. Hepatocytes are the primary source of ALT, a cytosolic enzyme that, when increased, frequently denotes hepatic damage or membrane leakage (Ozer *et al.,* 2008). Although AST is also found in the liver, it is less specific because it is also in skeletal and

cardiac muscle. Nevertheless, elevations of AST usually supplement ALT when evaluating hepatic stress. ALP mostly indicates biliary blockage or cholestasis.

ALT revealed a dose-dependent elevation in all groups, according to Table 4.1, with statistically significant increases in the higher-dose groups (300 mg/kg and 1000 mg/kg) as compared to the control ($P<0.05$). This implies that greater dosages of MSU may cause moderate hepatocellular stress. This result is consistent with recent botanical toxicity studies that found biochemical signs of hepatic strain in high-dose herbal extracts, such as Lepidium sativum seed oil and other hydro-alcoholic seed formulations (Aragie et al., 2025; Mbaka et al., 2014). In the meantime, there were no significant differences ($P>0.05$) in AST, ALP, albumin, and bilirubin (total and direct) between the groups, suggesting that the hepatocellular response remained mild rather than severe and that the hepatic changes did not escalate to cholestatic or synthetic dysfunction.

While total and direct bilirubin increases frequently indicate compromised conjugation or excretion mechanisms, albumin and total protein are significant markers of synthetic liver function. These parameters were assessed in the current investigation to ascertain whether consumption of MSU affected metabolic processes or hepatocellular integrity.

There were no statistically significant difference changes in albumin, total and direct bilirubin. Since isolated increases in protein without concurrent pathogenic enzyme elevations are frequently regarded as non-hazardous, the substantial rise in total protein seen in the 100 mg/kg group may indicate an adaptive or metabolic response rather than toxicity (Sanpinit et al., 2023).

Total cholesterol, triglycerides, LDL, HDL, and VLDL were measured as lipid function markers to see if MSU changed lipid production, transport, or clearance. Significant

deviations from these measures, which are important markers of metabolic stability, may indicate systemic metabolic stress or lipid dysregulation.

There were no statistically significant changes (P>0.05) in total cholesterol, triglycerides, Low Density Lipoprotein(LDL), High Density Liprotein (HDL), or Very Low Density Lipoprotein (VLDL) for the lipid profile parameters (Table 4.2).

This suggests that over the 28-day exposure period, MSU did not interfere with lipid metabolism. In certain sub-acute trials, the lack of lipid disruption is similar to that of botanicals like Moringa oleifera and Nigella sativa, where lipid parameters remained normal despite other biochemical alterations (Omodanisi *et al.,* 2017). This implies that while greater doses of MSU may affect hepatocellular enzymes, In the short-term sub-acute model employed in this investigation, its impact on systemic lipid metabolism is low. This study's general toxicological pattern confirms the scientific consensus that herbal compositions frequently exhibit dose-dependent toxicity rather than universal toxicity. In this instance, higher dosages of MSU led to a notable increase in ALT but no discernible changes in other liver or lipid parameters, whereas lower doses produced negligible changes in biochemical markers. This pattern is consistent with the traditional toxicological theory that "the dose makes the poison," which has been observed in numerous investigations employing extracts derived from seeds (Silva *et al.,* 2024). The results further emphasize how crucial it is to assess branded herbal combinations separately because their composition could be very different from previously studied individual plant extracts. In the short-term sub-acute model employed in this investigation, its impact on systemic lipid metabolism is low.

This study's general toxicological pattern confirms the scientific consensus that herbal compositions frequently exhibit dose-dependent toxicity rather than universal toxicity. In this instance, higher dosages of MSU led to a notable increase in ALT but no discernible changes

in other liver or lipid parameters, whereas lower doses produced negligible changes in biochemical markers. This pattern is consistent with the traditional toxicological theory that "the dose makes the poison," which has been observed in numerous investigations employing extracts derived from seeds (Silva *et al.,* 2024). The results further emphasize how crucial it is to assess branded herbal combinations separately because their composition could be very different from previously studied individual plant extracts (Krishnan *et al.,* 2012).

5.2 Conclusion

In conclusion, Miracle Seed Ultima is generally well tolerated at lower doses, as demonstrated by the 28-day sub-acute administration of the product in male Wistar rats, which showed no appreciable changes in AST, ALP, albumin, bilirubin, or lipid indices. Higher dosages, however, significantly raised ALT, indicating mild hepatic stress. All treatment groups' lipid parameters stayed normal, suggesting that metabolic lipid processing was unaffected. These findings show that although MSU does not seem to cause widespread systemic toxicity, care should be taken, especially at higher dosages when early signs of liver stress were noted. To confirm long-term safety and determine a suitable NOAEL for Miracle Seed Ultima, more research incorporating histopathology, extended exposure duration, and human-equivalent dosage concerns is advised.

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APPENDIX

APPENDIX I: Plant Material (Miracle Seed Ultima)

Figure A1.1: Miracle Seed Ultima used for the study

APPENDIX II: EXPERIMENTAL DESIGN

Table A2.1 : Grouping of Experimental Animals and Dosage Administration

Group	Number of Rats	Dosage (mg/kg)	Description
Group 1 (Control)	5	Group 1(Control)	Received distilled water only
Group 2 (Low dose)	5	100 mg/ kg	Received low dose of Miracle Seed Ultima
Group 3 (Medium dose)	5	300 mg/kg	Received medium dose of Miracle Seed Ultima
Group 4 (High dose)	5	1000 mg/kg	Received high dose of Miracle Seed Ultima

A2.2 :28-days Administration Schedule

Day	Treatment Activity	Observation
Day 1	Daily oral administration began	Rats active normal feedings
Day 2	Continued dosing	Rats active
Day 3	Continued dosing	Rats active consistent feeding
Day 4	Continued dosing	Rat active
Day 5	Continued dosing	Rats active ,consistent feeding
Day 6	Continued dosing	Rats active
Day 7	Continued dosing	Slight weight increase in all groups
Day 8	Continued dosing	Rats active, increased feedings
Day 9	Continued dosing	Rats active , consistent feeding
Day 10	Continued dosing	Rats active , consistent feeding
Day 11	Continued dosing	Rats active
Day 12	Continued dosing	Rats active
Day 13	Continued dosing	Rats active consistent feeding
Day 14	Continued dosing	No mortality, normal behavior , slight weight increase in all groups
Day 15	Continued dosing	Rats active , consistent feeding
Day 16	Continued dosing	Rats active, no abnormality

Day 17	Continued dosing	Rats active, consistent feeding
Day 18	Continued dosing	Rats active, consistent feeding
Day 19	Continued dosing	Rats active,no abnormal behavior
Day 20	Continued dosing	Rats active
Day 21	Continued dosing	Slight weight increase in all groups,no mortality
Day 22	Continued dosing	Rats active
Day 23	Continued dosing	Rats active
Day 24	Continued dosing	No abnormal behavior
Day 25	Continued dosing	Rats active, consistent feeding
Day 26	Continued dosing	Rats active no abnormal behavior
Day 27	Continued dosing	Rats active ,no abnormality
Day 28	Final dosing	Rats calm

APPENDIX III: Raw Data Sheets

A3.1 : Weekly body weights

Week	Start Weight	Week 1	Week 2	Week 3	Week 4
Control (1)	163.19	173.07	187.10	189.94	203.07
(2)	134.77	153.41	165.96	165.90	171.17
(3)	183.29	192.43	210.3	221.81	238.20
(4)	154.97	159.14	180.91	182.62	198.23
(5)	142.32	150.31	153.64	166.20	189.71
Low dose (1)	153.84	157.63	161.24	171.69	181.62
(2)	180.94	192.15	195.17	210.32	224.59
(3)	135.29	147.44	159.35	150.64	159.67
(4)	161.69	157.75	176.25	183.55	187.52
(5)	145.11	144.25	137.81	152.19	160.73
Medium dose (1)	161.58	169.25	176.34	187.80	205.33
(2)	179.53	178.60	188.73	203.61	225.73
(3)	135.80	143.82	157.24	166.94	167.76
(4)	145.83	141.40	151.42	161.11	173.79
(5(	153.58	154.02	172.09	174.82	185.85
High dose (1)	161.57	175.65	181.08	182.34	198.85
(2)	176.29	173.03	180.66	184.64	194.29
(3)	136.01	149.09	161.25	162.75	169.72
(4)	146.04	147.09	137.12	158.16	139.12
(5)	152.85	157..06	148.32	158.70	177.25

A3.2 Raw Absorbance Readings For Liver Function Tests

SAMPLE ID	ALT	ALP	AST	PROTEIN	ALB	T.BIL	D.BIL
	U/L	UI	U/L	g/dL	g/dL	mg/dL	Mg/dL
CTRL 1	161.12	35.33	143.67	4.30	1.76	0.51	0.45
CTRL 2	288.51	33.53	263.50	4.42	1.74	0.46	0.35
CTRL 3	215.80	35.18	127.97	3.88	1.58	0.49	0.36
CTRL 4	132.04	36.23	116.92	4.07	1.56	0.43	0.33
CTRL 5	112.26	33.08	115.75	3.63	1.52	0.50	0.35
MSU(1) 100mg	146.58	33.53	116.33	3.49	1.56	0.43	0.33
MSU (2)100mg	187.88	32.34	154.72	3.49	1.35	0.57	0.46
MSU (3)100mg	139.02	32.19	146.58	3.57	1.72	0.63	0.55
MSU (4)100mg	129.71	32.34	138.44	3.48	1.42	0.57	0.55
MSU (5)100mg	174.5	32.04	123.31	2.92	1.64	0.63	0.49

MSU (1)300mg	**111.68**	**33.53**	**93.07**	**4.30**	**1.57**	**0.53**	**0.46**
MSU (2)300mg	**170.43**	**32.64**	**143.67**	**3.44**	**1.42**	**0.60**	**0.52**
MSU (3)300mg	**166.94**	**30.84**	**182.64**	**3.49**	**1.62**	**0.68**	**0.52**
MSU (4)300	**118.66**	**40.87**	**163.45**	**3.44**	**1.62**	**0.64**	**0.43**
MSU (5)300mg	**237.32**	**30.69**	**189.04**	**3.50**	**1.62**	**0.56**	**0.46**
MSU (1)1000mg	**155.89**	**33.83**	**127.97**	**3.75**	**1.63**	**0.63**	**0.52**
MSU (2)1000mg	**147.16**	**33.08**	**141.35**	**3.77**	**1.39**	**0.41**	**0.32**
MSU (3)1000mg	**198.93**	**32.64**	**165.78**	**3.62**	**1.64**	**0.76**	**0.68**
MSU (4)1000mg	**255.35**	**35.63**	**223.94**	**3.82**	**1.48**	**0.56**	**0.46**
MSU (5)1000mg	**245.46**	**30.99**	**216.38**	**4.62**	**1.59**	**0.56**	**0.45**

A3.3 : Raw Absorbance Readings for Lipid Profile

SAMPLE ID	CHOL	TRIGGS	LDL	HDL
mg	mg/dL	mg/Dl	mg/dL	mg/dL
CTRL 1	80.77	176.47	82.6	33.46
CTRL 2	68.59	158.29	71.07	29.18
CTRL 3	88.46	128.34	82.22	31.91
CTRL 4	72.44	112.30	65.71	29.18
CTRL 5	87.82	96.26	77.11	29.96
MSU (1) 100	80.13	102.67	72.65	28.02
MSU (2) 100	103.21	97.33	92.71	29.96
MSU (3) 100	112.82	139.04	107.55	33.07
MSU (4) 100	73.08	142.25	72.34	29.18
MSU (5) 100	64.10	94.12	54.91	28.02
MSU (1) 300	93.59	144.39	94.84	27.63
MSU (2) 300	87.18	81.28	75.03	28.41
MSU (3) 300	82.05	161.50	85.95	28.41
MSU (4) 300	86.54	135.83	74.41	39.3
MSU (5) 300	85.26	148.66	86.58	28.41
MSU (1) 1000	71.80	87.70	60.93	28.41
MSU (2) 1000	78.21	93.05	68.41	28.41
MSU(3)1000	104.49	164.71	106.3	31.13
MSU (4) 1000	95.51	168.98	99.35	29.96
MSU (5)1000	76.92	161.50	81.60	27.63