SUBCHRONIC TOXICITY OF ETHANOL EXTRACT OF *Icacina trichantha* ON THE BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF THE LIVER IN WISTAR RATS

BY

AITEREBHE FAITHFULNESS

LSC1906429

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

APRIL 2024

SUBCHRONIC TOXICITY OF ETHANOL EXTRACT OF *Icacina trichantha* ON THE BIOCHEMICAL AND HISTOLOGICAL PARAMETERS IN THE LIVER OF WISTAR RATS

BY

AITEREBHE FAITHFULNESS

LSC1906429

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

APRIL 2024

CERTIFICATION

This is to certify that this research work was carried out by **AITEREBHE FAITHFULNESS** with matriculation number **LSC1906429** as part of the requirements for the award of a Bachelor of Science degree (BSc) in the Department of Biochemistry, University of Benin.

---------- ----------

Dr. O. C. Ugbeni **Date**

(Project Supervisor)

---------- ----------

Dr. S.I. Ojeaburu **Date**

(Project Coordinator)

---------- ----------

Prof. E.C. Oyeneke **Date**

(Head Of Department)

---------- ----------

External Supervisor **Date**

DEDICATION

TO MY FATHER, MR. H.F AITEREBHE

I experience your guidance every day.

ACKNOWLEDGEMENT

First and Foremost, heartfelt appreciation to God Almighty for His unending grace to complete this exercises successfully.I am grateful to Dr. O. C. Ugbeni, my Project Supervisor for his guidance and support during the course of the project. I also wish to especially appreciate Prof. E. C. Onyeneke, H.O.D. Biochemistry Department, University of Benin; Dr. S. Ojeaburu, Project Coordinator; Dr. Usifo my Course Adviser, and the entire academic and non-academic staff of this great and prestigious department. Your combined efforts have made all the differences.To Dad, Mum and Victory, my deepest gratitude.Finally, to Williams, Prince, Victor, and Miracle, I am grateful for the vital friendships and support.God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF PLATES

ABSTRACT

This experiment aimed to investigate the subchronic toxicity of *Icacina trichantha* ethanolic extract and its impact on the liver's biochemical and histological parameters in male Wistar rats. The research specifically focused on examining the potential positive or negative effects of the extract. The main objective was to evaluate liver function by analyzing various biochemical parameters, including liver enzymes (ALT, AST, ALP, and GGT), total protein, albumin, bilirubin, and lipid profile. Over a period of 28 days, twelve male Wistar rats were divided into four groups, with the first group serving as the control. The rats in the other groups were orally administered the extract in different solutions. After the specified time, the rats were sacrificed, and blood samples were collected via cardiac puncture. The collected blood samples were then used to analyze the biochemical parameters. The histological examinations of the liver showed vasodilation, normal hepatocytes and activation of Kupffer cells. The results of this study indicate that *Icacina trichantha* has specific effects on Wistar rats, particularly in terms of regulating liver function, lipid metabolism, and oxidative stress. These specific effects suggest a complex interaction between the active compounds in the extract and the physiological processes of the rats. The observed changes in important biochemical markers demonstrate the potential of Icacina trichantha as a source of therapeutic bioactive compounds. However, caution should be exercised when using it, and further research is needed to fully understand its mechanisms of action and assess its safety and effectiveness in clinical settings. Nevertheless, these findings offer valuable insights into the specific effects of *Icacina trichantha* and provide a foundation for future investigations into its pharmacological potential. aminations of the liver revealed vasodilation, normal hepatocytes, and activation of Kupffer cells.

CHAPTER ONE

1.1.1 Background of the study

Icacina trichantha, a perennial shrub belonging to the Icacinaceae family, is an indigenous plant species native to various regions of Africa, particularly West and Central Africa. It has been an integral part of traditional medicinal practices in these regions for centuries, owing to its diverse pharmacological properties and cultural significance (Asuzu *et al*., 2017; Zhao *et al*., 2014).In traditional African medicine, *Icacina trichantha* holds a revered status for its purported medicinal properties. Various parts of the plant, including the roots and leaves are utilized in the preparation of herbal remedies to treat a wide array of ailments. Its traditional uses range from treating fevers, respiratory infections, and gastrointestinal disorders to serving as a remedy for snakebites and as an aphrodisiac. Moreover, it is often employed in rituals and ceremonies due to its perceived spiritual significance (Okwuosa *et al*., 2020; Osadebe *et al*., 2019).While the traditional uses of *Icacina trichantha* have been documented anecdotally, scientific investigations into its pharmacological properties are gaining momentum. Several studies have reported the presence of bioactive compounds in *Icacina trichantha*, including alkaloids, flavonoids, saponins, and phenolic compounds. These phytochemical constituents have been associated with various pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects. Furthermore, recent research has highlighted the potential of *Icacina trichantha* as a source of novel drug candidates. For instance, extracts derived from the plant have shown promising inhibitory effects against microbial pathogens, suggesting its utility in the development of antimicrobial agents. Additionally, the antioxidant properties of *Icacina trichantha* extracts have sparked interest in their potential application in combating oxidative stress-related diseases (Adou *et al*., 2005).*Icacina trichantha* stands as a

botanical treasure with immense therapeutic potential. Its rich history in traditional medicine coupled with emerging scientific evidence underscores the importance of further exploration into its pharmacological properties and potential clinical applications. By bridging traditional knowledge with modern scientific methodologies, researchers aim to harness the therapeutic benefits of *Icacina trichantha* for the betterment of global health. (Onakpa *et al* 2016)

1.1.2 Statement of the Problem

The use of herbal remedies, once considered traditional, has now become widespread. *Icacina trichantha* has been traditionally used for various medicinal purposes. However, the effects of its ethanol extract on the liver's biochemical and histological parameters are still not fully understood. It is crucial to investigate the impact of *Icacina trichantha* extract on the liver's biochemical and histological parameters, as well as its structure and function in Wistar rats. This research is essential due to the significance of maintaining liver health and the potential implications of herbal treatments. By addressing this knowledge gap, we can gain valuable insights into the safety and effectiveness of this herbal remedy, enabling informed decision-making regarding its therapeutic use.

1.1.3 Aim of the Study

The aim of this research is to examine the subchronic toxicity of the ethanol extract derived from Icacina trichantha of the liver on Wistar rats.

1.1.4 Specific Objectives of the study

The specific objectives of this study are as follows;

1 To investigate the impact of the ethanol extract derived from *Icacina trichantha* on the liver's biochemical and histological aspects in male Wistar rats over an extended duration.

2 To evaluate the levels of biochemical enzymes (ALT, ASP, and GGT) and overall tissue in the liver of Wistar rats subjected to treatment with the ethanol extract of *Icacina trichantha*.

3 To analyze and compare the biochemical and histological parameters between the control group and the group administered with the ethanol extract of *Icacina trichantha*, aiming to identify any significant variances.

4 To offer valuable insights into the potential advantages or risks associated with the utilization of *Icacina trichantha* as an herbal remedy for general and cardiovascular health, based on the observed effects on biochemical and histological parameters in Wistar rats.

1.1.5 Justification of the Study

There is a lack of research studies that focus on investigating the effects of *Icacina trichantha* on the histological and biochemical aspects of the liver. Bridging this research gap has the potential to significantly improve our understanding of the potential health advantages linked to this plant extract. Histological and biochemical parameters are vital in maintaining liver health and overall well-being. Examining the influence of *Icacina trichantha* on these factors could provide valuable insights into its therapeutic capabilities for liver diseases and conditions associated with liver damage.

1.2 Literature review

1.2.1 Scientific Classification of *Icacina trichantha*

Kingdom……. Plantae

Phylum……... Tracheophyta

Class…… Magnoliopsida

Order……. Gentianales

Family…….. Icacinaceae

Genus……..... Icacina

Species…… *trichantha*

1.2.2 Nomenclature

The plant is scientifically known as Icacina trichantha. It is known as “Urumbia” or “Eriagbo” (referring to its emetic effect) among the Igbos of Nigeria, or “Gbegbe” (meaning to cleanse) by the Yoruba of western Nigeria (Guo *et al*)

1.2.3 Description

Icacina trichantha, a drought-resistant shrub native to West and Central Africa, is a medicinal plant utilized by indigenous tribes in Nigeria. Referred to as "Urumbia" or "Eriagbo" by the Igbos, and "Gbegbe" by the Yoruba, this plant has shown promising chemical and pharmacological properties in recent research (Shagal *et al,2013*). This review aims to summarize the scientific findings on I. trichantha, focusing on its biological activities and chemical composition. Notably, unique pimarane-type diterpenes have been discovered in the plant's tubers. The Icacinaceae family, to which Icacina trichantha belongs, was initially identified by Miers and later revised by Karehed in 2001 through DNA analysis. The genus consists of six recognized species and eight synonyms, with I. oliviformis being well-known as a

food plant in West Africa. Icacina trichantha is a shrub that can reach heights of up to two meters, characterized by its large, yam-like underground tubers. These tubers, rich in starch, are consumed fresh or processed into flour for various dishes. Preparation involves cleaning, slicing, soaking, drying, and grinding the tubers, which serve as a vital source of nutrition and emergency food during droughts. The tubers are also known to contain certain "anti-nutritional factors" like hydrogen cyanide and tannins, which are removed through traditional preparation methods. . The flour has been examined, revealing the existence of carbohydrates (mainly starch), lipids, and proteins, as well as mineral elements like potassium, sodium, and calcium(Onakpa *et al,*2013). The fruit of this plant is a drupe with a tender sweet outer pulp that is consumable. In western Nigeria and nearby regions, I. trichantha is utilized as a popular household remedy for emergency and first-aid care for food poisoning. Supposedly, the tubers and leaves of the plant are aphrodisiacs.

Fig 1.1

The flower of *Icacina trichantha*

Source:iNaturalist

Fig 1.2

The tubers of *Icacina trichantha*

Source:SawitSecure

Fig 1.3

The leaves of *Icacina trichantha*

Source: SawitSecure

Fig 1.4

The fruits of *Icacina trichantha*

Source:iNaturalist

1.2.4 Photochemistry.

Phytochemical analysis of I. trichantha leaf extract revealed the presence of alkaloids, tannins, phenols, and saponins. Fatty acid components like stearolic acid, oleic acid, and erucic acids were also identified. In the tuber, alkaloids, tannins, saponins, steroids, carbohydrates, and cardiac glycosides were detected. Early studies on Icacina plants showed diterpenes and diterpene alkaloids in I. claessensii, I. mannii, and I. guessfeldtii. New diterpene alkaloids icaceine and demethylicaceine were isolated from I. guessfeldtii leaf and root. Icacinol was found in the root of I. claessensii [36], and icacenone was obtained from the root of I. mannii . These compounds have a (9βH)-pimarane or (9βH)-17-nor-pimarane skeleton, unique in the pimarane class of diterpenes. Recently, novel (9βH)-pimarane and (9βH)-17-nor-pimarane structures were isolated from I. trichantha tuber in our labs These (9βH)-pimaranes are a subset of pimarane-type diterpenes with a 9β-H instead of the typical 9α-H. Many of these metabolites feature a 19,6β-γ-lactone, a 3β,20-epoxy bridge, and a furan or tetrahydrofuran moiety.

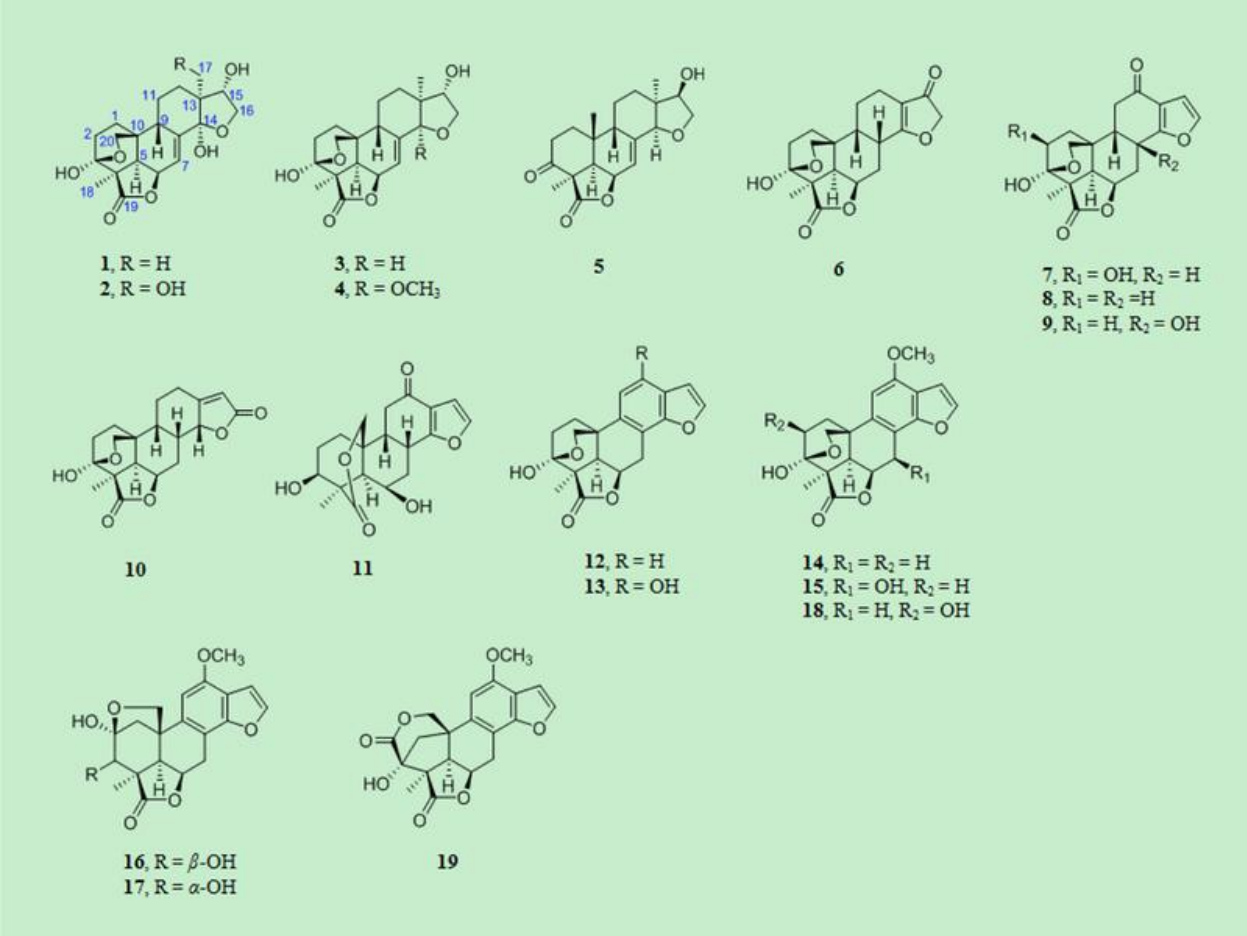

Fig 1.5 Diterpene compounds isolated from *Icacina trichantha*

Source: Google

1.2.5 Pharmacological Characteristics

- **Antimalarial Properties:** Icacina trichantha has garnered attention for its potential as a source of antimalarial agents, contributing to the ongoing efforts to combat malaria, a devastating parasitic disease affecting millions of people worldwide. Several studies have investigated the antimalarial properties of *Icacina trichantha* extracts, revealing promising results that underscore its therapeutic potential in malaria treatment (Nwodo *et al*., 2014; Ojiako *et al*., 2019; Adamu *et al*., 2020; Amadi *et al*., 2017).The mechanism of action underlying the antimalarial activity of *Icacina trichantha* involves multiple pathways, reflecting the complex interactions between the plant's bioactive constituents and the malaria parasite. One of the key mechanisms is the inhibition of parasite growth and replication. Studies have demonstrated that extracts derived from Icacina trichantha exhibit potent inhibitory effects against Plasmodium species, the causative agents of malaria. These inhibitory effects are attributed to the ability of the plant's bioactive compounds to interfere with essential metabolic processes within the parasite, such as nucleic acid synthesis, protein synthesis, and mitochondrial function (Nwodo et *al*., 2014; Ojiako *et al*., 2019). *Icacina trichantha* extracts have been shown to modulate the host immune response against malaria infection. The plant's immunomodulatory properties enhance the host's ability to combat the parasite by stimulating innate and adaptive immune mechanisms. This includes the activation of macrophages, natural killer cells, and T lymphocytes, which play critical roles in parasite clearance and immune defense (Adamu *et al*., 2020; Amadi *et al*., 2017).

- **Anti-Oxidant Properties:** Icacina trichantha exhibits notable antioxidant properties, making it a potential candidate for combating oxidative stress-related diseases and promoting overall health. Extensive research has been conducted to elucidate the antioxidant mechanisms of Icacina trichantha, with several studies providing insights into its efficacy and therapeutic potential (Onyeyili *et al*., 2018; Amusa *et al*., 2016; Nwosu *et al*., 2017; Ojiako *et al*., 2019).The antioxidant activity of Icacina trichantha is attributed to its rich phytochemical composition, which includes flavonoids, phenolic compounds, and other bioactive constituents. These phytochemicals function as antioxidants by scavenging free radicals, neutralizing reactive oxygen species (ROS), and inhibiting oxidative damage to biomolecules such as lipids, proteins, and DNA (Onyeyili *et al*., 2018; Amusa *et al*., 2016).Moreover, *Icacina trichantha* extracts have been shown to enhance the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes play crucial roles in the cellular defense against oxidative stress by catalyzing the breakdown of ROS and detoxifying harmful reactive intermediates (Nwosu *et al*., 2017; Ojiako *et al*., 2019).The plant exerts its antioxidant effects through modulation of intracellular signaling pathways involved in oxidative stress and inflammation. Studies have demonstrated that the plant extracts inhibit the activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which are implicated in the regulation of inflammatory responses and oxidative stress-related gene expression (Onyeyili *et al*., 2018; Amusa *et al*., 2016).

- **Anti-Inflammatory Properties:** *Icacina trichantha* exhibits significant anti-inflammatory properties, which have been extensively studied for their potential therapeutic applications in various inflammatory conditions. Research into the anti-inflammatory effects of *Icacina trichantha* has provided valuable insights into its mechanism of action and therapeutic potential (Onyeyili *et al*., 2018; Amusa *et al*., 2016; Nwosu *et al*., 2017; Ojiako et al., 2019).One of the primary mechanisms underlying the anti-inflammatory activity of *Icacina trichantha* is the inhibition of pro-inflammatory mediators and cytokines. Studies have shown that extracts derived from the plant inhibit the production and release of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and prostaglandin E2 (PGE2). By suppressing the expression of these inflammatory molecules, *Icacina trichantha* mitigates the inflammatory response and reduces tissue inflammation (Onyeyili *et al*., 2018; Amusa *et al*., 2016).*Icacina trichantha* exerts its anti-inflammatory effects by modulating intracellular signaling pathways involved in the regulation of inflammation. Experimental evidence indicates that the plant extracts inhibit the activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which are key signaling pathways implicated in the initiation and propagation of inflammatory responses. By blocking the activation of these pro-inflammatory signaling pathways, *Icacina trichantha* attenuates the production of inflammatory cytokines and mediators, thereby suppressing inflammation (Nwosu *et al*., 2017; Ojiako *et al*., 2019). *Icacina trichantha* exhibits antioxidant properties that contribute to its anti-inflammatory activity. Oxidative stress plays a pivotal role in the pathogenesis of inflammation by promoting the production of reactive oxygen species (ROS) and oxidative damage to cellular

components. *Icacina trichantha* extracts scavenge free radicals, inhibit lipid peroxidation, and enhance the activity of antioxidant enzymes, thereby reducing oxidative stress and attenuating inflammation (Onyeyili *et al*., 2018; Amusa *et al*., 2016).The anti-inflammatory properties of *Icacina trichantha* have been linked to its modulation of immune responses. The plant extracts regulate the activity of immune cells such as macrophages, T lymphocytes, and dendritic cells, which play crucial roles in the initiation and regulation of inflammatory responses. By modulating immune cell function, *Icacina trichantha* suppresses excessive inflammation and promotes immune homeostasis (Nwosu *et al*., 2017; Ojiako *et al*., 2019).

- **Anti-Microbial Properties:** *Icacina trichantha* has long been recognized for its medicinal properties, including its antimicrobial effects. Research into the antimicrobial properties of *Icacina trichantha* has provided valuable insights into its mechanism of action and potential therapeutic applications in combating microbial infections (Amadi et al., 2017; Adamu *et al*., 2019; Onyeyili *et al*., 2018; Amusa *et al*., 2016).The antimicrobial activity of *Icacina trichantha* by its ability to disrupt microbial cell membranes. Studies have demonstrated that extracts derived from Icacina trichantha exhibit membrane-damaging effects on a wide range of microbial pathogens, including bacteria, fungi, and protozoa. This membrane-disrupting activity leads to leakage of intracellular contents, loss of membrane integrity, and ultimately, microbial cell death (Amadi *et al*., 2017; Adamu *et al*., 2019).The antimicrobial effects are exerted through the inhibition of microbial growth and proliferation. Experimental evidence indicates that the plant extracts inhibit the growth of pathogenic microorganisms by interfering with

essential metabolic processes, such as nucleic acid synthesis, protein synthesis, and cell wall biosynthesis. By targeting these vital microbial functions, *Icacina trichantha* disrupts microbial growth and replication, thereby inhibiting the spread of infections (Onyeyili *et al*., 2018; Amusa *et al*., 2016).Furthermore, *Icacina trichantha* exhibits synergistic interactions with conventional antimicrobial agents, enhancing their antimicrobial efficacy. Studies have reported potentiation of the antimicrobial activity of antibiotics and antifungal drugs in combination with *Icacina trichantha* extracts. This synergistic effect is attributed to the complementary mechanisms of action between the plant extracts and conventional antimicrobial agents, resulting in enhanced microbial inhibition and suppression of resistance development (Amadi *et al*., 2017; Adamu *et al*., 2019).

- **Anti-Diabetic Properties:** In recent years, there has been a growing interest in researching the antidiabetic properties of Icacina trichantha. conducted studies that have shown the hypoglycemic effects of extracts from different parts of the plant, such as leaves, tubers, and roots These effects play a crucial role in managing diabetes by regulating blood sugar levels.Additionally.There is evidence suggesting that compounds found in I. trichantha may either mimic the action of insulin or enhance insulin sensitivity in cells.This insulin-mimetic action has the potential to improve glucose uptake by cells and reduce insulin resistance, which is a characteristic of type 2 diabetes.Icacina trichantha also contains antioxidants that scavenge free radicals and reduce oxidative damage to cells, including the pancreatic beta cells responsible for insulin production.This antioxidant activity is important in mitigating oxidative stress, which is a

key factor in diabetes-related complications. Extracts of I. trichantha can improve lipid profiles by reducing LDL cholesterol and triglyceride levels while increasing HDL cholesterol (Azuzu *et al*). These lipid-lowering effects are significant in managing dyslipidemia, which is commonly associated with diabetes.Histopathological studies conducted also revealed that extracts of I. trichantha can alleviate structural abnormalities in pancreatic tissues associated with diabetes.This suggests that there is a potential to preserve pancreatic function and prevent further deterioration..

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Reagent and Chemicals

The reagents used in this study were of analytical grade. They are as follows;

Alanine Transaminase Reagent, Aspartate Transaminase Reagent, Gamma-Glutamyl Transferase Reagent, High-Density Lipoprotein, Cholesterol Reagent, Bilirubin Reagent. Total Cholesterol Reagent, Low-Density Lipoprotein Cholesterol Reagent, Total Protein Reagent, Triacylglyceride Reagent, Alkaline Phosphatase Reagent, Albumin Reagent. Chloroform. Ethanol, Formalin, Distilled water, Ethylenediaminetetraacetic acid (EDTA),10% Formalin. TCA, Phosphate buffer, Ellaman's reagent, Pyrogallol, Carbonate Buffer.

2.1.2 Equipment and Apparatus

Beakers, Water bath, Incubator, Sample containers (EDTA),Garvage, Refrigerator, Cotton woolTest Tubes Test Tube Rack, Measuring Cylinder, Micro pipettes. pH meter Mortar and Pestle, UV/Visible spectrophotometer, Centrifuges.

2.2 Methods.

2.2.1. Plant Sample

Fresh roots of *Icacina trichantha* (false yam) was collected on February2024 from Ewu, Esan Central local government area, Edo state. The leaves were taxonomically authenticated and documented at the herbarium.

2.2.2 Preparation of the extract

The roots of the plant were collected from the tree of the plant by cutting out the required part. The roots were then washed with running water and rinsed with ionized water. The rinsed roots were air-dried at room temperature for some days and pulverized. The already air-dried roots were reduced to powder by grinding and stored in a polyethene bag before use. The extract was prepared by soaking a known quantity (in gram) of the powder in ethanol. 100 gram of the powder (solute) was mixed with 500 ml of 40% ethanol (solvent). It was immersed for 72 hours with regular stirring (Olumese *et al.,* 2018). The extract was filtered using muslin fabric and Whatman filter paper and concentrated using a rotary evaporator. It was further freeze-dried and concentrated to dryness using desiccators.

The yield was calculated as:

$$\frac{\text{Weight of dried extract}}{\text{Weight of plant root}} \times 100$$

2.2.3 Preparation of Stock Solution

After the extracts were prepared, it was necessary to find the dose to be administered.

Since the rats were divided into 4 groups to be administered with the extract, the volume to be administered was dependent on gram/kilogram (body weight/B.w). The workings below were done to get the required dose, taking stock solution as 120ml/150g:

Administration Concentration:

For group 2A, a concentration of 200 mgl/kg B.w

For group 3A, a concentration of 500 mgl/kg B.w

For group 4A, a concentration of 1000 mgl/kg B.w

The volume administered was 0.75ml using a 2ml syringe for an extended period of 28 days.

2.3 Animal handling

Male Wistar rats with an average weight of 150 grams each were obtained from the animal house in the Department of Pharmacy, University of Benin, Benin City. They were kept in wooden cages.

There was no need for acclimatization since they were bred and kept in the same environment where the research was done. They had access to feed and clean drinkable water *ad libitum*. The animals were exposed to natural conditions of temperature and lighting. For safety of the rats, the students were assigned to maintain a healthy environment. This also aided in providing a comfortable environment for work to be carried out by the students and keeping the rats in a good state.

2.4 Experimental design

Twelve male wistar rats were randomly allotted into four groups labelled 1A, 2A, 3A and 4A. Each group contained a number of 3 male wistar. Group 1A was used as the control experiment/group. Hence, only 9 rats were given the extracts orally.

All the groups were allowed access to water and feed, and every group was administered with the plant's extract and the required dose except for group 1 which was the control group.

Group 2A, 3A and 4A were administered with the required dose of 0.75ml of the extract in respect to the prepared solution for twenty-eight (28) days before the commencement of the sacrifice to carry out the assays.

Table 2.1: Table showing the grouping of test animals and the treatment they received

Groups	Description	Treatment
1A	Positive Control	No treatment
2A	Administered with the extract	Conc. of 200 mgl/kg B.w
3A	Administered with the extract	Conc. of 500 mgl/kg B.w
4A	Administered with the extract	Conc. of 1000 ml/kg B.w

At the end of administration, the rats were denied access to feed and water over the night (they were fasted) and later sacrificed. Blood samples were collected from the rats through cardiac puncture into already labeled ethylenediaminetetracetate (EDTA), heparin and plain containers for analysis. The liver was also dissected out and weighed.

2.5 Samples Collection

At the end of administration, the rats were made to fast overnight and later sacrificed.

The rats were anaesthetized with chloroform and placed on its back, the abdomen was cut open using a surgical scissor and forceps to hold firm. The collection of blood samples was done using 5 ml syringes with 23 G needles. Blood samples were collected from the rats through cardiac puncture into already labeled ethylenediaminetetracetate (EDTA), heparin and plain containers for analysis. The sample was placed in an iced medium to prevent denaturation. The liver was dissected out and weighed. The liver was collected into 10% formalin and subject to histological and histopathological investigations.

2.6 Measurement of Biochemical and Histological Indices

The biochemical analysis was done after the supernatant was gotten from the blood samples by centrifugation using a centrifuge at the Department of Biochemistry, University of Benin, Benin City. The parameters determined were: ALT, AST, ALP, GGT, Bilirubin levels (total and direct), Albumin levels, Total Protein, High and Low Density Lipoproteins, Cholesterol and Triglcerides. The liver samples were taken to the University of Benin Teaching Hospital (UBTH) for analysis.

2.7 Biochemical Assays

The following assays were carried out using specialized kits, analytical grade chemicals/reagents and their respective standards, as directed by the manufacturer:

2.7.1 Alkaline Phosphatase (ALP)

Principle: The enzyme ALP exerts its action on the sodium thymolphthalein monophosphate that is buffered with AMP. When an alkaline reagent is introduced, it not only halts the activity of the enzyme but also triggers the formation of a blue chromogen, which can be quantified at a wavelength of 590 nm.

Procedure: 250 µL of reagent one (R1) substrate of the kit was pipetted into a test tube. Afterwards, 25 µL of DDH_2O was then added to the the test tube and 25 µL of the sample was measured and also added. Lastly, 1250 µL of the second reagent (R2) which is also known as the colour reagent was added. The gotten solution was allowed to mix well and the absorbance was read at 590 nm.

Calculation

$$\text{ALP Concentration (IU/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Value of Standard (IU/L)}$$

2.7.2 Alanine Aminotranferase (ALT)

Principle:

α-oxoglutarate + L-aspartate $\xrightarrow{\text{GPT}}$ L-glutamate + Pyruvate

Procedure: 100 µL of the sample was pipetted into test tube. Thereafter, 500µL of R1 Buffer (reagent 1) was added to the test tube. The sample was incubated for exactly 30mins at 37ºC in a water bath. After incubation, 500µL of R2 Dye Reagent was added. The mixture was stirred and allowed to stand for 20 minutes at room temperature (20-25ºC). 5ml of dilute NaOH was the pipetted into the test tube. The absorbnce was read at 546 nm.

Calculation:

The GPT /ALT activity in the serum was obtained with the aid of a graph via extrapolation. A graph of Absorbance against value(U/I) gave the respective ALT activity in the serum.

2.7.3 Aspartate Aminotransferse (AST)

Principle:

-oxoglutarate + L-asppartate $\xrightarrow{\text{GOT}}$ L-glutamate + oxaloacetate

Procedure: 0.1ml of sample was mixed with 0.5ml of the R1 AST Buffer and incubated for 30mins at 37ºC. 0.5ml of the R2 Dye Reagent was then added to the mixture and allowed to stand for another 20 minutes at room temperature. Thereafter, 5.0ml dilutd NaOH was then put into the mixture. After 5 minutes, the absorbance was read against the reagent blank.

Calculation:

A graph of absorbance against concentration was plooted to get AST activity in the serum.

2.7.4 Gamma-Glutamyl Transferase (GGT)

Principle: The substrate L-Ɣ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted to 5-amino-2-nitrobenzoate by Ɣ-GT measured at 405 nm. Increase in absorbance is proportional to Ɣ-GT activity.

Procedure: 50µL of sample was mixed with 500µL of the working reagent. The mixture was allowed to stand for 1 minute and the absorbance was read at interval of one minute thrice (i.e 3 minutes).

Calculation:

GGT Conc. (U/L) = 1158 x ΔA 405 nm/min.

2.7.5 Albumin

Principle: The assay for serum albumin utilized the colorimetric method outlined by Doumas *et al*. (1971). This method relies on the quantitative interaction between serum albumin and the indicator 3,3',5,5'-tetrabromo-m cresol sulphonaphthalein (bromocresol green, BCG). The albumin-BCG complex exhibits maximum absorption at 578 nm, with the absorbance directly correlating to the albumin concentration in the sample.

Procedure: 10 µL sample of serum and standard were combined with 3 mL of working reagent, which consisted of succinate buffer (75 mmol/l; pH 4.2) and Bromocresol green (1.7 mmol/l). The resulting mixture was thoroughly mixed and allowed to incubate for a duration of 5 minutes at a temperature of $+25^0$C. Subsequently, the absorbance readings of both the standard and sample were measured at a wavelength of 578 nm.

Calculation:

$$\text{Albumin Concentration (mg/dl)} = \frac{\text{Abs. of Sample}}{\text{Abs. of Std.}} \times \text{Conc. of Standard}$$

2.7.6 Bilirubin

Principle: The assay utilized the colorimetric technique outlined by Jendrassik and Grof (1938). In an alkaline environment, direct (conjugated) bilirubin interacts with diazotized sulphanilic acid to produce a blue complex. The determination of total bilirubin, in the presence of caffeine to release albumin-bound bilirubin, was carried out through the reaction with diazotized sulphanilic acid.

Procedure: Sample (serum) of 200 µL was added with working reagent of R1 (sulphanilic acid and hydrochloric acid), 200 µL, R2 (sodium nitrite) 50 µL, R3 (caffeine and sodium benzoate) 1000 µL. The mixture was mixed and incubated for 10 min at 25ºC, after which R4 (tartrate) 1000 µL was added and mixed, which was incubated for further 30 min at 25^0C. Absorbance reading was taken at 578 nm.

Calculation:

Bilirubin (mg/dl) = (Abs. of Sample – Abs. of Blank) x 20.2

2.7.7 Total Protein

Principle: Proteins interact with cupric ions in an alkaline environment to form a color complex. This color complex absorbs light at 546 nm (530-570nm). The strength of the color is directly linked to the protein concentration in the sample.

Protein $\xrightarrow[\text{Alkaline pH}]{Cu^{2+}}$ Blue colour complex

Procedure: Sample (serum) of 20 µL was added to 1000 µL of Biuret reagent (sodium hydroxide 100 mmol/l Na-K-tartrate 16 mmol/l potassium iodide 15 mmol/l cupric sulphate) and mixed thoroughly. After which, it was left to incubate for 30 min at +25°C and the mixture was read at 546 nm.

Calculation:

$$\text{Tot. Prot. Conc. (gm\%)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 6$$

2.7.8 Triglycerides

Principle:

Enzymatic calorimetric test:

$$\text{Triglcerides} + 3H_2O \longrightarrow \text{Glycerol} + 3RCOOH$$

$$\text{Glycerol} + ATP \longrightarrow \text{G-3-P} + ADP$$

$$\text{G-3-P} + O_2 \longrightarrow \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipynne} + \text{p-chlorophenol} \longrightarrow \text{4-(p-benzaquinone-monoiminol)-phenazone} + 2H_2O + HCL$$

Procedure: 10µL of sample was mixed with 1000µL of triglyceride reagent. The mixture was then incubated at 37^0C for 5 minutes. The absorbance was then read against sample blank.

Calculation:

$$\text{Triglyceride conc.(mg/dL)} = \frac{\text{Abs. of Sample}}{\text{Abs. of Std.}} \times 2.28$$

$$\text{mmol/L} = \text{mg/Dl} \times 0.02586$$

2.7.9 Cholesterol, HDL and LDL

Principle: The chylomicrons; very low density lipoproteins and low density lipoproteins of serum are precipitated by phosphotungstic acid and magnesium ions. After centrifugation, HDL are in the supernatant. HDL content of supernatants are measured by enzymatic method.

Procedure: 1000µL of cholesterol reagent was added to 50µL of HDL supernatant. The mixture was incubated for 5 mins at 37^0C and then, the absorbance was measured.

Calculation:

$$\text{Cholesterol (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of Std.}} \times 200$$

$$\text{H.D.L (mg/dL)} = \frac{\text{Abs. of Sample}}{\text{Abs. os Std.}} \times 50$$

mmol/L = 0.02586 x mg/Dl

$$\text{L.D.L (mmol/L)} = \text{Cholesterol} - \frac{\text{Triglceride}}{2.2} - \text{HDL}$$

2.8 Histopathology

To achieve this, the Wissowzky (1876) method was employed, which utilizes the H&E combination technique. Hematoxylin and Eosin (H&E) staining is the standard method used to examine histopathological changes in animal tissues and organs during toxicity studies. Hematoxylin, a basic dye, has an affinity for acid structures within the cell, particularly nucleic acids present in the cell nucleus. On the contrary, Eosin, an acidic dye, binds to cytoplasmic structures. As a result, H&E staining produces nuclei that appear blue and cytoplasm that appears orange-red.

2.8.1 Staining Principle

Staining techniques rely on the attraction between ions of opposite charges. Hematoxylin, which carries a positive charge, binds to nucleic acids that are negatively charged. Conversely, eosin, which has a negative charge, binds to acidophilic substances that are positively charged, such as amino groups in cytoplasmic proteins. The following is a comprehensive manual for executing the H&E staining method.

1. Prepare the working solution for Harris hematoxylin. Filter the solution to eliminate any metallic residue that may form as it settles. Then, create a 0.1% aqueous solution of eosin Y by

dissolving 1 g of eosin Y in 1 L of deionized water. Adjust the pH to a range of 4 to 5 by adding four drops of HCL. Include a thymol crystal to prevent mold growth.

2. Proper identification of solutions is essential, therefore, it is recommended to label and date them accordingly. The stability of the aqueous eosin solution has been noted to last for at least two months when stored at room temperature. To prepare the acid alcohol differentiation solution, mix 1 mL of 37% hydrochloric acid with 100 mL of 70% ethanol.

3. The process should commence by dewaxing the sections to remove paraffin using xylene. Subsequently, hydrate the sections in 100% ethanol, followed by further hydration in 95% ethanol. Once the sections have been rehydrated in 95% ethanol, rinse them in running tap water for 3 minutes. 4. After completing step 3, immerse the sections in Harris hematoxylin and allow them to stain for 3 to 5 minutes. Then, rinse the sections once again in running tap water for 3 minutes.

5. Following step 4, briefly decolorize the sections in acid alcohol for 2 seconds. Subsequently, ensure thorough washing and bluing of the sections in running tap water for 3 minutes. To stain the sections, submerge them in a 0.1% aqueous eosin Y solution for a duration ranging from 2 to 5 minutes. Finally, rinse the sections in tap water for 30 seconds.

6. Carry out two rounds of dehydration on the sections using 95% ethanol, each lasting for duration of 2 minutes. Repeat this process with 100% ethanol, also for two rounds of 2 minutes each. To ensure optimal clarity, employ a clearing agent to clear the sections, repeating this step twice for duration of 2 minutes each time.

7. Prior to cover slipping, it is advisable to store the slides in a pristine clearing agent to maintain their cleanliness. During the staining process, the nuclei will exhibit a blue shade, while the cytoplasm and other tissue components will appear in a pink-orange hue.

8. When the staining intensity of the sections diminishes, typically after approximately 500 slides, it indicates the need to replace the staining solutions.

9. After the staining process, a thin glass cover is placed on top of the tissue sample to protect it and improve the visual examination of the tissue. This step also aids in preserving the tissue sample for an extended period. Since the mounting solution is usually not soluble in water, it is necessary to dehydrate the tissue once again using alcohol and xylene solutions of increasing concentrations before applying the cover slip.

2.9 Statistical Analysis

All Biochemical assays were carried out in triplicates and the results were presented as Mean ± standard error of mean (S.E.M.). Statistical significance was determined through the use of Analysis of Variance (ANOVA)

.

CHAPTER THREE

RESULTS

3.1 Effect of ethanolic root extract of Icacina trichantha on Biochemical Parameters of the Liver on Wistar Rats.

3.1.1 Effect of ethanolic root extract of *Icacina trichantha* on Liver Enzymes

The effects of ethanolic root extract of *Icacina trichantha* on (ALP), (ALT), (AST) and (GGT) activities in male Wistar rats are presented in Table 3.1.

Test Groups	ALP (IU/L)	ALT (U/I)	AST (U/I)	GGT (U/L)
Group 1	30.35±0.69	3.40±0.53	21.21±2.29	1.93±0.39
Group 2	26.07±0.48	60.67±0.87^{A}	12.28±0.42^{A}	2.70±0.38
Group 3	30.39±3.95	67.90±0.17^{A}	18.75±0.41	4.24±0.77
Group 4	40.88±2.89^{A}	67.87±0.67^{A}	17.96±0.76	1.93±0.39

Data reported as Mean ± Standard Error of Mean, n = 3. Values with superscripts are significantly different ($P<0.05$) from the control group.

Table 3.2. Effect of ethanolic root extract of Icacina trichantha on Total Protein, Albumin, Direct Bilirubin and Total Bilirubin levels in male Wistar rats.

Test Groups	Total Protein (g/dL)	Albumin (g/dL)	Direct Bilirubin (mg%)	Total Bilirubin (mg%)
Group 1	4.29±0.68	3.53±0.06	0.13±0.02^{A}	0.23±0.18
Group 2	4.58±0.68	4.70±0.0^{A}	0.27±0.02^{A}	0.28±0.03
Group 3	4.62±0.79	3.73±0.29	0.20±0.02^{A}	0.16±0.12
Group 4	3.55±0.24	3.98±0.15	0.20±0.01^{A}	0.19±0.03

Data reported as Mean ± Standard Error of Mean, n = 3. Values with superscripts are significantly different ($P<0.05$) from the control group.

Table 3.3. Effect of ethanolic root extract of Icacina trichantha on Triglycerides, Cholesterol, High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL) in male Wistar rats.

Test Groups	Triglycerides (mmol/L)	Cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Group 1	0.20±0.16	9.26±0.29	1.92±0.12	6.23±0.56
Group 2	0.04±0.00	9.69±0.59^{A}	2.42±0.01^{A}	7.11±0.04
Group 3	0.21±0.27	9.44±0.27^{A}	2.35±.056^{A}	6.93±0.17
Group 4	0.06±0.00	9.54±0.40^{A}	2.35±.056^{A}	6.90±0.16

Data reported as Mean ± Standard Error of Mean, n = 3. Values with superscripts are significantly different ($P<0.05$) from the control group.

3.1.2 Result Analysis

The impact of the ethanolic root extract of Icacina trichantha on the liver's biochemical parameters in male Wistar rats is detailed in tables 3.1-3.3. The findings indicate that there were no notable differences ($P>0.05$) in ALP and AST activities in the experimental groups compared to the control group. However, there was a significant change ($P<0.05$) in GGT activities in the experimental groups compared to the control group. Additionally, there was a significant alteration ($P<0.05$) in ALT activities compared to the control group. The levels of Total Protein, Albumin, Direct Bilirubin, and Total Bilirubin in the liver did not show significant changes ($P>0.05$) compared to the control group. Furthermore, there were no significant differences ($P>0.05$) in Triglyceride, Cholesterol, and LDL content when compared to the control group. Notably, there was a significant increase ($P<0.05$) in HDL levels compared to the control group.

3.2. HISTOLOGICAL ANALYSIS OF THE LIVER

Histological examination of the liver of male wistar rats administered ethanolic root extract of Icacina trichantha showed no adverse effect on the liver (plate 3.0-3.4).

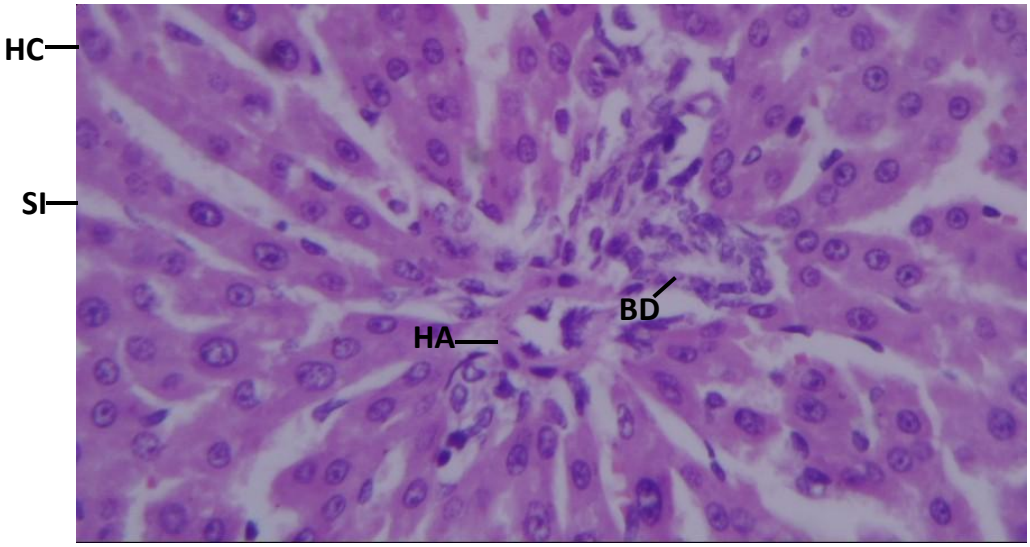

Plate 3.1 Rat liver from group 1 showing: normal architecture: hepatocytes (HC), sinusoids (SI), hepatic artery (HA) and bile ducts (BD): H&E x 400

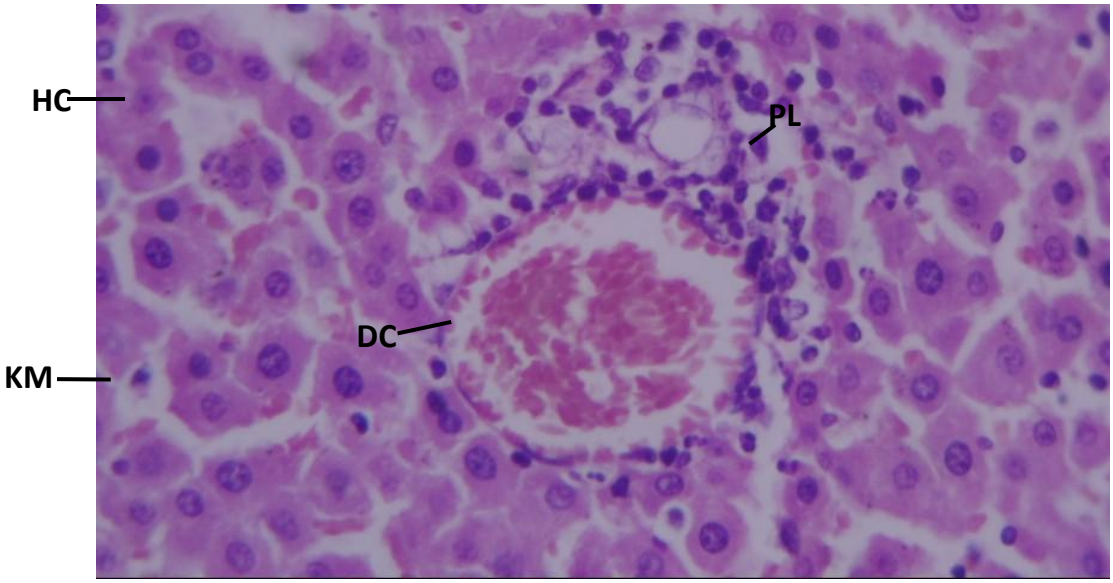

Plate 3.2 Rat liver from group 2B showing: normal hepatocytes (HC), sinusoidal Kupffer cell mobilization (KM), vasodilatation and active congestion (DC), periportal mobilization of lymphocytes (PL): H&E x 400

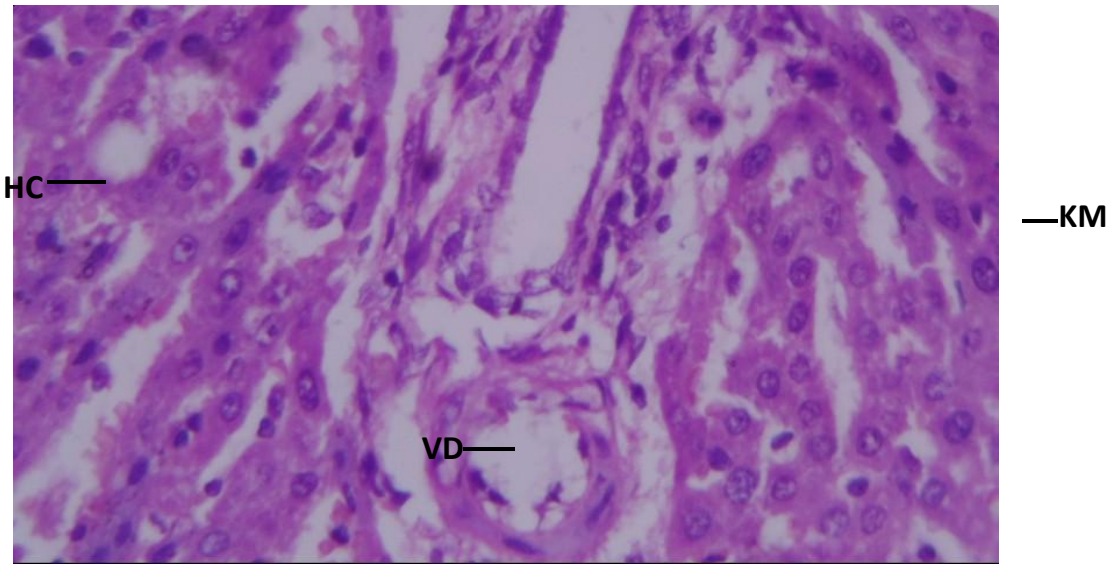

Plate 3.3 Rat liver from group 3B showing: normal hepatocytes (HC), sinusoidal Kupffer cell activation (KM) and vasodilatation (VD): H&E x 400

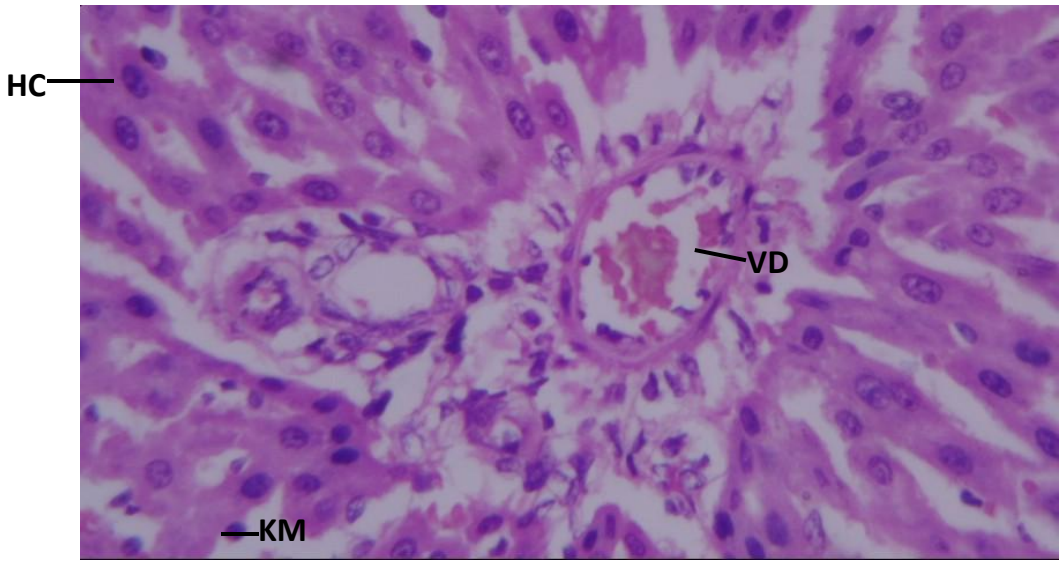

Plate 3.4 Rat liver from group 4B showing: normal hepatocytes (HC), Kupffer cell mobilization (KM) and vasodilatation (VD): H&E x 400

3.2.1 Effect of ethanolic root extract of *Icacina trichantha* on the Histological Parameters of The Liver In Male Wistar Rats.

Based on the histological examination presented in the various Plates above, with Plate 1 serving as the control, it is clear that the dosage given to each rat did not result in any negative impacts on the liver. The liver appeared normal and continued to function properly without any abnormalities. Although minor variances were observed in the microscopic analysis of the liver across different groups, none of these variances suggested any liver dysfunction.

CHAPTER FOUR

DISCUSSION

The measurement of biochemical parameters in rats after the administration of a chemical compound containing a plant extract can provide valuable insights into the impact of such a compound on blood composition (Yakubu *et al*., 2007). Assessing hepatofunctional indices through biochemical evaluation is crucial due to the documented occurrence of liver toxicity following the use of phytotherapeutic products (Isnard et al., 2004; Saad *et al*., 2006). Liver-specific synthetic products, such as albumin and protein, can serve as indicators for evaluating the functional capabilities of the organs (Jesse, 1982). The absence of any significant effects on the liver-body weight ratios subsequent to the administration of the extracts suggests that the extract did not induce organ swelling, atrophy, or hypertrophy (Amresh *et al*., 2008).

Albumin, total bilirubin, and globulin are combinations of molecules utilized to assess the liver's normal functioning in animals (Rasekh *et al*., 2008). A decrease in serum albumin levels could suggest a decline in the liver's synthetic function due to hepatocellular damage (Woodman, 1996). However, the experiment with Icacina trichantha did not show a significant impact on serum albumin concentration, indicating the absence of hepatocellular damage. Bilirubin is a crucial metabolic product of blood with both biological and diagnostic significance. Elevated levels of total and conjugated bilirubin may indicate a decrease in liver function capacity (Woodman, 1996). Numerous enzymes present in the serum do not actually originate from extracellular fluid. In cases of tissue damage, some of these enzymes may enter the serum, likely due to leakage (Reichling and Kaplan, 1988). Serum enzyme measurements play a crucial role in clinical diagnosis by providing insights into the impact and characteristics of pathological damage to various tissues. The absence of any significant impact on ALP and GGT levels in the

serum of the subjects indicates that the extract did not induce damage to the plasma membrane. Furthermore, the lack of effect on ALT activity, along with changes in AST levels, further supports the selective influence on enzyme activity (Nakanishi and Goto, 1975). findings from this study revealed no liver or liver tissue damage. These findings reveal that the plant extract did not compromise liver integrity or inflict damage on the liver. Changes in the levels of key lipids such as cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides can offer valuable information on lipid metabolism and the susceptibility of subjects to atherosclerosis and related coronary artery diseases (Yakubu *et al*., 2008). Higher total cholesterol levels are linked to an increased risk of atherosclerosis. The comparison with the control group revealed no liver or liver tissue damage in the histological findings of this study. These results demonstrate that the plant extract did not compromise liver integrity or cause any harm to the liver. The analysis of the liver histological report is as follows: Plate 2, belonging to group 2 with a stock solution of 2.4g/120ml, showed the highest vasodilation compared to other groups. This suggests that a lower dosage of the drug can enhance blood flow by expanding blood vessels optimally. Hepatocytes in Plate 1 remained normal compared to the control group. Additionally, there was mild activation of Sinusoidal Kupffer cells, indicating a mildly activated immune system. In Plate 3, with a stock solution of 6g/120ml, vasodilation decreased compared to Plate 2, but there was an increase in Kupffer Cell activation. Hepatocytes maintained their normal structure compared to Plate 1 (control Plate). Plate 4, with a stock solution of 12g/ml, showed the least vasodilation compared to other Plates that received the extract, but more than the control group. Hepatocytes remained normal, and there was maximum activation of Kupffer Cells, indicating an activated immune system in the liver compared to all other groups, including the control. These

findings illustrate the effects of the plant extract at both low and high concentrations, and also highlight that a specific dosage is associated with vasoconstriction.

CONCLUSION

The investigation conducted in this study has shown that the extract derived from the roots of *Icacina trichantha* has caused specific changes in the liver functional parameters of male Wistar rats. It has been observed that the extract possesses mild hepatotoxic effects, which are dependent on the dosage administered. Therefore, it is important to consider the potential risks associated with the oral use of this extract as a remedy for male rats, as it may not be entirely safe.

REFERENCES

Ajibesin, K.K., Ekpo, B.A., Bala, D.N., Essien, E.E. and Adesanya, S.A., (2008). Ethnobotanical survey of Akwa Ibom State of Nigeria. *J Ethnopharmacol*, **115**,pp. 387–408.

Akubue, P.I., (1990). Nigerian Medicinal Plants.Pharmacology and Toxicology. In: The State of Medicinal Plant Research in Nigeria (Edited by Abayomi, Sofowora); pp.53-54.

ALUKO, J.B., (2021). ANTIMICROBIAL ASSESSMENT of Icacina trichantha Oliv Leaf Extract.

Anon, (2008) Icacina In *Lost Crops of Africa: Volume* National Academies Press, Washington, DC, pp.281–290.

Ariwaodo, J.O., Chukwuma, E.C. and Adeniji, K.A.,(2012). Some medicinal plant species of Asamagbe stream bank vegetation, Forestry Research Institute of Nigeria, Ibadan. *Ethnobotany Research and Applications*, **10**, pp.541–549.

Asuzu, I.U. and Abubakar II,(1995).The effects of Icacina trichantha tuber extract on the nervous system. *Phytotherapy research*, **9**, pp.21–25.

Asuzu, I.U. and Abubaker II, (1995). The emetic, antihepatotoxic, and antinephrotoxic effects of an extract from Icacina trichantha. *Journal of Herbs, Spices and Medicinal Plants*, **3**,pp. 9–20.

Asuzu, I.U. and Aforonwa, C.U., (1998). The pharmacological effects of Icacina trichantha leaf methanol extract. *Journal of Herbs, Spices and Medicinal Plants*, **6**, pp.49–56.

Asuzu, I.U. and I.I. Abubakar, (1995). The effects of Icacina trichantha tuber extract on the nervous system. Phytother. Res.,**9**,pp. 21-25.

Asuzu, I.U. and O.K. Egwu, (1998). Search for the centrally active component of Icacina trichantha tuber. Phytomedicine, **53**,pp. 35-39.

Asuzu, I.U. and Ugwueze, E.E., (1990). Screening of Icacina trichantha extracts for pharmacological activity. *Journal of ethnopharmacology*, ***28***(2), pp.151-156.

Asuzu, I.U. and Ugwueze, E.E., (1990). Screening of Icacina trichantha extracts for pharmacological activity. *Journal of ethnopharmacology*, ***28***(2), pp.151-156.

Asuzu, I.U., Sosa, S. and Della Loggia, R.O.B.E.R.T.O., (1999). The antiinflammatory activity of Icacina trichantha tuber. *Phytomedicine*, ***6***(4), pp.267-272.

Burkill, H.M., (1994). *The Useful Plants of West Tropical Africa*, Ed. 2; Vol. 2 The Royal Botanical Garden, Kew, U.K., pp. 418–419.

Ekeke, C., Joseph, T.O. and Mensah, S.I., (2021). Morphological, anatomical and histological studies on the genus Icacina (Icacinaceae) from Nigeria, West Africa. *Phytologia Balcanica*, ***27***(1).

El-Sayed, S.A., Salih, A.B., Mohamed, M.S., Mortada, M.E. and Eman, A,E.,(2012). Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of Conocarpus erectus L. Growing in Taif, Saudi Arabia. European Journal of Medicinal Plants, **2**(2),pp.93-112

Fatokun, F.K., (2010). *Investigation of the possible anti-diabetic activity of Icacina trichantha, Ananas cosmos and Uraria picta in a rat model* (Doctoral dissertation).

Fay J.M., (1987). Icacina oliviformis : a close look at an *III - Fruits*, underexploited food plant. I. Overview and ethnobotany. *Economic Botany*, **41**, pp.512–522.

Guo, B.C., (2020). *Phytochemical Investigation of a Traditional West African Ethnomedicinal Plant, Icacina trichantha* (Doctoral dissertation, University of Illinois at Chicago).

KADİRİ, A., ASEKUN, O., ANITA, A., AYODELE, A. and OLOWOKUDEJO, J., (2020). Morpho-anatomical and phytochemical evaluation of Icacina trichantha Oliver (Icacinaceae). *Anatolian Journal of Botany*, ***4***(2), pp.100-105.

Karehed, J.,(2001). Multiple origin of the tropical forest tree family Icacinaceae. *American Journal of Botany*, **88**,pp. 2259–2274.

Mbatchou, V.C. and Dawda, S., (2012). Phytochemical and pharmacological profile of genus Icacina. *Phytopharmacology*, **2**,pp. 135–143.

Miers, J., (1864). Observations on the affinities of the Icacinaceae. *Annals and Magazine of Natural History, Second series* **9**,pp. 218–226.

Ojatula, A.O., Tawose, F.O. and Erinoso, S.M., (2023). Studies on in-vitro bioactivity of Icacina trichantha methanol seed extract. *International Journal of Science for Global Sustainability*, ***9***(1), pp.13-13.

Oliver-Bever, B., (1986). Medicinal Plants in Tropical West Africa. Cambridge University Press, pp.940

Orimisan, O.A., Olakunle, T.F. and Morenikeji, E.S., (2023). Studies on In-vitro Bioactivity of Icacina trichantha Methanol Seed Extract. *International Journal of Science for Global Sustainability (IJSGS)*, ***9***(1).

Quattrocchi, U., 2012. Icacina In *CRC World Dictionary of Medicinal and Poisonous Plants*, CRC Press, Boca Raton, Florida, 2055.

Shagal, M.H. and Kubmarawa, D., (2013). Antimicrobial and phytochemical screening of Icacina trichantha. *Am J Biomed Life Sci*, ***1***(2), pp.37-40

Suriname rainforest. *Bioorganic and Medicinal Chemistry*, 13, 6009–6014.

Swain T.,(1972). Plants in the Development of Modern Medicine Harvard University Press, Cambridge, pp. 125 -129

Timothy, O. and Idu, M., (2011). Preliminary phytochemistry and in vitro antimicrobial properties of aqueous and methanol extracts of Icacina trichantha Oliv. leaf.

Ubom, R.M.,(2010). Ethnobotany and biodiversity conservation in the Niger Delta, Nigeria. *Int. J. Bot*, **6**,pp. 310–322.

Udofia, S.I., Uluocha, O.B. and Asuquoekpo, C.R.,(2014). Evaulation of two indigenous multipurpose shrub species for agroforestry practices in Nigeria. *AFRREV STECH: An International Journal of Science and Technology*, **3**, pp.16–26.

Umoh EO and Iwe MO. (2014) Effects of processing on the nutrient composition of false yam (Icacina trichantha) flour. *Nigerian Food Journal*, **32**,pp. 1–7.

Umoh, E.O., (2013). Anti-nutritional factors of false yam (Icacina trichantha) flour. *Inernet Journal of food Safety*, **15**, pp.78–82.

Zhao, M., Onakpa, M.M., Santarsiero, B.D., Chen, W.L., Szymulanska-Ramamurthy, K.M., Swanson, S.M., Burdette, J.E. and Che, C.T., (2015). (9βH)-Pimaranes and Derivatives from the Tuber of Icacina trichantha. *Journal of natural products*, ***78***(11), pp.2731-2737.

APPENDICES

TOTAL PROTEIN

UNIT: g/dL

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	4.2942	1.17862	.68048	1.3664	7.2220	3.18	5.53
2.00	3	4.5767	1.59982	.92366	.6025	8.5508	3.55	6.42
3.00	3	4.6267	1.38385	.79896	1.1890	8.0643	3.03	5.48
4.00	3	3.5500	.42000	.24249	2.5067	4.5933	3.13	3.97
Total	12	4.2619	1.14017	.32914	3.5375	4.9863	3.03	6.42

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.28247	1.00333	.992	-3.4955	2.9305
		3.00	-.33247	1.00333	.987	-3.5455	2.8805
		4.00	.74420	1.00333	.878	-2.4688	3.9572
	2.00	1.00	.28247	1.00333	.992	-2.9305	3.4955
		3.00	-.05000	1.00333	1.000	-3.2630	3.1630
		4.00	1.02667	1.00333	.741	-2.1863	4.2397
	3.00	1.00	.33247	1.00333	.987	-2.8805	3.5455
		2.00	.05000	1.00333	1.000	-3.1630	3.2630
		4.00	1.07667	1.00333	.714	-2.1363	4.2897
	4.00	1.00	-.74420	1.00333	.878	-3.9572	2.4688
		2.00	-1.02667	1.00333	.741	-4.2397	2.1863
		3.00	-1.07667	1.00333	.714	-4.2897	2.1363
LSD	1.00	2.00	-.28247	1.00333	.785	-2.5961	2.0312
		3.00	-.33247	1.00333	.749	-2.6461	1.9812

		4.00	.74420	1.00333	.479	-1.5695	3.0579
	2.00	1.00	.28247	1.00333	.785	-2.0312	2.5961
		3.00	-.05000	1.00333	.961	-2.3637	2.2637
		4.00	1.02667	1.00333	.336	-1.2870	3.3403
	3.00	1.00	.33247	1.00333	.749	-1.9812	2.6461
		2.00	.05000	1.00333	.961	-2.2637	2.3637
		4.00	1.07667	1.00333	.315	-1.2370	3.3903
	4.00	1.00	-.74420	1.00333	.479	-3.0579	1.5695
		2.00	-1.02667	1.00333	.336	-3.3403	1.2870
		3.00	-1.07667	1.00333	.315	-3.3903	1.2370

TOTAL BILIRUBIN

UNIT: mg%

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	.2333	.31770	.18342	-.5559	1.0225	.04	.60
2.00	3	.2753	.05200	.03002	.1461	.4045	.22	.32
3.00	3	.1600	.02000	.01155	.1103	.2097	.14	.18
4.00	3	.1933	.02517	.01453	.1308	.2558	.17	.22
Total	12	.2155	.14515	.04190	.1233	.3077	.04	.60

Multiple Comparisons

Dependent Variable: VAR00002

			Mean			95% Confidence Interval	
	(I) VAR00001	(J) VAR00001	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.04200	.13208	.988	-.4650	.3810
		3.00	.07333	.13208	.943	-.3496	.4963
		4.00	.04000	.13208	.990	-.3830	.4630
	2.00	1.00	.04200	.13208	.988	-.3810	.4650
		3.00	.11533	.13208	.819	-.3076	.5383
		4.00	.08200	.13208	.923	-.3410	.5050

	3.00	1.00	-.07333	.13208	.943	-.4963	.3496
		2.00	-.11533	.13208	.819	-.5383	.3076
		4.00	-.03333	.13208	.994	-.4563	.3896
	4.00	1.00	-.04000	.13208	.990	-.4630	.3830
		2.00	-.08200	.13208	.923	-.5050	.3410
		3.00	.03333	.13208	.994	-.3896	.4563
LSD	1.00	2.00	-.04200	.13208	.759	-.3466	.2626
		3.00	.07333	.13208	.594	-.2312	.3779
		4.00	.04000	.13208	.770	-.2646	.3446
	2.00	1.00	.04200	.13208	.759	-.2626	.3466
		3.00	.11533	.13208	.408	-.1892	.4199
		4.00	.08200	.13208	.552	-.2226	.3866
	3.00	1.00	-.07333	.13208	.594	-.3779	.2312
		2.00	-.11533	.13208	.408	-.4199	.1892
		4.00	-.03333	.13208	.807	-.3379	.2712
	4.00	1.00	-.04000	.13208	.770	-.3446	.2646
		2.00	-.08200	.13208	.552	-.3866	.2226
		3.00	.03333	.13208	.807	-.2712	.3379

LOW DENSITY LIPOPROTEINS

UNIT: (mmol/L)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	6.2303	.98335	.56774	3.7876	8.6731	5.30	7.26
2.00	3	7.1133	.08622	.04978	6.8992	7.3275	7.02	7.19
3.00	3	6.9333	.29704	.17150	6.1954	7.6712	6.60	7.17
4.00	3	6.9067	.34356	.19835	6.0532	7.7601	6.51	7.11
Total	12	6.7959	.58128	.16780	6.4266	7.1652	5.30	7.26

Multiple Comparisons							
Dependent Variable: VAR00002							
			Mean			95% Confidence Interval	
	(I) VAR00001	(J) VAR00001	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.88300	.44360	.267	-2.3036	.5376
		3.00	-.70300	.44360	.437	-2.1236	.7176
		4.00	-.67633	.44360	.467	-2.0969	.7442
	2.00	1.00	.88300	.44360	.267	-.5376	2.3036
		3.00	.18000	.44360	.976	-1.2406	1.6006
		4.00	.20667	.44360	.964	-1.2139	1.6272
	3.00	1.00	.70300	.44360	.437	-.7176	2.1236
		2.00	-.18000	.44360	.976	-1.6006	1.2406
		4.00	.02667	.44360	1.000	-1.3939	1.4472
	4.00	1.00	.67633	.44360	.467	-.7442	2.0969
		2.00	-.20667	.44360	.964	-1.6272	1.2139
		3.00	-.02667	.44360	1.000	-1.4472	1.3939
LSD	1.00	2.00	-.88300	.44360	.082	-1.9059	.1399
		3.00	-.70300	.44360	.152	-1.7259	.3199
		4.00	-.67633	.44360	.166	-1.6993	.3466
	2.00	1.00	.88300	.44360	.082	-.1399	1.9059
		3.00	.18000	.44360	.696	-.8429	1.2029
		4.00	.20667	.44360	.654	-.8163	1.2296
	3.00	1.00	.70300	.44360	.152	-.3199	1.7259
		2.00	-.18000	.44360	.696	-1.2029	.8429
		4.00	.02667	.44360	.954	-.9963	1.0496
	4.00	1.00	.67633	.44360	.166	-.3466	1.6993
		2.00	-.20667	.44360	.654	-1.2296	.8163
		3.00	-.02667	.44360	.954	-1.0496	.9963

ALBUMIN

UNIT: g/dL

Descriptives							
VAR00002							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
1.00	3	3.5250	.10851	.06265	3.2554	3.7946	3.40	3.60
2.00	3	4.7000	.34641	.20000	3.8395	5.5605	4.30	4.90
3.00	3	3.7333	.51316	.29627	2.4586	5.0081	3.30	4.30
4.00	3	3.9833	.26577	.15344	3.3231	4.6435	3.73	4.26
Total	12	3.9854	.54689	.15787	3.6379	4.3329	3.30	4.90

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid Lower Bour
Tukey HSD	1.00	2.00	-1.17500*	.27861	.012	-2.0672
		3.00	-.20833	.27861	.875	-1.1005
		4.00	-.45833	.27861	.408	-1.3505
	2.00	1.00	1.17500*	.27861	.012	.2828
		3.00	.96667*	.27861	.034	.0745
		4.00	.71667	.27861	.122	-.1755
	3.00	1.00	.20833	.27861	.875	-.6839
		2.00	-.96667*	.27861	.034	-1.8589
		4.00	-.25000	.27861	.807	-1.1422
	4.00	1.00	.45833	.27861	.408	-.4339
		2.00	-.71667	.27861	.122	-1.6089
		3.00	.25000	.27861	.807	-.6422
LSD	1.00	2.00	-1.17500*	.27861	.003	-1.8175
		3.00	-.20833	.27861	.476	-.8508
		4.00	-.45833	.27861	.139	-1.1008
	2.00	1.00	1.17500*	.27861	.003	.5325
		3.00	.96667*	.27861	.008	.3242
		4.00	.71667*	.27861	.033	.0742
	3.00	1.00	.20833	.27861	.476	-.4341
		2.00	-.96667*	.27861	.008	-1.6091
		4.00	-.25000	.27861	.396	-.8925
	4.00	1.00	.45833	.27861	.139	-.1841
		2.00	-.71667*	.27861	.033	-1.3591

		3.00	.25000	.27861	.396	-.3925

*. The mean difference is significant at the 0.05 level.

TRIGLYCERIDES

UNIT: mmol/L

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	.2030	.27127	.15662	-.4709	.8769	.04	.52
2.00	3	.0393	.00289	.00167	.0322	.0465	.04	.04
3.00	3	.2050	.27032	.15607	-.4665	.8765	.04	.52
4.00	3	.0553	.00289	.00167	.0482	.0625	.05	.06
Total	12	.1257	.18275	.05276	.0096	.2418	.04	.52

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	.16367	.15635	.729	-.3370	.6644
		3.00	-.00200	.15635	1.000	-.5027	.4987
		4.00	.14767	.15635	.783	-.3530	.6484
	2.00	1.00	-.16367	.15635	.729	-.6644	.3370
		3.00	-.16567	.15635	.722	-.6664	.3350
		4.00	-.01600	.15635	1.000	-.5167	.4847
	3.00	1.00	.00200	.15635	1.000	-.4987	.5027
		2.00	.16567	.15635	.722	-.3350	.6664
		4.00	.14967	.15635	.776	-.3510	.6504
	4.00	1.00	-.14767	.15635	.783	-.6484	.3530
		2.00	.01600	.15635	1.000	-.4847	.5167
		3.00	-.14967	.15635	.776	-.6504	.3510
LSD	1.00	2.00	.16367	.15635	.326	-.1969	.5242
		3.00	-.00200	.15635	.990	-.3625	.3585
		4.00	.14767	.15635	.373	-.2129	.5082

	2.00	1.00	-.16367	.15635	.326	-.5242	.1969
		3.00	-.16567	.15635	.320	-.5262	.1949
		4.00	-.01600	.15635	.921	-.3765	.3445
	3.00	1.00	.00200	.15635	.990	-.3585	.3625
		2.00	.16567	.15635	.320	-.1949	.5262
		4.00	.14967	.15635	.366	-.2109	.5102
	4.00	1.00	-.14767	.15635	.373	-.5082	.2129
		2.00	.01600	.15635	.921	-.3445	.3765
		3.00	-.14967	.15635	.366	-.5102	.2109

ALKALINE PHOSPHATASE

UNIT: (IU/L)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	30.3473	1.18890	.68641	27.3939	33.3007	29.02	31.32
2.00	3	26.0733	.82513	.47639	24.0236	28.1231	25.39	26.99
3.00	3	30.3967	6.84328	3.95097	13.3970	47.3963	23.05	36.59
4.00	3	40.8833	5.01293	2.89421	28.4305	53.3361	35.44	45.31
Total	12	31.9252	6.78276	1.95801	27.6156	36.2347	23.05	45.31

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	4.27400	3.51317	.634	-6.9764	15.5244
		3.00	-.04933	3.51317	1.000	-11.2997	11.2011
		4.00	-10.53600	3.51317	.067	-21.7864	.7144
	2.00	1.00	-4.27400	3.51317	.634	-15.5244	6.9764
		3.00	-4.32333	3.51317	.626	-15.5737	6.9271
		4.00	-14.81000*	3.51317	.013	-26.0604	-3.5596
	3.00	1.00	.04933	3.51317	1.000	-11.2011	11.2997
		2.00	4.32333	3.51317	.626	-6.9271	15.5737
		4.00	-10.48667	3.51317	.068	-21.7371	.7637

	4.00	1.00	10.53600	3.51317	.067	-.7144	21.7864
		2.00	14.81000*	3.51317	.013	3.5596	26.0604
		3.00	10.48667	3.51317	.068	-.7637	21.7371
LSD	1.00	2.00	4.27400	3.51317	.258	-3.8274	12.3754
		3.00	-.04933	3.51317	.989	-8.1507	8.0521
		4.00	-10.53600*	3.51317	.017	-18.6374	-2.4346
	2.00	1.00	-4.27400	3.51317	.258	-12.3754	3.8274
		3.00	-4.32333	3.51317	.253	-12.4247	3.7781
		4.00	-14.81000*	3.51317	.003	-22.9114	-6.7086
	3.00	1.00	.04933	3.51317	.989	-8.0521	8.1507
		2.00	4.32333	3.51317	.253	-3.7781	12.4247
		4.00	-10.48667*	3.51317	.017	-18.5881	-2.3853
	4.00	1.00	10.53600*	3.51317	.017	2.4346	18.6374
		2.00	14.81000*	3.51317	.003	6.7086	22.9114
		3.00	10.48667*	3.51317	.017	2.3853	18.5881

*. The mean difference is significant at the 0.05 level.

DIRECT BILIRUBIN

UNIT: mg%

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	.1333	.04163	.02404	.0299	.2368	.10	.18
2.00	3	.2733	.03055	.01764	.1974	.3492	.24	.30
3.00	3	.2000	.04000	.02309	.1006	.2994	.16	.24
4.00	3	.2000	.01000	.00577	.1752	.2248	.19	.21
Total	12	.2017	.05890	.01700	.1642	.2391	.10	.30

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.14000*	.02698	.004	-.2264	-.0536
		3.00	-.06667	.02698	.140	-.1531	.0197
		4.00	-.06667	.02698	.140	-.1531	.0197
	2.00	1.00	.14000*	.02698	.004	.0536	.2264
		3.00	.07333	.02698	.099	-.0131	.1597
		4.00	.07333	.02698	.099	-.0131	.1597
	3.00	1.00	.06667	.02698	.140	-.0197	.1531
		2.00	-.07333	.02698	.099	-.1597	.0131
		4.00	.00000	.02698	1.000	-.0864	.0864
	4.00	1.00	.06667	.02698	.140	-.0197	.1531
		2.00	-.07333	.02698	.099	-.1597	.0131
		3.00	.00000	.02698	1.000	-.0864	.0864
LSD	1.00	2.00	-.14000*	.02698	.001	-.2022	-.0778
		3.00	-.06667*	.02698	.039	-.1289	-.0045
		4.00	-.06667*	.02698	.039	-.1289	-.0045
	2.00	1.00	.14000*	.02698	.001	.0778	.2022
		3.00	.07333*	.02698	.026	.0111	.1355
		4.00	.07333*	.02698	.026	.0111	.1355
	3.00	1.00	.06667*	.02698	.039	.0045	.1289
		2.00	-.07333*	.02698	.026	-.1355	-.0111

		4.00	.00000	.02698	1.000	-.0622	.0622
	4.00	1.00	.06667*	.02698	.039	.0045	.1289
		2.00	-.07333*	.02698	.026	-.1355	-.0111
		3.00	.00000	.02698	1.000	-.0622	.0622

*. The mean difference is significant at the 0.05 level.

CHOLESTEROL

UNIT: mmol/L

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	9.2583	.50045	.28894	8.0151	10.5015	8.68	9.58
2.00	3	9.6997	.10263	.05925	9.4447	9.9546	9.58	9.78
3.00	3	9.4400	.47948	.27683	8.2489	10.6311	8.89	9.77
4.00	3	9.5367	.40464	.23362	8.5315	10.5418	9.07	9.79
Total	12	9.4837	.38325	.11063	9.2402	9.7272	8.68	9.79

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.44133	.33031	.568	-1.4991	.6164
		3.00	-.18167	.33031	.944	-1.2394	.8761
		4.00	-.27833	.33031	.833	-1.3361	.7794
	2.00	1.00	.44133	.33031	.568	-.6164	1.4991
		3.00	.25967	.33031	.859	-.7981	1.3174
		4.00	.16300	.33031	.958	-.8948	1.2208
	3.00	1.00	.18167	.33031	.944	-.8761	1.2394
		2.00	-.25967	.33031	.859	-1.3174	.7981
		4.00	-.09667	.33031	.991	-1.1544	.9611
	4.00	1.00	.27833	.33031	.833	-.7794	1.3361
		2.00	-.16300	.33031	.958	-1.2208	.8948
		3.00	.09667	.33031	.991	-.9611	1.1544

LSD	1.00	2.00	-.44133	.33031	.218	-1.2030	.3204
		3.00	-.18167	.33031	.597	-.9434	.5800
		4.00	-.27833	.33031	.424	-1.0400	.4834
	2.00	1.00	.44133	.33031	.218	-.3204	1.2030
		3.00	.25967	.33031	.454	-.5020	1.0214
		4.00	.16300	.33031	.635	-.5987	.9247
	3.00	1.00	.18167	.33031	.597	-.5800	.9434
		2.00	-.25967	.33031	.454	-1.0214	.5020
		4.00	-.09667	.33031	.777	-.8584	.6650
	4.00	1.00	.27833	.33031	.424	-.4834	1.0400
		2.00	-.16300	.33031	.635	-.9247	.5987
		3.00	.09667	.33031	.777	-.6650	.8584

ALANINE AMINOTRANSFERASE

UNIT: (U/I)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	3.4000	.91652	.52915	1.1233	5.6767	2.40	4.20
2.00	3	66.6667	1.50111	.86667	62.9377	70.3956	65.20	68.20
3.00	3	67.9000	.30000	.17321	67.1548	68.6452	67.60	68.20
4.00	3	67.8667	1.15470	.66667	64.9982	70.7351	67.20	69.20
Total	12	51.4583	28.99907	8.37131	33.0332	69.8835	2.40	69.20

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-63.26667*	.86763	.000	-66.0451	-60.4882
		3.00	-64.50000*	.86763	.000	-67.2784	-61.7216
		4.00	-64.46667*	.86763	.000	-67.2451	-61.6882

	2.00	1.00	63.26667*	.86763	.000	60.4882	66.0451
		3.00	-1.23333	.86763	.521	-4.0118	1.5451
		4.00	-1.20000	.86763	.542	-3.9784	1.5784
	3.00	1.00	64.50000*	.86763	.000	61.7216	67.2784
		2.00	1.23333	.86763	.521	-1.5451	4.0118
		4.00	.03333	.86763	1.000	-2.7451	2.8118
	4.00	1.00	64.46667*	.86763	.000	61.6882	67.2451
		2.00	1.20000	.86763	.542	-1.5784	3.9784
		3.00	-.03333	.86763	1.000	-2.8118	2.7451
LSD	1.00	2.00	-63.26667*	.86763	.000	-65.2674	-61.2659
		3.00	-64.50000*	.86763	.000	-66.5008	-62.4992
		4.00	-64.46667*	.86763	.000	-66.4674	-62.4659
	2.00	1.00	63.26667*	.86763	.000	61.2659	65.2674
		3.00	-1.23333	.86763	.193	-3.2341	.7674
		4.00	-1.20000	.86763	.204	-3.2008	.8008
	3.00	1.00	64.50000*	.86763	.000	62.4992	66.5008
		2.00	1.23333	.86763	.193	-.7674	3.2341
		4.00	.03333	.86763	.970	-1.9674	2.0341
	4.00	1.00	64.46667*	.86763	.000	62.4659	66.4674
		2.00	1.20000	.86763	.204	-.8008	3.2008
		3.00	-.03333	.86763	.970	-2.0341	1.9674

*. The mean difference is significant at the 0.05 level.

GAMMA-GLUTAMYL TRANSFERASE

UNIT: (U/L)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	1.9327	.67088	.38733	.2661	3.5992	1.16	2.32
2.00	3	2.7033	.66395	.38333	1.0540	4.3527	2.32	3.47
3.00	3	4.2433	1.33945	.77333	.9159	7.5707	3.47	5.79
4.00	3	3.8600	1.33368	.77000	.5470	7.1730	2.32	4.63
Total	12	3.1848	1.31618	.37995	2.3486	4.0211	1.16	5.79

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.77067	.86253	.809	-3.5328	1.9915
		3.00	-2.31067	.86253	.105	-5.0728	.4515
		4.00	-1.92733	.86253	.194	-4.6895	.8348
	2.00	1.00	.77067	.86253	.809	-1.9915	3.5328
		3.00	-1.54000	.86253	.346	-4.3021	1.2221
		4.00	-1.15667	.86253	.565	-3.9188	1.6055
	3.00	1.00	2.31067	.86253	.105	-.4515	5.0728
		2.00	1.54000	.86253	.346	-1.2221	4.3021
		4.00	.38333	.86253	.969	-2.3788	3.1455
	4.00	1.00	1.92733	.86253	.194	-.8348	4.6895
		2.00	1.15667	.86253	.565	-1.6055	3.9188
		3.00	-.38333	.86253	.969	-3.1455	2.3788
LSD	1.00	2.00	-.77067	.86253	.398	-2.7597	1.2183
		3.00	-2.31067*	.86253	.028	-4.2997	-.3217
		4.00	-1.92733	.86253	.056	-3.9163	.0617
	2.00	1.00	.77067	.86253	.398	-1.2183	2.7597
		3.00	-1.54000	.86253	.112	-3.5290	.4490
		4.00	-1.15667	.86253	.217	-3.1457	.8323
	3.00	1.00	2.31067*	.86253	.028	.3217	4.2997
		2.00	1.54000	.86253	.112	-.4490	3.5290
		4.00	.38333	.86253	.669	-1.6057	2.3723
	4.00	1.00	1.92733	.86253	.056	-.0617	3.9163
		2.00	1.15667	.86253	.217	-.8323	3.1457
		3.00	-.38333	.86253	.669	-2.3723	1.6057

*. The mean difference is significant at the 0.05 level.

ASPARTATE AMINOTRANSFERASE
UNIT: U/I

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	21.2133	3.96189	2.28740	11.3715	31.0552	16.64	23.60

2.00	3	12.2833	.72127	.41643	10.4916	14.0751	11.50	12.92
3.00	3	18.9500	.70548	.40731	17.1975	20.7025	18.23	19.64
4.00	3	17.9600	1.30985	.75624	14.7062	21.2138	16.47	18.93
Total	12	17.6017	3.89272	1.12373	15.1284	20.0750	11.50	23.60

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	8.93000*	1.75263	.004	3.3175	14.5425
		3.00	2.26333	1.75263	.593	-3.3492	7.8759
		4.00	3.25333	1.75263	.317	-2.3592	8.8659
	2.00	1.00	-8.93000*	1.75263	.004	-14.5425	-3.3175
		3.00	-6.66667*	1.75263	.022	-12.2792	-1.0541
		4.00	-5.67667*	1.75263	.047	-11.2892	-.0641
	3.00	1.00	-2.26333	1.75263	.593	-7.8759	3.3492
		2.00	6.66667*	1.75263	.022	1.0541	12.2792
		4.00	.99000	1.75263	.940	-4.6225	6.6025
	4.00	1.00	-3.25333	1.75263	.317	-8.8659	2.3592
		2.00	5.67667*	1.75263	.047	.0641	11.2892
		3.00	-.99000	1.75263	.940	-6.6025	4.6225
LSD	1.00	2.00	8.93000*	1.75263	.001	4.8884	12.9716
		3.00	2.26333	1.75263	.233	-1.7782	6.3049
		4.00	3.25333	1.75263	.101	-.7882	7.2949
	2.00	1.00	-8.93000*	1.75263	.001	-12.9716	-4.8884
		3.00	-6.66667*	1.75263	.005	-10.7082	-2.6251
		4.00	-5.67667*	1.75263	.012	-9.7182	-1.6351
	3.00	1.00	-2.26333	1.75263	.233	-6.3049	1.7782
		2.00	6.66667*	1.75263	.005	2.6251	10.7082
		4.00	.99000	1.75263	.588	-3.0516	5.0316
	4.00	1.00	-3.25333	1.75263	.101	-7.2949	.7882
		2.00	5.67667*	1.75263	.012	1.6351	9.7182
		3.00	-.99000	1.75263	.588	-5.0316	3.0516

*. The mean difference is significant at the 0.05 level.

HIGH DENSITY LIPOPROTEINS

UNIT: mmol/L

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	1.9270	.21419	.12366	1.3949	2.4591	1.77	2.17
2.00	3	2.4200	.02646	.01528	2.3543	2.4857	2.39	2.44
3.00	3	2.3567	.11846	.06839	2.0624	2.6509	2.22	2.43
4.00	3	2.3733	.09866	.05696	2.1283	2.6184	2.26	2.44
Total	12	2.2693	.23658	.06830	2.1189	2.4196	1.77	2.44

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.49300*	.10828	.008	-.8397	-.1463
		3.00	-.42967*	.10828	.017	-.7764	-.0829
		4.00	-.44633*	.10828	.014	-.7931	-.0996
	2.00	1.00	.49300*	.10828	.008	.1463	.8397
		3.00	.06333	.10828	.934	-.2834	.4101
		4.00	.04667	.10828	.971	-.3001	.3934
	3.00	1.00	.42967*	.10828	.017	.0829	.7764
		2.00	-.06333	.10828	.934	-.4101	.2834
		4.00	-.01667	.10828	.999	-.3634	.3301
	4.00	1.00	.44633*	.10828	.014	.0996	.7931
		2.00	-.04667	.10828	.971	-.3934	.3001
		3.00	.01667	.10828	.999	-.3301	.3634
LSD	1.00	2.00	-.49300*	.10828	.002	-.7427	-.2433
		3.00	-.42967*	.10828	.004	-.6794	-.1800
		4.00	-.44633*	.10828	.003	-.6960	-.1966
	2.00	1.00	.49300*	.10828	.002	.2433	.7427

		3.00	.06333	.10828	.575	-.1864	.3130
		4.00	.04667	.10828	.678	-.2030	.2964
	3.00	1.00	.42967*	.10828	.004	.1800	.6794
		2.00	-.06333	.10828	.575	-.3130	.1864
		4.00	-.01667	.10828	.881	-.2664	.2330
	4.00	1.00	.44633*	.10828	.003	.1966	.6960
		2.00	-.04667	.10828	.678	-.2964	.2030
		3.00	.01667	.10828	.881	-.2330	.2664

*. The mean difference is significant at the 0.05 level.