EFFECTS OF CELL LIFE IQ ON LIVER AND LIPID PROFILE OF MALE WISTAR RATS

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DEDICATION

I dedicate this work to God Almighty, my source of strength,inspiration, wisdom, knowledge 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

This study evaluated the subacute toxic effects of Cell Life IQ on liver function and lipid profile following 28-day repeated oral administration. Cell Life IQ is a widely used dietary supplement, but its safety profile during prolonged intake remains unclear. To assess potential toxicity, experimental animals were randomly assigned into four groups: a control group receiving distilled water and three treatment groups administered 20mg/kg(low dose) , 80mg/kg (medium doses) and 600mg/kg (high doses)of Cell Life IQ. At the end of the exposure period, blood samples were collected for biochemical analysis of liver function parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin. Lipid profile markers such as total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were also measured.

The results showed at high dose there was a significant increase in total and direct bilirubin when compared to other groups. Also there was also increase in AST at the higher dose while ALP and ALT was not significantly unchanged at all groups. There was no significant change in the lipid profile parameters (triglycerides, HDL, LDL etc) in all the treatment groups when compared to the control groups.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The liver is a central organ for xenobiotic metabolism, detoxification and vital biosynthetic functions including plasma protein and lipoprotein synthesis (Lala et al.,2023). Because orally administered compounds are first exposed to the liver via the portal circulation, the organ is particularly susceptible to injury from drugs, herbal preparations and dietary supplements. Standard biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total and direct bilirubin, and serum albumin/protein — together with histopathological assessment, provide the primary means of detecting hepatocellular injury and cholestasis in preclinical and clinical settings (Kalas et al.,2021).

Subacute (repeat-dose) toxicity studies are an essential early step in preclinical safety evaluation. These studies typically involve daily administration of the test article to rodents for approximately 14–28 days and are designed to identify target organs of toxicity, dose–response relationships, and a preliminary no-observed-adverse-effect level (NOAEL) for planning longer-term studies or human risk assessment (OECD, 2008). The Organisation for Economic Co-operation and Development (OECD) Test Guideline 407 specifies the core design elements for a 28-day repeated-dose oral toxicity study in rodents (e.g., group sizes, range of doses, endpoints such as clinical observations, body weight, food/water intake, hematology, clinical biochemistry, organ weights and histopathology) and remains the internationally accepted framework for subacute testing.

Lipid metabolism is intimately linked to liver function. The liver synthesizes cholesterol, assembles and secretes very-low-density lipoproteins (VLDL), and plays a major role in triglyceride and high-density lipoprotein (HDL) homeostasis. Consequently, hepatocellular dysfunction or metabolic interference caused by xenobiotics commonly results in measurable alterations in serum lipid parameters (total cholesterol, triglycerides, HDL-C, LDL-C) (Thakur et al., 2024). Including a lipid-profile panel alongside liver-function tests in subacute toxicology studies therefore provides a broader assessment of metabolic and hepatic safety.

The Wistar rat (Rattus norvegicus) is a standard and widely accepted rodent model in toxicology owing to its well-characterized physiology, availability of historical control data and reproducible responses to xenobiotic exposure. Male Wistar rats are frequently selected for subacute studies where the endpoints include metabolic measures (e.g., lipids, liver enzymes) to reduce hormonal cycle-related variability associated with females (Dossou-Agoin et al., 2023). Typical endpoints reported in recent subacute investigations in Wistar rats include clinical observations, body and organ weights (absolute and relative), hematology, clinical biochemistry (including ALT/AST/ALP and lipid profile), and liver histopathology — all of which together form the evidence base for identifying hepatotoxic or dyslipidemic effects after repeated exposure.

Herbal products and multi-ingredient dietary supplements have been associated with a spectrum of liver injuries — from asymptomatic enzyme elevations to fulminant hepatic failure — and thus represent an important class of products requiring rigorous toxicological evaluation (Phillips et al., 2020). Mechanisms implicated in herb- and supplement-induced hepatotoxicity include direct hepatocellular toxicity, idiosyncratic immune-mediated reactions, and oxidative stress leading to mitochondrial dysfunction. These mechanisms often manifest biochemically as

elevated transaminases and may be corroborated by histopathological findings such as hepatocellular necrosis, inflammation or steatosis (Gurley et al., 2022).

1 2 Statement of the Problem

The global use of herbal and nutritional supplements has increased markedly in recent years, with many consumers assuming that “natural” products are inherently safe. However, numerous studies have reported that some herbal formulations and nutraceuticals can induce toxic effects, particularly hepatotoxicity, due to the presence of bioactive compounds, contaminants, or formulation inconsistencies. The liver, being the primary organ responsible for xenobiotic metabolism, is especially vulnerable to such injuries, which may manifest as elevated liver enzymes, disruption of lipid metabolism, or histopathological change.Despite this, many commercially available supplements—such as Cell Life IQ, which is marketed as a natural cell-support and immune-enhancing formulation—are widely consumed in Nigeria and other regions without any published, peer-reviewed toxicological evaluation. The absence of scientific data on its safety profile raises significant public health concerns, particularly regarding potential subacute hepatotoxic and dyslipidemic effects following repeated use (OECD, 2008).Furthermore, toxicological investigations that evaluate both liver function markers (ALT, AST, ALP, bilirubin, total protein) and lipid profile indices (total cholesterol, triglycerides, HDL-C, LDL-C) provide more comprehensive insight into possible alterations in hepatic metabolism and lipid homeostasis. Such data are crucial to establish the No Observed Adverse Effect Level (NOAEL) and to determine whether continuous exposure to Cell Life IQ could pose health risks.The lack of empirical evidence regarding Cell Life IQ’s safety on vital organs, particularly the liver, highlights

an urgent need for controlled animal studies. Therefore, profile in male Wistar rats, providing experimental data that could contribute to risk assessment, product regulation, and safer public use.

1 3 Justification of study

The study is necessary to provide scientific evidence on the safety of Cell Life IQ, a product that is currently marketed and consumed without adequate toxicological evaluation. Determining its subacute toxicity profile using a standardized OECD 28- day repeated dose oral administration in male Wistar rats will help establish its potential effects on liver function and lipid metabolism. The findings will contribute to public health awareness, guide regulatory assessment., and promote the responsible use of herbal and nutritional supplements.

1.4 Research Questions

To achieve the aims and objectives of this study, the following research questions were formulated:

1. Does subacute administration of Cell Life IQ affect liver function biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, and total protein in male Wistar rats?

2. Does Cell Life IQ alter serum lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)?

3. What is the effect of Cell Life IQ on body weight, food intake, water intake, and relative liver weight of the treated rats compared with the control group?

4.What is the No Observed Adverse Effect Level (NOAEL) for Cell Life IQ based on biochemical and physiological parameters?

1.5 Aim and Objectives

1.5.1 Aim of the Study:

The overall aim of this study is to evaluate the subacute toxicological effects of Cell Life IQ on liver function and lipid profile in male Wistar rats following 28 days of oral administration, in order to establish its safety profile and determine any potential hepatotoxic or dyslipidemic effects.

1.5.2 Specific Objectives;

To achieve this aim, the study will pursue the following specific objectives:

1. To determine the effect of subacute administration of Cell Life IQ on liver function biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein in male Wistar rats.

2. To assess the effect of Cell Life IQ on serum lipid profile parameters — total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) — after 28 days of repeated oral dosing..

3. To evaluate changes in body weight, food and water intake, and relative liver weights as indicators of general systemic toxicity.

4. To estimate the No- Observed - Adverse- Effect Level (NOAEL) for Cell Life IQ based on biochemical and physiological findings.

CHAPTER TWO

LITERATURE EREVIEW

2.1 Overview Of Toxicities

Toxicity studies are fundamental to evaluating the safety profile of chemical substances, pharmaceuticals, herbal preparations, and food supplements before human or veterinary use. Among the different toxicity categories — acute, subacute, subchronic, and chronic. subacute studies serve as an essential bridge between single-dose (acute) and long-term (chronic) investigation (Mirza and Panchal,2019). They are typically designed to detect biochemical, physiological, or histopathological changes following repeated daily administration of a test substance for about 28 days, usually in rodents such as Wistar or Sprague–Dawley rats.

2.2 Types of Toxicities

2.2.1 Acute toxicities

Acute toxicity studies are among the first steps in toxicological evaluation of any new substance — such as a drug, herbal product, or supplement. They are conducted to determine the toxic effects that occur within a short time (usually 24 hours) after the administration of a single dose or multiple doses given within that short period. Jatoth et al.,2025 did an acute oral toxicity studies of novel polyherbal formulations by using wistar rats and Swiss albino mice as per OECD 425 TG. The results shows that the single-dose acute of these formulations in mice and rats was fine No signs of toxicity were observed in the animals treated at (2000 mg/kg.b. wt, Po), and revealed dullness, causing lethargy, and piloerections, which only persisted on the 1st day. At the end of the study, mild congestion in the liver, and lungs was observed in animals, treated with formulation compared to the vehicle control groups.

Abdu-aguye et al.,(1986) also reported on the acute toxicities of Jatropha curcas L. The seeds of Jatropha curcas L. ingested accidentally by two children aged 3 and 5 years led to a clinical syndrome of restlessness, severe vomiting and dehydration. A systematic study of the seeds indicated that they produced toxic effects in mice.

Objectives of Acute Toxicity Studies

The objectives are to:

1. Determine the toxicity level and LD_{50} value (the dose that kills 50% of the test animals).

2. Identify target organs affected by the toxicant.

3. Provide data for classification and labeling of chemicals or supplements for safety regulation.

4. Supply baseline information for designing repeated-dose (subacute/subchronic) studies.

5. Evaluate early signs of toxicity, such as behavioral, neurological, or biochemical changes.

Design and Methodology

Acute toxicity studies are typically conducted using laboratory animals such as Wistar rats or mice, under controlled laboratory conditions.

a. Test Guidelines

There are standard international methods used, including:

OECD Test Guideline 420: Acute oral toxicities. Fixed Dose Procedure.

OECD Test Guideline 423: Acute Toxic Class Method.

OECD guideline recommends giving a single dose of the test substance orally, dermally, or via inhalation, then observing the animals for 14 days for signs of toxicity or death.

b. Parameters Observed

Mortality (number of deaths)

Behavioral changes (e.g., tremors, salivation, lethargy)

Body weight and food intake.

Physical signs (fur, eyes, mucous membranes)

Post-mortem (necropsy) findings on vital organs such as liver, kidney, heart, and lung.

Dose Levels

If no mortality occurs at a specific dose (e.g., 2000 mg/kg), that dose is considered a "limit dose.

Importance of Acute Toxicity Studies

1. Safety Evaluation: Determines whether a compound is safe for human or

2. Guidance for Further Testing: Provides safe dose limits for subacute, subchronic, or chronic toxicity studies.

3. Regulatory Requirement: Required by agencies like OECD, WHO, and FDA for the approval of new drugs or herbal products.

4. Risk Assessment: Helps classify chemicals based on the Globally Harmonized System (GHS) of chemical classification.

Limitations

Only detects short-term or immediate effects — not long-term toxicity.

May not reveal cumulative, reproductive, or carcinogenic effects.

Ethical concerns exist because animal death is often an endpoint, though modern guidelines minimize animal use and suffering.

2.2.2 SUB-CHRONIC TOXICITES

Sub-chronic toxicity studies form a critical component of preclinical toxicological evaluation, designed to assess the health effects of repeated exposure to a substance over an intermediate duration, typically 90 days (OECD, 2018).These studies act as a bridge between subacute (28 day) and chronic (6–24-month) studies, helping to identify potential cumulative or delayed toxic effects before long-term exposure.Thus, sub-chronic toxicity describes the potential harmful effects of repeated daily dosing of a chemical, herbal product, or drug for about 90 days.The purpose is to determine the No-Observed-Adverse-Effect Level (NOAEL) — the highest dose at which no harmful effect is observed — and to provide data for risk assessment and dose selection in chronic or clinical studies. Yuet et al.,(2013) did a subchronic and acute toxicity study of euphorbia hirta L.methanol extract in rats. Patel et al.,2008 also did an acute and subchronic toxicity studies on the safety assessment of pomegranate fruit extract. Based on the results of this study, the no observed-adverse-effect level (NOAEL) for this standardized pomegranate fruit extract was determined as 600mg/kg body weight/day, the highest dose tested.

Purpose and Objectives

Sub-chronic studies are performed to:

1. Evaluate cumulative effects after prolonged exposure.

2. Identify target organs susceptible to toxic injury.

3. Determine the reversibility or progression of toxic effects.

4. Establish a NOAEL for regulatory and clinical safety decisions.

5. Provide data for risk assessment in humans and environmental safety.

Experimental Design

a. Test Guideline

The standard international procedure is the OECD Test Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD, 2018).

b. Species

The Wistar rat is the most frequently used experimental model because of its stable physiology, reproducibility, and sensitivity to toxic agents.

c. Study Duration

Exposure lasts 90 consecutive days, with daily oral administration (usually by gavage).

d. Dose Selection

Control group (no treatment)

Low, medium, and high-dose groups

Doses are based on findings from the acute and subacute toxicity tests.

e. Parameters Observed

1. Behavioral and physical signs – activity, posture, fur condition.

2. Body weight and food/water intake.

3. Hematology – RBC, WBC, hemoglobin, platelets.

4. Serum biochemistry – liver (ALT, AST, ALP), kidney (urea, creatinine), and lipid profile (cholesterol, triglycerides, HDL, LDL).

5. Organ weights and histopathology of liver, kidney, heart, spleen, and brain.

Significance

Sub-chronic studies are vital for:

Detecting organ-specific toxicity, particularly in the liver and kidneys, which metabolize most xenobiotics.

Understanding adaptive versus irreversible effects of a compound.

Supporting regulatory safety documentation for new products.

Predicting long-term risk before chronic exposure.

Limitations

Costly and time-consuming (about 3 months).

Species differences may limit direct extrapolation to humans.

Some delayed or cumulative effects may only appear in chronic studies.

2.2.3 CHRONIC TOXICITES

Chronic toxicity studies are long-term investigations designed to evaluate the adverse health effects of a chemical, drug, or herbal product after continuous or repeated exposure over a significant portion of the test animal's lifespan (OECD, 2018).They represent the highest level of preclinical toxicological assessment — following acute, subacute, and sub-chronic studies — and are essential for predicting long-term safety in humans.While acute and subacute studies determine short-term toxicity, chronic studies assess the cumulative, irreversible, and carcinogenic potential of substances. Sireeratawong et al.,2013 did an acute and chronic toxicity studies of the water extract from dried fruits of terminalia bellerica (gaertn.) roxb. in spargue-dawley rats. The study of chronic toxicity was determined by oral feeding both female and male rats (10 female, 10 male) daily with the test substance at the dose of 300, 600 and 1,200 mg/kg body weight continuously for 270 days. The examinations of signs of toxicity showed no abnormalities in the test groups compared to the controls. Also Afolabi et al.,2012 also did a 90 day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats.

Duration of Study

The duration depends on the test species:

Rats or mice: 6–24 months (often 12 months minimum)

Dogs or primates: Up to 12 months

Exposure route: Oral (by gavage or diet), inhalation, or dermal.

Objectives of Chronic Toxicity Studies

1. Determine the No-Observed-Adverse-Effect Level (NOAEL) for long-term use.

2. Identify cumulative or delayed toxic effects on organs such as the liver, kidney, heart, and spleen.

3. Detect carcinogenic, mutagenic, or teratogenic potential.

4. Assess metabolic adaptation and reversibility of damage after prolonged exposure.

5. Provide data for human risk assessment and regulatory classification.

Study Design and Methodology

a. Test Guideline :The most widely accepted protocol is OECD Test Guideline 452: Chronic Toxicity Studies (OECD, 2018).

b. Test Organisms : Rodents such as Wistar rats and Swiss mice are the preferred models due to their well-characterized metabolism and sensitivity to toxic agents.

c. Dosage and Grouping;

Control group (no treatment).

Three or more treatment groups with increasing doses.

Daily administration (oral, dermal, or inhalation) for 6–12 months.

d. Parameters Evaluated;

1. Clinical observations – signs of distress, mobility, behavior, fur texture.

2. Body weight and feed consumption.

3. Hematological and biochemical indices (ALT, AST, ALP, urea, creatinine, lipid profile).

4. Organ weights and histopathology of vital organs (liver, kidney, lungs, spleen, heart, reproductive organs).

5. Mortality and tumor incidence (in carcinogenicity studies).

Importance of Chronic Toxicity Studies

Establish safety limits for long-term human exposure.

Detect late-onset toxicities not seen in shorter studies.

Evaluate potential tumorigenic or carcinogenic risks.

Required for regulatory approval of pharmaceuticals, food additives, and herbal supplements.

Helps in predicting human health risks associated with chronic environmental exposure.

Limitations

Very time-consuming and expensive.

Ethical constraints due to the long exposure and animal welfare issues.

Species differences may limit extrapolation to humans.

Not suitable for every compound, especially those with short-term intended use.

2.2.4 SUBACUTE TOXICITES

Subacute toxicity refers to the adverse biological effects arising from repeated exposure to a chemical or natural compound for a short duration, usually spanning 2–4 weeks. The aim is to identify the target organs of toxicity, the dose–response relationship, and to determine the No-Observed-Adverse-Effect Level (NOAEL). These findings guide the selection of doses for subchronic or chronic studies and provide data for regulatory risk assessment. Wang et al.,2019 did an acute and subacute toxicity of oxyclozanide in wistar rats they observed that during the 28-days time period, no obvious adverse effects or death were detected. Histopathological changes were observed in the heart, liver, and kidney of animals treated with high dose of oxyclozanide**.**

OECD Test Guideline 407 (28-Day Repeated-Dose Study)

The Organisation for Economic Co-operation and Development (OECD) developed Guideline 407, which outlines the procedures for conducting subacute oral toxicity studies in rodents. According to this protocol, animals are exposed daily to the test substance at three or more dose levels plus a control for 28 consecutive days. Key endpoints include clinical signs, body and organ weights, hematology, clinical biochemistry, and histopathology of major organs such as the liver, kidney, heart, and spleen (OECD, 2008). The liver is particularly emphasized because of its central role in xenobiotic metabolism, and its enzymes — alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) — are sensitive indicators of hepatocellular damage (Lala et al., 2023).

Importance of Subacute Toxicity Studies

Subacute toxicity studies are vital for several reasons:

They provide early warning signals of systemic toxicity before irreversible damage occurs.

They help identify safe dose ranges and potential target organs affected by the compound.

They assist in predicting human safety margins by extrapolating from animal data.

They support regulatory submissions for food supplements, herbal drugs, and pharmaceuticals, especially where chronic data are unavailable (OECD, 2008; Thakur et al., 2024).

In herbal and nutraceutical research, subacute studies help verify whether claimed health benefits (e.g., antioxidant or hepatoprotective effects) are accompanied by hidden toxicities when administered repeatedly.

Parameters Assessed in Subacute Toxicity Studies

The assessment includes biochemical, hematological, and histopathological parameters.,

Biochemical tests: Evaluate liver (ALT, AST, ALP, bilirubin) and kidney (urea, creatinine) functions (Lala et al., 2023).

Lipid profile: Alterations in total cholesterol, triglycerides, HDL-C, and LDL-C may indicate disturbances in lipid metabolism or hepatic dysfunction (Thakur et al., 2024).

Histopathology: Examines tissue architecture for inflammation, necrosis, steatosis, or fibrosis.

Hematology: Includes red and white blood cell counts, hemoglobin, and platelet indices, useful for detecting anemia or immune modulation.

Subacute Toxicity Studies in Herbal and Nutraceutical Research

Natural products are increasingly marketed as safe, yet many contain bioactive phytochemicals capable of causing hepatotoxicity or nephrotoxicity at high or prolonged doses. Subacute studies using Wistar rats have been applied to evaluate the safety of various plant extracts and supplements. Figueredo et al.,2018 did an acute and subacute oral toxicity showing the safety assessment of Morus nigra L. the results from the subacute toxicity there was a decrease in AST in males (750 and 1000 mg/kg) and females (1000 mg/kg), reduction of total cholesterol in females (750 and 1000 mg/kg), and increase in renal and hepatic change the LPO levels.

Han et al.,(2015) also reported on the Acute and 28-Day Subacute Toxicity Studies of Hexane Extracts of the Roots of Lithospermum erythrorhizon in Sprague-Dawley rats and the results showed that In the subacute toxicity study, LEH was administered orally to male and female rats for 28 days at dose levels of 25, 100, and 400 mg/kg/day. There was no LEH-related toxic effect in the body weight, food consumption, ophthalmology, hematology, clinical chemistry and organ weights. Benrahou et .,(2022) did a subacute toxicities study of Erodium guttatum Extracts by Oral Administration in Rodents. The subacute toxicity study of E. guttatum extracts showed no significant changes in body or organ weights. Zhang et al.,2025 did a subacute toxicities studies as well as safety pharmacology in beagle dogs of total lignans from Arctii Fructus and it was reported however that repeated oral administration of TLAF (540 mg/kg) to beagle dogs for 28 days can

cause 50% of administered animals to die. The toxic reactions are mainly seen in the digestive system, heart, liver, and kidneys. Kharchoufa et al., 2020 did a subacute toxicity of Haloxylon scoparium Pomel extract at doses 500, 1000, and 2000 mg/kg did not produce any observable symptoms of toxicity and no significant variation in body weight, organ weights, food, and water consumption or mortality in all treated rats. It was reported by Hanif et al.,2025 that the 28 day administration of molybdenum disulfide nanoflowers in rats resulted in increased levels of ALT and AST, decreased levels of CAT, SOD and GSH and increased MDA and urea levels.

2.3 LIVER

The liver is the largest internal organ in mammals, constituting about 2–5% of total body weight in adult rats and approximately 1.5 kg in adult humans (Trefts et al., 2017). It is a highly vascularized organ, situated in the upper right quadrant of the abdomen, just below the diaphragm, and enclosed by a fibrous capsule known as Glisson's capsule. The liver is divided into two major lobes — right and left — in humans, while in Wistar rats, it typically consists of four lobes: median, left lateral, right lateral, and caudate lobes (Marieb & Hoehn, 2019).Histologically, the liver is composed of numerous hexagonal structural and functional units called lobules. Each lobule contains hepatic cords of hepatocytes radiating from a central vein (Trefts et al., 2017). The hepatocytes are arranged around sinusoids, which are specialized capillaries lined by Kupffer cells (resident macrophages) and endothelial cells. The portal triad—comprising a branch of the hepatic artery, portal vein, and bile duct—is located at each corner of the lobule. Blood from the hepatic artery (oxygen-rich) and portal vein (nutrient-rich) flows through the sinusoids toward the central vein, where it drains into the hepatic vein and subsequently into the inferior vena cava.

2.3.1 Functions of the Liver

The liver performs a wide range of metabolic, synthetic, detoxifying, and excretory functions, making it central to homeostasis. Key functions include:

a. Metabolic Functions ; The liver plays a central role in carbohydrate, lipid, and protein metabolism.

Carbohydrate metabolism: It regulates blood glucose through glycogenesis, glycogenolysis, and gluconeogenesis.

Lipid metabolism: It synthesizes cholesterol, phospholipids, and lipoproteins, and facilitates fatty acid oxidation for energy.

Protein metabolism: The liver synthesizes albumin, clotting factors, and other plasma proteins, while also converting toxic ammonia to urea via the urea cycle (Lala et al., 2023).

b. Detoxification and Biotransformation ; One of the liver's most vital functions is detoxification of endogenous and exogenous substances. It metabolizes drugs, alcohol, and xenobiotics through phase I (oxidation, reduction, hydrolysis) and phase II (conjugation) reactions, primarily via the cytochrome P450 enzyme system . This detoxification ensures harmful compounds are converted into less toxic, water-soluble metabolites that can be excreted via urine or bile

c. Bile Production and Excretion ; The liver continuously secretes bile, which is stored in the gallbladder and released into the duodenum. Bile contains bile acids, cholesterol, phospholipids, and bilirubin, playing essential roles in fat digestion and absorption of fat-soluble vitamins (A, D, E, K). Bile also serves as an excretory route for bilirubin, the end-product of hemoglobin breakdown.

d. Storage Function; The liver acts as a storage depot for several vital substances, including glycogen, vitamins (A, D, B12, K), iron (in ferritin form), and copper. These stores are mobilized according to the body's metabolic needs (Marieb & Hoehn, 2019).

e. Hematological Functions; During fetal development, the liver serves as a major site of hematopoiesis (blood cell production). In adults, it contributes to iron recycling from hemoglobin degradation and synthesis of clotting factors such as fibrinogen, prothrombin, and factors V, VII, IX, and X (Lala et al., 2023).

f. Immunological Function; The liver also acts as an immunological organ, housing numerous Kupffer cells that play a role in the mononuclear phagocyte system. These cells filter bacteria, endotoxins, and debris from the portal blood, contributing to innate immune defense (Trefts et al.,2017).

2.3.2 Liver Function Tests (LFTs)

Biochemical assessment of liver function is commonly used in toxicological studies to evaluate hepatocellular integrity and functional capacity. Key serum enzymes include:

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST): Indicators of hepatocellular injury.

Alkaline phosphatase (ALP): Elevated levels suggest cholestasis or biliary obstruction.

Total protein, albumin, and bilirubin: Reflect synthetic and excretory functions of the liver (Lala et al., 2023).

Alterations in these parameters are often the first indication of hepatic damage in subacute and chronic toxicity studies, such as those involving herbal or nutraceutical formulations.

2.3.3 The Liver in Toxicology

Because the liver is the primary site of xenobiotic metabolism, it is also the major target organ for drug- or chemical-induced toxicity. Prolonged exposure to toxicants may result in fatty liver (steatosis), hepatocellular necrosis, fibrosis, or cirrhosis. Therefore, assessment of hepatic histopathology, enzyme activity, and lipid metabolism is a cornerstone in subacute and chronic toxicity evaluations in experimental models like male Wistar rats.

2.4 Lipid Profile

Lipids are a diverse group of hydrophobic or amphipathic molecules that play vital roles in energy storage, membrane structure, and cellular signaling. The term “lipid profile” refers to the quantitative measurement of key serum lipids, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The lipid profile serves as a biochemical indicator of lipid metabolism and cardiovascular risk, but it is also an important biomarker in toxicological and pharmacological studies, especially those involving hepatotoxic or metabolic disturbances.

2.4.1 Structure and Classification of Lipids

Lipids are classified into major groups based on their structure and biological role. These include:

1. Simple lipids (e.g., triglycerides, waxes) — esters of fatty acids with alcohols.

2. Compound lipids (e.g., phospholipids, glycolipids, lipoproteins) — contain additional groups such as phosphate or carbohydrate moieties.

3. Derived lipids (e.g., sterols such as cholesterol, and fat-soluble vitamins).

Cholesterol is an essential sterol lipid synthesized primarily in the liver and intestines. It is a key component of cellular membranes and a precursor for bile acids, steroid hormones, and vitamin D

2.4.2 Lipid Metabolism and Transport

Because lipids are insoluble in water, they are transported in the bloodstream as lipoprotein complexes. These are spherical structures composed of a lipid core (triglycerides and cholesterol esters) surrounded by phospholipids, free cholesterol, and apolipoproteins (Tall & Yvan-Charvet, 2015). The major lipoproteins are:

Chylomicrons: Transport dietary triglycerides from the intestines to tissues.

Very low-density lipoproteins (VLDL): Carry endogenous triglycerides synthesized in the liver.

Low-density lipoproteins (LDL): Deliver cholesterol to peripheral tissues ("bad cholesterol").

High-density lipoproteins (HDL): Remove excess cholesterol from tissues and return it to the liver ("good cholesterol").

The liver regulates lipid homeostasis by synthesizing, packaging, and excreting lipids, and disturbances in its function often manifest as altered serum lipid profiles (Trefts et al., 2017).

2.4.3 Lipid Profile Parameters and Their Physiological Roles

1. Total Cholesterol (TC): Represents the sum of all cholesterol fractions in serum. It is essential for membrane stability and steroid hormone synthesis but becomes pathological when elevated.

2. Triglycerides (TG): Serve as the primary energy reserve, stored in adipose tissue and mobilized during fasting. Elevated TG levels can indicate impaired lipid metabolism or hepatic dysfunction.

3. HDL-C: Plays a protective role by facilitating reverse cholesterol transport from peripheral tissues to the liver for excretion. High HDL-C levels are associated with reduced cardiovascular risk (Tall & Yvan-Charvet, 2015).

4. LDL-C: Transports cholesterol to peripheral tissues. Elevated LDL-C contributes to atherogenesis and is often linked with hepatic lipid metabolism disturbances and oxidative stress.

2.4.4 Lipid Profile in Toxicological Studies

In toxicology, the lipid profile serves as an indicator of metabolic and hepatic integrity. Substances that disrupt liver function or lipid metabolism may cause:

Hyperlipidemia: Increased TC, TG, and LDL-C due to impaired lipid clearance or enhanced synthesis.

Hypolipidemia: Reduced lipid levels resulting from hepatocellular injury, defective synthesis, or increased oxidation.

For example, hepatotoxins such as carbon tetrachloride or certain herbal extracts can cause oxidative stress and peroxidation of lipid membranes, leading to altered lipid homeostasis (Thakur et al., 2024). Conversely, antioxidant-rich compounds may restore lipid balance by protecting hepatocytes and normalizing lipid synthesis and export pathways.Changes in lipid profile therefore provide indirect evidence of hepatic or systemic toxicity in subacute and chronic studies. When combined with liver enzyme assays (ALT, AST, ALP), lipid analysis offers a comprehensive

understanding of how a test substance such as Cell Life IQ influences both hepatic integrity and metabolic function.

2.4.5 Mechanistic Link Between Liver Function and Lipid Profile

The liver plays a central role in maintaining lipid equilibrium through β-oxidation, lipoprotein synthesis, and cholesterol metabolism. Toxic injury to hepatocytes impairs these pathways, resulting in:

Accumulation of fat in hepatocytes (steatosis).

Increased release of VLDL and LDL into circulation.

Reduced synthesis of HDL (Trefts et al., 2017).

Hence, evaluating both liver enzyme activities and lipid profiles in subacute toxicity studies provides a more holistic picture of toxicant-induced metabolic disruption.

2.5 Overview Of Cell Life IQ

What is CellifeIQ? CellifeIQ is an advanced dietary supplement with a blend of 30 unique ingredients including minerals, fruits, vegetables and herbs all backed by rigorous scientific approvals. CellifeIQ does not have side effects. It's organically produced with natural materials like fruits, herbs and vegetables. Anyone above 18 years whether there is a health challenge or not can take CellifeIQ. However, this product is very effective for diseases such as cancer, ulcers, diabetes, stroke, arthritis, skin infection, dementia, Parkinson's disease and other brain disorders, heart and eye problems. CellifeIQ is more than an immune booster. It helps to enhance the immune

system at the same generally work to enhance the body's optimum function. The powerful 30 ingredients in CellifeIQ help restore youthfulness, thereby helping to fight against diseases connected to aging.

2.5.1 How does CellifeIQ work?

The supplement raises the level of Glutathione and Superoxide Dismutase in the body system. Glutathione is known as the mother of all antioxidants. It detoxifies and protects the cells and enables optimal function of the body. While Superoxide dismutase (SOD) is an enzyme found in all living cells. Superoxide dismutase helps break down potentially harmful oxygen molecules in cells. This prevents cellular and tissue damage. When glutathione increases in the cells then the miracle happens to the body, the body will naturally begin to heal itself of every disease, and a healthy cell is a healthy life. Hence, the supplement is not responsible for the healing, rather the supplement increases glutathione which is responsible for the healing of all kinds of diseases in the body. However, CellifeIQ is a product of Upward International (a United States Health Company). CellifeIQ is an advancement of RiboCeine Technology which makes it more efficient(tested and trusted) as it is made with 30 unique ingredients including Glutathione that is master of all antioxidants.

2.5.2 Who should take CellifeIQ?

Everyone can benefit from supplementing with CellifeIQ. Even if you do not suffer from any known health problems or chronic illness, you are still aging like everyone else. CellifeIQ can help to slow down your aging process and keep you healthy and younger looking.CellifeIQ will be especially beneficial to you if you have health problems such as: Arthritis, High Blood Pressure, Diabetes, Glaucoma, Prostate issues, Fibromyalgia, Brain defect, Hormone Over-expression/

Under-expression, Hepatitis and other liver diseases, Back Pain, Diabetes, Kidney Disease, Sickle Cell Disease and over 60 other diseases regain their quality of life. If you are also an athlete, or play any kind of vigorous sports, it will enhance your performance.

2.5.3 Some Key Benefits of CellifeIQ

1. Combating Tumors: CellifeIQ possesses Cordyceps mushroom which has anti-tumor properties, offering potential support in maintaining overall health.

2. Blood Purification: CellifeIQ contains S-Acety Glutathione and N-Acetyl-L-cysteine which support the natural production of Glutathione. These ingredients have the ability to clean/detoxify the body system. It also helps purify the blood, promoting healthy circulation.

3. Reduced Inflammation: Say goodbye to chronic inflammation with the potential anti-inflammatory effects of CellifeIQ. Pomegranate fruit present in CellifeIQ is known for its antioxidants properties that supports heart health and reduce inflammation.

4. Cardiovascular Support: CellifeIQ possesses grape seed extract which has powerful antioxidants properties that offer support for a healthy cardiovascular system, promoting overall heart health.

5. Liver Protection: Shield your liver from harmful toxins with the potential protective properties of CellifeIQ. Milk thistle seed extract has the full ability to naturally enhance liver function.

6. Alleviating Diarrhea: Experience relief from occasional diarrhea with the potential benefits of CellifeIQ. The antioxidants effect of Glutathione is capable of treating constipation and colon issues.

7. Combating Bacterial Effects: CellifeIQ has loads of antimicrobial properties, offering strong potential support for your immune system against infections.

8. Neurological Support: Experience potential alleviation of symptoms associated with neurological diseases with CellifeIQ. With vitamin B12 and other ingredients that supports blood formation, brain and other nervous system functions.

9. Wound healing: Experience fast wound healing with CellifeIQ. The supplement contains zinc and manganese minerals necessary for bone formation and wound healing.

10. Anti-aging effect: Grow up and experience a youthful vitality with CellifeIQ. Always appear looking radiant with glowing skin with the help of vitamin complex in CellifeIQ.

2.5.4 Constituents Of Cell Life IQ

L-Glutathione.

N-acetyl-L- cystine.

Organic aloe vera leaf powder.

Alpha lipoic acid.

Horse radish (moringa) oleifera (leaf) extract 10:1.

Turmeric root extract.

Melon fruit concentrate.

Irish moss whole plant powder.

Noni fruit powder.

N, N dimethylglycine HCL.

Cordeceps.

Mushroom whole plant powder.

Quercetin Dihydrate.

Milk thistle seed powder.

Blue berry fruit powder.

Schisandra berry powder.

Grape seed extract.

Pomegranate fruit hull extract.

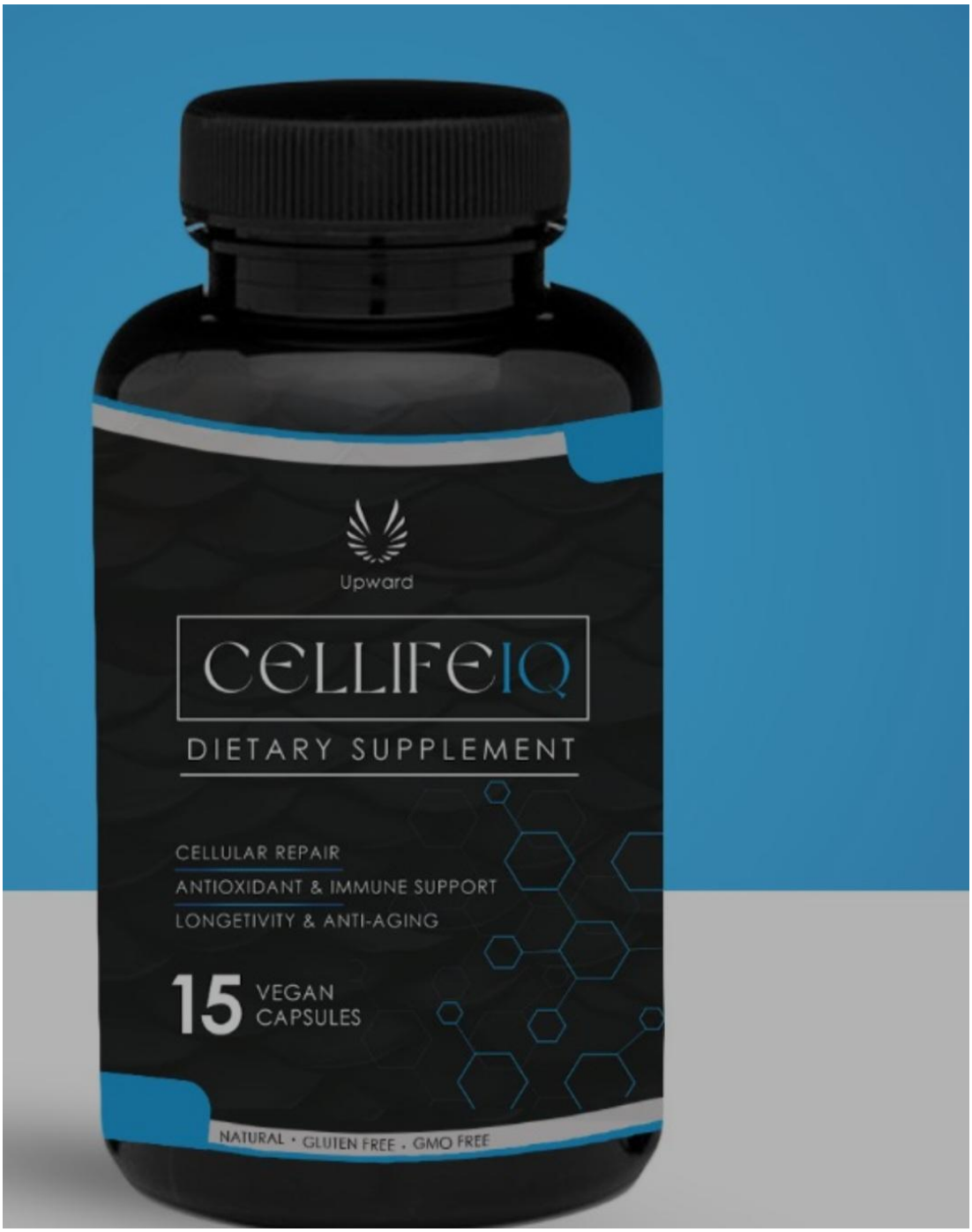
Black pepper fruit extract (Bioperine).

(S-acetyl)-L- Glutathione (Empthione).

L-glutamine.

Sulphoraphane(From broccoli sprout extract).

Other ingredients include; Gelatin(capsule), dicalcium phosphate,rice flour and silicon dioxide.



Source; upwardints.com**.** **fig 2.55 image of Cell Life IQ**

CHAPTER THREE

MATERIALS

3.1.1 Test Substance

CellifeIQ an advanced dietary supplement with a blend of 30 unique ingredients including minerals, fruits, vegetables and herbs all backed by rigorous scientific approvals. It was purchased from a local distributor in lagos state, Nigeria

3.1.2. APPARATUS AND EQUIPMENTS

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

Apparatus and Equipment.	Producer/maker
Beakers (50, 150 and 250ml)	Pyrex (England)
Retort Stand	
Tripod Stand, Bunsen Burner and Gas Supply	
Pippettes (1,10 and 25ml)	Pyrex (England)
Automated micropippette (0-100 µl, 0-1000µl)	Micropet and Accumax PRO

Conical flasks

flasks .

Filter paper (0.45µm and 125mm) Whatman (England)

Cuvettes Pyrex (England)

Needles and syringes (1ml, 2ml, 5ml, 10ml)

Paper tapes, cardboard papers and pins

Cotton wool and Methylated spirit

Animal cages UNIBEN MEDBCH Dept. (Nigeria)

Oro-gastric Gavage UNIBEN MEDBCH Dept. (Nigeria)

Stop watch

Test tube racks and test tubes MEDBCH Dept. (Nigeria)

Volumetric flasks (100, 250 and 500ml) Technics (England)

HH-W Constant Temperature Water Bath Bran Sc. Inst. Company, England.

Analytical weighing balance. Metter H-80 (Germany)

Water distiller. Bran Sc. Inst. Company, England.

Simple Weighing Balance Adventurer OHAUS AR1530

T70UV/VISSpectrophotometer PG Instruments Ltd., UK.

microplate reader PG Instruments Ltd., UK.

Refrigerator Citizen PRC424**6**

80-2 model Electric Centrifuge. B.Bran Scientific and. Instrument Company, England

3.1.3 Reagents

Reagent/Enzyme kits and other reagentsused were of standard quality and were purchased from qualified/accredited dealers/suppliers or their manufacturers' representative in Nigeria. The Chemicals used were of analytical grade and an accredited dealer - Pyrex Laboratories, Benin, Nigeria they include;

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

2. Distilled water [Trigas, UNIBEN].

3. Formalin [BDH, USA].

4. Methylated spirit [Ivee Pharmaceutical Limited].

5.Iodine.

6.Reagents/chemicals used for the assessment of liver function status;

a. Total protein kit (RandoxLab.UK; Purchased from manufacturer's representative in Nigeria). The kit contains the following reagents:

- R1: Biuret reagent: Sodium hydroxide (100mmol/l); Na-k-tartrate (16mmol/l); Potassium iodine (15mmol/l); Cupric sulphate (6mmol/l).

- R2: Blank reagent: Sodium hydroxide (100mmol/l); Na-K-tartrate (16mmol/l);
- Protein standard: TP- Standard (59.80g/l or 5.98g/dl)

b. Bilirubin kit (Randox Lab.UK; Purchased from manufacturers representative in Nigeria). The kit contains the following reagents: • RI. Sulphanilic acid (29mmol/l); Hydrochloric acid (0.17N)• R2. Sodium Nitrite (38.5mmol/l)• R3. Caffeine (0.26mol/l); Sodium benzoate (0.52mol/l)• R4. Tartrate (0.93mol/l); Sodium hydroxide 1.9N

c. Aspartate aminotransferase (AST) kit: (RandoxLab.UK;Purchased from manufacturers representative in Nigeria). The kit contains the following reagents:

- R1: Buffer: Phosphate buffer (100mmol/l, pH7); L-aspartate (100mmol/l); α-oxoglutarate (2mmol/l)
- R2: 2,4-dinitrophenlhydrazine (2mmol/l).

d. Alanine aminotransferase (ALT) kit: (RandoxLab.UK;Purchased from manufacturers representative in Nigeria). The kit contains the following reagents: •

R1. Buffer: Phosphate buffer (100mmol/l, pH7.4); L-alanine (200mmol/l); α-oxoglutarate (2.0mmol/l)• R2.2,4 – dinitrophenylhydrazine (2.0mmol/l).

e. Alkaline phosphatase (ALP) kit: (RandoxLab.UK;Purchased from manufacturers representative in Nigeria). The kit contains the following reagents:

- RIa. Buffer: Diethanolamine buffer (1mol/l; pH9.8); MgCl2 (0.5mmol/l).
- R1b. Substrate: p-nitrophenylphosphate(10mmol/l).

7. Reagents/chemicals used for the assessment of lipid profile

a. Total cholesterol: The total cholesterol level is determined using the (Randox Lab.UK; purchased from manufacturer's representative in Nigeria). The kit contains the following reagents:Cholesterol standard{(5.04mmol/l or 195mg/dl)} and SolutionR1-4-aminoantipyrine (0.30mm/l), Phenol (6mmol/l), Peroxidase (≥0.5IU), Cholesterol esterase (≥0.15IU), Cholesterol oxidase (≥0.1IU),Pipes buffer (80mmol/l pH 6.8).

b. HDL-cholesterol:

The HDL-cholesterol level is determined using the(RandoxLab.UK; purchased from manufacturer representative in Nigeria).The kit contains the following Reagents:

HDL-cholesterol standard (5.04mmol/l or 195mg/dl); Precipitant R1.Phosphotungstic acid (0.55mmol/l),Magnesium chloride (25mmol/l); CHOL Reagent.

c. Triglyceride:

The triglyceride level is determined using the(RandoxLab.UK;Purchased from manufacturers representative in Nigeria). The kit contains the following reagents.

Standard (2.21 mmol/l or 196mg/dl); R1a- Buffer(Pipes Buffer 40mm/l, pH 7.6; 4-chlorophenol 5.5mmol/l; magnesium ions 17.5mm/l); R1b-Enzyme reagent (4-aminophenazone 0.5mm/l; ATP 1.0; Lipases ≥ 150IU; Glycerol kinase≥0.4IU; Glycerol-3-phosphate oxidase ≥1.5IU; Peroxidase ≥0.5IU).

3.2. Methods

Sub acute toxicities

3.2.1 Experimental methods

20 Male Wistar rats weighing an average of 90g were obtained from the department of Anatomy, Faculty Of Basic Medical Sciences,University Of Benin,Benin-City,Edo State. The animals were placed under controlled conditions and 12-hours light- dark cycles. The animals were acclimatised for 2 weeks before commencement of study. The rats were put in cages and they had free access to water and feed. The rats were fed with pellets and water daily for about 28 days of these study. The rats were divided into four groups. Three cages had different doses of the drug and one cage was for the control. The animals were stained with iodine for easy identification. They were stained in the regions of their body like their head, hand ,leg,tail,abdomen. The cages were in an enclosed environment (animal house) and they were disinfected daily.

3.2.2. Experimental design

20 male wistar rats were arranged into four different with about five rats per cage.

Group 1: Control group they were given only distilled water.

Group 2: low dose (20mg/kg of Cell Life iq).

Group 3: Medium dose (80mg/kg of Cell Life Iq).

Group 4: High dose(600mg/kg of Cell Life iq).

3.2.3 Observation And Collection Of Data

The rats were observed twice daily for mortality and morbidity signs. Morning after administration and evening also. They were observed daily for toxicity signs like itching, dizziness, change of colour of the fur, overactiveness. The feed and water was measured daily before giving it to the rats and also their left overs was also measured. The weights of the rats was measured weekly.

3.2.4. Blood Collection And Biochemical Analyses

On the 29th day, blood samples was collected under the use of anaesthetic (chloroform) on the rats.The blood sample were taken through cardiac puncture and put into heparin bottles and plain bottles for biochemical analyses. Clinical biochemistry parameters measured included:

- Liver function: The liver biomarkers that are analyzed are

1. Alkaline phosphatase

2. Aspartate aminotransferase

3. Alanine aminotransferase

4.Total bilirubin

5. Direct Bilirubin

6. Total protein

- Lipid profile.

1. Cholesterol

2. Triglyceride

3. Very-low-density lipoprotein

4. High- density lipoprotein

5. Low-density lipoprotein

3.2.5. Assessment of the lipid profile

Determination of Plasma Total Cholesterol (Trinder, 1969).

Assay Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Trinder, 1969).

Cholesterol ester+H_20 Cholesterol Cholesterol + Fatty Acids

esterase

Cholesterol + O2 cholesterol Cholestene – 3-one+H2O2

oxidase

2H2O2+ phenol + 4-aminoantipyrine peroxidase

Quinoneimine + 4H2O

Procedure:

The following were pipetted into curvette:

	Reagent blank (µl)	Standard (µl)	Sample (µl)
Distilled H20	10µl	-----	--------
Standard.	----	10µl	--------
Sample	-----	------	10µl
Reagent	1000µl	1000µl	1000µl

The solution was mixed and incubated for 5 minutes at 370c. The absorbance of the sample (Asample) was measured against the reagent blank within 60 minutes.

Calculation

Conc. Of Cholesterol in sample

$$= \frac{\text{Asample}}{\text{Astandard}} \times \text{Conc. of standard (mg/dl)}$$

Determination of Plasma HDL-Cholesterol (Trinder, 1969; Tietz, 1990).

Assay Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined (Tietz, 1990).

Procedure:

1. Precipitation

The following were pipetted into centrifuge tubes;

Sample/standard	500µl
Diluted precipitant (R1)	1000µl

The solution was mixed and allowed to sit for 10 minutes at room temperature, after which it was centrifuged for 10 minutes at 4,000rpm. The clear supernatant was separated off within two hours and the cholesterol content was determined by the CHOD-PAP method.

2. Cholesterol CHOD-PAP Assay

The following were pipetted into test tube;

	Reagent blank	Standard	Sample
Distilled H20	100µl	------	------
Standard Supernatant	------	100µl	-------
Sample Supernatant	-------	-------	100µl
CHOL reagent	1000µl	1000µl	1000µl

The solutions are mixed for 5 minutes at 370c. The absorbance of the sample (Asample) and standard (Astandard) was measured against the reagent blank within 60 minutes at a wavelength of 500nm.

Calculation:

1. HDL Cholesterol

Concentration of HDL cholesterol in supernatant

= A sample x Concentration of Standard

Astandard

Determination of Plasma Triglycerides (Trinder, 1969; Tietz, 1990).

Principle:

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase

Triglycerides + H2O -lipases------->glycerol + fatty acids

Glycerol + ATP glycerol -3- phosphate + ADP

Glycerol -3- phosphate + 02 dihydroxyacetone + phosphate + H_2O2

2H2O2 + 4 – aminophenazone + 4 chlorophenol

quinoneimine + HCL + $4H_2O$

Note: to correct for free glycerol, subtract 0.11mmol/l (10mg/dl) from the triglyceride value obtained.

Procedure:

The following were pipetted into test tubes:

	Reagent Blank	Standard	Sample
Standard	--------	10µl	------
Sample	--------	------	10µl
Reagent R1	1000µl	1000µl	1000µl

Solution was mixed and incubated for 5 minutes at 370c, and the absorbance of the sample (A sample) and standard (A standard) was measured against the reagent blank within 60 minutes at wavelength of 500nm/546nm.

Calculation:

Triglyceride concentration= Asamplex Standard Conc.

(mmol/l or mg/dl) Astandard

Determination of serum LDL-cholesterol (Trinder, 1969; Tietz, 1990).

LDL-cholesterol is calculated using the Frieldewald equation as follows:

In mmol/l:

LDL = Total - Triglycerides - HDL

Cholesterol Cholesterol 2.2 Cholesterol

In mg/dl:

LDL = Total - Triglycerides - HDL

Cholesterol Cholesterol 5 Cholesterol

Determination of serum VLDL-cholesterol (Frieldewald et al., 1972).

VLDL-cholesterol will be calculated using the Frieldewald's formula:

In mmol/l:

$$\text{VLDL- cholesterol} = \frac{\text{Triglycerides}}{2.2}$$

In mg/dl:

$$\text{VLDL- cholesterol} = \frac{\text{Triglycerides}}{5}$$

3.2.6 Assessment of liver function status

a. Determination of plasma total protein: Randox lab. kit, UK, was used (Tietz, 1995).

Principle:

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex which will be measured spectrophotometrically at a wavelength of 546nm (Tietz, 1995). The intensity of the coloured complex formed is proportional to the concentration of total protein in the sample" (Tietz, 1995);

The concentration of total protein is then calculated using the formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \text{Concentration of standard (g/l)}$$

Procedure:

Three cuvettes were labelled standard, sample and blank respectively. 0.02ml of the standard solution (CAL) was pipetted into the cuvette labelled standard, 0.02ml of the sample plasma solution was also pipetted into the cuvette labelled sample, while 0.02ml of distilled water was put in the cuvette labelled reagent blank. 1ml of the solution labelled R1 (made up of a mixture of sodium hydroxide; Na-k-tartrate; potassium iodine and cupric sulphate, was then added to the standard and sample cuvettes sequentially and the R2: blank reagent (made up of a mixture of sodium hydroxide andNa-K-tartrate) and the resulting solutions mixed and incubated for 30 minutes at 250C before readings were taken. The absorbance of the sample (Asample) and of standard (Astandard) were measured against the reagent blank at a wavelength of 546nm" and 1cm light path.

$$\text{Total protein concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{Conc. of standard (g/l)}$$

b. **Determination of plasma total bilirubin:** Randox lab. kit, UK, was used (Jendrassik and Grof, 1938).

Principle:

The colorimetric method used was based on that described by Jendrassik and Grof, (1938). Total bilirubin was determined in the presence of caffeine, which releases albumin bound to bilirubin, by the reaction with diazotized sulphanilic acid. Both this released bilirubin and direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. The resulting solution was measured spectrophotometrically at a wavelength of 578nm (Jendrassik and Grof, 1938) with the level of absorbance being indirectly proportional to the concentration of bilirubin in the sample.

The concentration of bilirubin is then calculated using the formula:

Total bilirubin in mg/dl = 10.8 x absorbance of sample at 578nm

Procedure:

Into two cuvettes labelled “Sample Blank” and “Sample”, 200µl of Reagent 1 (made up of a mix of sulphanilic acid andhydrochloric acid) was added, followed by 1 drop (50µl) of Reagent 2 (sodium nitrite) being added to the sample cuvette only. This was closely followed by the addition of 1000µl of Reagent 3 (caffeine and sodium benzoate) and 200µl of the sample to the two cuvettes. Mixing and incubating for 10 minutes at 250C was done, followed by the addition of 1000µl of Reagent 4 (tartrate and sodium hydroxide) to the two cuvettes. Mixing and incubating for a further 5-30 minutes at 250C was done. The absorbances of the sample against sample blank at 578nm were read.

Calculation:

Total bilirubin in mg/dl = 10.8 x absorbance of sample at 578nm

c. Determination of plasma direct/conjugated and indirect/unconjugated bilirubin: The Randox lab. kit, UK, was also used (Jendrassik and Grof, 1938).

Principle: The blue coloured complex formed by the reaction of the sample with diazotized sulphanilic acid is indicative of conjugated (direct) bilirubin alone, since caffeine has not been added (indirect bilirubin is only added to make up total bilirubin when caffeine breaks down the bilirubin-albumin complex to release free bilirubin). Its absorbance is measured spectrophotometrically at 546nm.

The direct bilirubin is calculated using the formula:

Direct bilirubin in mg/dl = 14.4 x absorbance of sample at 546nm

While the indirectly reacting bilirubin (unconjugated bilirubin or indirect bilirubin) is calculated by subtracting the directly reacting bilirubin (direct bilirubin) from the total bilirubin value (Kirk, 2008).

Procedure:

Into two cuvettes labelled “Sample Blank” and “Sample”, 200µl of Reagent 1 (made up of a mix of sulphanilic acid andhydrochloric acid) was added, followed by 1 drop (50µl) of Reagent 2 (sodium nitrite) being added to the sample cuvette only. This was closely followed by the addition of 2000µl of 0.9% NaCl and 200µl of the sample to the two cuvettes. Mixing and incubating for 10 minutes at 250C was done. The absorbances of the sample against sample blank at 546nm was

then determined.

Calculation:

Direct bilirubin in mg/dl = 14.4 x absorbance of sample at 546nm

The indirectly reacting bilirubin (unconjugated bilirubin or indirect bilirubin) is calculated by subtracting the directly reacting bilirubin (direct bilirubin) from the total bilirubin value:

Indirect bilirubin = total bilirubin – direct bilirubin (Kirk, 2008).

d. Determination of plasma aspartate aminotransferase (AST), EC 2.6.1.57. Randox lab. kit, UK, was used(Reitman and Frankel, 1957).

Principle:

α- oxoglutarate + L-aspartate AST L- glutamate+ oxaloacetate.

oxaloacetate + 2,4-DNPH oxaloacetate hydrazone

Aspartate aminotransferase (AST) catalyses the reaction between α- oxoglutarate and L-aspartate to yield L- glutamate and oxaloacetate. AST activity is then measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4- Dinitrophenylhydrazine (2, 4-DNPH).

The absorbance of the resulting solution will be measured spectrophotometrically at a wavelength of 546nm (Reitman and Frankel, 1957). AST activity in plasma is obtained by extrapolating it from a standard curve derived from known AST standard activities and their corresponding absorbance's.

Procedure:

Two test tubes were labelled “sample blank” and “sample”. 0.1ml of the sample was put into the sample tube only, followed by the addition of 0.5 ml of Reagent 1(made up of a mix of phosphate buffer,L-aspartate andα-oxoglutarate into both tubes and 0.1ml of distilled water into the sample blank tube. The solutions were mixed and incubated for exactly 30 minutes at 370 C, then into the tubes was added 0.5ml of Reagent 2 (2, 4-dinitrophenlhydrazine). The solutions were mixed again and allowed to stand for exactly 20 minutes at250C, then 5.0ml of Sodium hydroxide was added. The solutions were mixed and the absorbance of Samples (Asample) read against the sample blank after 5 minutes at a wavelength of 546nm, 1cm light path.

Calculation:

The AST activity in plasma samples were obtained by extrapolating them from the standard curve derived from known standard AST activities and their corresponding absorbances

e. Determination of plasma alanine aminotransferase (ALT), EC 2.6.1.2: Randox lab. kit, UK, was used(Reitman and Frankel, 1957).

Principle:

α-oxoglutarate + L-alanine ALT L-glutamate + pyruvate.

pyruvate + 2,4-DNPH pyruvate hydrazone

Alanine aminotransferase (ALT) catalyses the reaction between α- oxoglutarate and L-alanine to yield L- glutamate and pyruate. Alanine aminotrasferase is measured by monitoring the concentration of pyrurate hydrazone formed with 2, 4-dinitrophenylhydrazine.

The absorbance of the resulting solution is measured spectrophotometrically at a wavelength of 546nm" (Reitman and Frankel, 1957). ALT activity in plasma is obtained by extrapolating it from a known standard curve derived from known standard ALT activity values and their absorbances.

Procedure:

Two test tubes were labelled "sample blank" and "sample". 0.1ml of the sample was put into the sample tube only, followed by the addition of 0.5 ml of Solution R1 (a mix of phosphate buffer, L-alanine andα-oxoglutarate) into both tubes and 0.1ml of distilled water into the sample blank tube only. The solutions were mixed and incubated for exactly 30 minutes at 370 C, then into the tubes was added 0.5ml of Solution R2 (2, 4 – Dinitrophenylhydrazine). The solutions were mixed again and allowed to stand for exactly 20 minutes at250C, then 5.0ml of sodium hydroxide was added. The solutions were mixed and the absorbance of samples (Asample) read against the sample blank after 5 minutes at a wavelength of 546nm, 1cm light path

Calculation:

Using the absorbance results for the samples, the activity of ALT in plasma was obtained by extrapolating it from a standard curve of the known standard ALT activity levels and their corresponding absorbances.

f.Determination of plasma alkaline phosphatase (ALP), EC 3.1.3.1: Randox lab. kit, UK, was used(DGKC, 1972).

Principle:

This is an optimized standard method according to the recommendations of the deutsche gesellschaft fur klinische chemie (DGKC). Alkaline phosphatase (ALP) enzyme reacts with disodium para-nitrophenylphosphate and liberates phenol which forms a yellow coloured complex at alkaline pH.

p-nitrophenylphosphate + H20 --ALP---------> Phosphate + p-nitrophenol

The absorbance of the resulting yellow solution is measured spectrophotometrically at a wavelength of 405nm (DGKC, 1972). The absorbance is again read after 1, 2, and 3 minutes at wavelength of 405nm, 1cm light path, at 250C.

To calculate ALP activity, the following formula is used:

IU/l = 3300 x A at 405nm/min;

Procedure:

Fresh distilled H2O was aspirated and used as water blank. The following solutions were then pipetted into a test tube, Sample (0.05ml) and Reagent (3.00ml) made up of a mix of diethanolamine buffer,MgCl2 and substrate: p-nitrophenylphosphate. After mixing, the initial absorbance was read and timer was simultaneously started. The absorbance was again read after 1, 2, and 3 minutes at wavelength of 405nm, 1cm light path, at 250C.

Calculation:

To calculate ALP activity, the following formula was used:

IU/l = 3300 x A at 405nm/min

Where the mean change in absorbance per minute was represented by A

3.2.7 STATISTICAL DATA ANALYSES

Results were expressed as mean ± SEM (standard error of mean) from each experimental group of n=5 rats. Analysis of variance (ANOVA) was computed among the mean values of the experimental data to ascertain the level of significance at 95% confidence interval using graph pad prism and SPSS statistical package and data and data with comparative mean values of $p<0.05$ were significant.

3.2.8. ETHICAL CONSIDERATION

All experimental procedures involving the male wistar rats were performed in strict accordance with ethical guidelines for animal research. Efforts were made to reduce the animal suffering,ensure human handling, and follow the principles of replacement, reduction and refinement as outlined by National Research Council (2011).

CHAPTER FOUR

RESULTS

4.1 CELLIFEIQ SUBACUTE TOXICITES ON LIVER FUNCTION STATUS

The subacute administration of Cell Life IQ at 600mg/kg caused a significant increase ($p<0.05$) in total and direct bilirubin levels in the rats when compared to the control and lower dose groups (20 and 80 mg/kg) (Table 4.1). However, the lower doses (20 and 80 mg/kg) did not cause significant changes ($p>0.05$) in bilirubin levels when compared to the control, showing that the mild hepatic stress was only shown at the highest dose. The subacute administration of the test substance,caused a relevant increase ($p<0.05$) in AST levels in the rats of the 600 mg/kg group when compared to the control and lower dose groups (Table 4.1). ALT and ALP levels were not significantly altered ($p>0.05$) in all treatment groups when compared to the control, showing that hepatocellular and bile duct functions were largely preserved at low and moderate doses. Identically, total protein levels were significantly decreased ($p<0.05$) in the 600 mg/kg group when compared to the 20 mg/kg group, while control and 80 mg/kg groups were not significantly different ($p>0.05$). Albumin levels remained constant ($p>0.05$) across all groups Table 4.1), showing that hepatic synthetic function for albumin remained intact despite high-dose administration. In sum total, sub-acute exposure to the test compound was well-tolerated at low to moderate doses (20–80 mg/kg) with mild hepatic alterations observed only at the high dose (600 mg/kg).

Table 4.1a Liver Function indices of rats subjected to subacute toxicity study of celllife iq

Groups	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
Control	0.48 ± 0.01	0.37 ± 0.02	4.06 ± 0.14
20 mg/kg.	0.54 ± 0.04	0.42 ± 0.04	4.33 ± 0.19
80 mg/kg	0.49 ± 0.02	0.38 ± 0.02	4.04 ± 0.09
600 mg/kg	0.65 ± 0.03	0.50 ± 0.02	3.48 ± 0.20

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

Table 4.1b Liver Function Tests indices of rats subjected to subacute toxicity study of celllife iq

Groups	**ALT(U/L)**	**AST(U/L)**	**ALP (U/L)**
Control	147.35 ± 12.98	133.56 ± 9.01.	34.67 ± 0.59

20mg/kg	142.53 ± 10.29	125.12 ± 9.17	31.78 ± 0.97
80mg/kg	141.44 ± 3.85	140.64 ± 6.36	31.85 ± 1.18
600mg/kg	172.38 ± 12.87	181.60 ± 7.92.	33.58 ± 0.66

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

The administration of Cell Life IQ at different doses caused no important changes ($p>0.05$) in total cholesterol, triglycerides, HDL, LDL, and VLDL levels in the rats when compared to the control group (Tables 4.1–4.5). In general, all doses of the test compound maintained these lipid parameters at levels comparable to the control, and their effects were not significantly different ($p>0.05$) from each other.

Table 4.2 Lipid Profile indices of rats subjected to subacute toxicity study of celllife iq

Groups	Total Cholesterol	Triglycerides	HDL.	LDL	VLDL
Control	179.62 ± 4.00	130.33 ± 12.15	30.74 ± 0.84	122.81 ± 5.44	26.07 ± 2.43
20 mg/kg	187.43 ± 6.20.	127.27 ± 9.24	29.80 ± 0.81	132.18 ± 4.57.	25.45 ± 1.85
80 mg/kg.	178.46 ± 4.69	117.25 ± 7.11	28.33 ± 0.23	126.68 ± 4.26.	23.45 ± 1.42
600 mg/kg	178.07 ± 1.69	129.88 ± 5.28	29.61 ± 0.51	122.49 ± 2.37	25.98 ± 1.06

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

4.3 FEED CONSUMED AND WATER INTAKE DAILY

The daily feed per rats are varied among the different groups. The control group maintained the highest feed intake while the lowest was seen in the highest dose groups of Cell Life IQ. The remaining treatment groups showed intermediate consumption. Generally, cell life IQ caused a dose- dependent decrease in feed consumption with higher doses associated with lower intake. Overall, all groups maintained consistent feed intake within their respective group throughout the measurement period. Water consumed daily per rats differed among groups.The control groups had a moderate intake, while the highest intake was observed in the highest dose group.The other two treatment groups demonstrated intermediate water consumption. Overall, the test compound caused a dose-dependent increase in water consumption, with higher doses associated with higher intake. Generally, all groups maintained consistent water intake within their respective group throughout the measurement period.

Table 4.3. Feed Consumed per Day per Rats (g/day)

Groups	Feed Consumed
Control	25.01 ± 0.00
20 mg/kg	22.17 ± 0.00
80 mg/kg	24.00 ± 0.00
600 mg/kg	18.41 ± 0.00

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

Table 4.4. Water Consumed per Day per Rat (mL/day)

Groups	Water Consumed
Control	36.72 ± 0.00
20 mg/kg	33.67 ± 0.00
80 mg/kg	39.69 ± 0.00
600 mg/kg	41.44 ± 0.00
Total (n=20)	37.88 ± 0.68

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

The body weight of the rats at the beginning of the experiment (Day 0) was not different from all groups ($p>0.05$) indicating similar base line weights (Table 4.5). Similarly at the end of first week (Day7) there was no significant differences in the body weight among the different groups($p>0.05$),showing that the treatment has not affected the weight gain. By the second week (Day 14), although the highest dose group (600 mg/kg) had a lower mean body weight when compared with the other groups, the differences were not statistically significant ($p>0.05$). During the third week (Day 21), there was still no relevant difference in body weight between the groups ($p>0.05$), although the 80 mg/kg group showed a slightly higher mean weight. At the end of the fourth week (Day 28), the highest dose group (600 mg/kg) had a lower body weight compared with the control and other treated groups, while the 80 mg/kg group had the highest mean weight; these differences approached significance but were not statistically significant at $p<0.05$. In

general, all treatments maintained body weight within a comparable range to the control group throughout the study period, with no treatment causing a significant decrease or increase in weight gain.

4.4 Table 4 5. Body Weight (g)

Group	DAY_0	DAY_7	DAY_14	DAY_21	DAY_28
Control	155.71 ± 8.47	65.67 ± 7.75	179.58 ± 9.64	185.29 ± 10.25	200.08 ± 10.97
20mg/kg	154.49 ± 5.31	163.77 ± 6.60	173.41 ± 7.61	185.06 ± 9.37	194.91 ± 10.40
80 mg/kg	154.38 ± 4.91	170.62 ± 7.22	185.85 ± 6.00	204.75 ± 9.14	216.68 ± 10.17
600 mg/kg	153.18 ± 3.81	155.07 ± 6.90	162.09 ± 9.27	176.40 ± 13.16	175.68 ± 12.11

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

The subacute administration of Cell Life IQ did not cause any important change in relative kidney weight in the rats when compared to the control group ($p>0.05$) (Table 4.8). In general, all treatment groups (20 mg/kg, 80 mg/kg, and 600 mg/kg) maintained relative kidney weight similar to the control, with no significant differences observed among the groups ($p>0.05$).Identically, the treatments did not cause any significant change in relative heart weight in the rats when compared to the control group ($p>0.05$) (Table 4.8). All treated groups showed a relative heart weights within the range of the control group, and there were no significant differences in efficacy among the groups ($p>0.05$). However, the treatments preserved organ weight indices, showing no adverse

effect on kidney or heart weight in the rats. The treatments did not cause any relevant change in relative liver weight in the rats when compared to the control group ($p>0.05$) (Table 4.8). In general, all treatment groups (20 mg/kg, 80 mg/kg, and 600 mg/kg) maintained relative liver weight similar to the control, with no important differences observed among the groups ($p>0.05$).Overall, the treatments did not adversely affect liver weight, indicating preserved hepatic organ indices across all groups.

4.5 Table 4.6. Relative Organ weights

Groups	Relative Kidney Weight	Relative Heart Weight	Relative Liver Weight
Control	0.35 ± 0.01	0.36 ± 0.02	3.77 ± 0.19
20 mg/kg	0.31 ± 0.02	0.37 ± 0.01	3.45 ± 0.15
80 mg/kg	0.32 ± 0.02	0.34 ± 0.01	3.65 ± 0.12
600 mg/kg	0.32 ± 0.01	0.38 ± 0.02	3.85 ± 0.10

The subacute administration caused irregular effects of body weight gain in rats(Table 4.9). Rats treated with moderate dose exhibited the highest weight gain, which was significantly greater ($p < 0.05$) than that observed in the highest dose group. The highest dose group showed the lowest weight gain among all groups, indicating a possible adverse effect at this dose. No relevant differences ($p > 0.05$) were noticed between the control and the lower doses, suggesting that mild effects on body weight occurred only at the highest dose.

4.6 Table 4.7 Daily weight Change (g)

Group	Daily Weight Change (g)
Control	1.584 ± 0.114
20 mg/kg	1.444 ± 0.299
80 mg/kg	2.224 ± 0.338
600 mg/kg	0.806 ± 0.324

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

Feed efficiency varied slightly among the groups during the study period. The control group maintained baseline feed efficiency, while the treatment groups exhibited minor differences in efficiency across the doses. Similarly, there was no significant differences ($p>0.05$) among the groups. CellifeIQ at the different doses did not significantly alter feed efficiency compared with the control.

4.7 Table 4.8: Feed Efficiency

Group	Feed Efficiency (%)
Control	6.336 ± 0.457
20 mg/kg	6.508 ± 1.349
80 mg/kg	9.272 ± 1.412
600 mg/kg	4.368 ± 1.76

All research results are expressed as mean ± SEM (=standard error of mean of five determination (n=5).

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The study aimed to investigate the subacute toxicity of Cell Life IQ on liver function status and lipid profile. Conversely (Table 4.1a) showed a significant increase ($p<0.05$) in total and direct bilirubin levels at high dose in the rats when compared.to the control and lower dose groups . This indicates that a lenient hepatic stress was exhibited at the higher dose . Table 4.1b AST also showed relevant increase at high dose compared to the control and lower dose groups. Although AST is not liver specific, it is also present in heart or other organs. So the increase of the AST levels at this particular dose indicates a mild stress on liver tissues. While other liver biomarkers like ALP and ALT at all treatment groups compared to the control were not altered. This indicates that the drug is hepatologically safe at these tested doses.This results is in corelation with the studies on the toxicity effects of gluten extracts on albino rats which the results was no alterations in ALT and ALP (Yahaya *et al.,*2025). A decrease in total protein levels was observed at the high dose indicating a dose related hepatotoxicity affecting total protein synthesis even if ALP and AST are normal.

Moreover, there was no important changes in the cholesterol,triglycerides, HDL,VLDL and LDL levels when compared from the treatment groups to the control group (Table 4.2).This indicates that Serum lipid profile parameters, remained within the normal physiological range across all treatment groups, indicating that the intervention did not adversely affect lipid metabolism.

However, cell life IQ showed a dose- dependent decrease relationship where the highest dose group has the least food intake. The control group has the highest food intake and the other groups showed a moderate consumption. Water intake daily was at its highest in the high-dose group while it was low for the control group.The remaining groups had a moderate intake of water intake daily. High water intake alongside low food intake could signal mild stress on the liver, kidneys, or other organs at that particular dose.This is often seen in subacute toxicity studies when the body tries to maintain homeostasis.(Table 4.3-4.4).

Moreover, body weight of rats showed no significant changes from day 0 to day 7. But at Day 14 the rats in the higher dose groups had a low mean body weight when compared to other groups. By Day 21, there was still no significant changes but the 80mg/kg had a slightly higher mean body weight. At Day 28, the rats that were given the higher dose had a low mean body weight while rats given 80mg/kg still had a slightly higher mean weight. During the course of the Study all the rats had maintained their weight when compared to the control group and there was no significant increase or decrease that was noticed.(Table 4.5)

Furthermore, the was no significant alterations in the liver, kidney and heart weight across all groups when compared to the control groups. It shows that that there was no adverse on the kidney and heart on subsequent administration. Weight of the liver was also maintained indicating there is preserved hepatic indices across all groups.(Tqble 4.6)

Additionally, subacute administration of Cell Life IQ caused variable effects in the weight gain of the rats. This kind of pattern is often expected in subacute toxicity studies, where low/moderate doses may be safe or beneficial, but high doses induce adverse effects. Feed efficiency was normal

across all groups indicating no impairment of metabolism/nutrient utilisation and weight changes likely due to in take differences.(Table 4.7-4.8)

5.2 CONCLUSION

I recommend studies like chronic toxicity and subchronic toxicity to know the toxicity levels at long and subsequent administration and also the histopathological examinations on both the liver and lipid profile to ascertain more toxicological evaluation of the supplement cell life IQ.

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APPENDIX

SAMPLE PREPARATION

A suspension of Cell Life IQ was made by dissolving the powdered supplement with each of three different doses mixed with distilled water. Each rats in the three different cages received a daily dose of approximately 1ml by oral gavage to make sure that there is uniformity in the doses administered and to remain within the recommended safe oral volume limit (≤ 2 mL/100 g body weight). The rats were given the doses daily through a sterile syringe blunt-ended gavage needle. The supplement were given once daily for about 28 days.