ACUTE TOXICITY STUDIES OF CELLIFEIQ IN MALE WISTAR RATS

BY

Precious EHIGBAI-JEREMIAH

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC) IN MEDICAL BIOCHEMISTRY

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CERTIFICATION

We the undersigned hereby certify that EHIGBAI-JEREMIAH PRECIOUS (BMS2101393) carried out this work ,in the department of Medical Biochemistry, University of Benin,Benin-city and we approve same as adequate in scope and quality for the reward of Bachelor of Science degree in Medical Biochemistry.

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External Examiner Date

DEDICATION

I dedicate this work to God Almighty, my parents, my colleagues, my supervisor, and the rest of my family for the inspiration, encouragement and support given towards the successful completion of this work.

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ABSTRACT

Acute toxicity studies is essential for determining the immediate safety of substances following a single high-dose exposure, providing early indicators of potential adverse effects or lethality. CellifeIQ, a multi-component nutraceutical formulated with antioxidant-rich herbal extracts, vitamins, and minerals, is widely promoted for supporting cellular health, yet its toxicological safety has not been scientifically evaluated . This study evaluated the acute oral toxicity of CellifeIQ in male Wistar rats. Using Lorke's method,male wistar rats received single oral doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg and were observed for 14 days for mortality, behavioural changes, body-weight trends, feed and water intake, and gross pathological alterations in major organs. No mortality occurred at any tested dose, indicating an LD_{50} greater than 5000 mg/kg. Mild and transient effects, such as slight restlessness or sedation, were observed at higher doses but resolved within hours, while delayed mild itching was noted only at doses ≥1000 mg/kg. Body-weight progression, feed consumption, water intake, and feed efficiency showed no significant differences compared to controls ($p>0.05$). Gross necropsy revealed no visible abnormalities in the liver, kidneys, heart, lungs, or spleen. CellifeIQ demonstrated very low acute oral toxicity and may be considered practically non-toxic under single-dose exposure conditions in male Wistar rats. However, further studies including sub-acute and chronic toxicity, biochemical assays, histopathology, and genotoxicity evaluations are recommended to fully characterize its long-term safety profile.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

In recent years, the use of nutraceutical and functional food supplements has increased significantly as individuals seek natural means to promote health, enhance immunity, and prevent chronic illness. Functional foods and nutraceuticals are products derived from natural sources that provide physiological or health benefits beyond basic nutrition (Dable-Tupas e*t al* 2020). Their popularity is largely driven by the perception that "natural" substances are inherently safer than synthetic pharmaceuticals (Ekor, 2014). However, despite this assumption, several herbal and nutraceutical formulations possess biologically active components that may produce adverse effects, especially when taken in high doses, over prolonged periods, or without standardized toxicity evaluation (Ekor, 2014). Therefore, assessing the safety profiles of such formulations is essential to ensure appropriate consumer protection.

CellifeIQ is a nutraceutical supplement formulated from a blend of fruit, vegetable, mineral, and herbal extracts. The formulation is designed to enhance cellular antioxidant defense by supporting the activity of glutathione and superoxide dismutase (SOD)—two major endogenous antioxidant systems. Glutathione serves as a central intracellular antioxidant involved in detoxification, free radical neutralization, and protection of cellular components from oxidative damage (Forman *et al,* 2009). Similarly, SOD plays a fundamental role in the antioxidant defense system by catalyzing the conversion of harmful superoxide radicals into less reactive species, thereby minimizing oxidative stress (Birben *et al.*, 2012).

Oxidative stress occurs as a result of production of reactive oxygen species (ROS) exceeding the capacity of antioxidant defenses, which can lead to cellular injury, inflammation, and the progression of chronic diseases. Antioxidant-supportive supplements like CellifeIQ are therefore promoted for their potential to maintain cellular balance and protect tissues from oxidative damage. However, the biological effectiveness and safety of multi-component herbal-antioxidant formulations depend on dosage, bioavailability, metabolic interactions, and organism-specific responses. Without experimental validation, assumptions of safety may be misleading and potentially harmful, particularly when supplements are used repeatedly or in high concentrations (Ekor, 2014).

Superoxide dismutase (SOD) contributes to cellular protection by catalyzing the breakdown of superoxide radicals into less harmful molecules, helping to maintain oxidative balance (Birben *et al.*, 2012).

When the body's antioxidant defenses are overwhelmed by the production of reactive oxygen species (ROS), oxidative stress occurs. This imbalance has been widely associated with inflammation, tissue injury, metabolic dysfunction and disease progression. Although supplements like CellifeIQ are promoted for their possible protective effects against oxidant damage, the biological safety and systemic tolerance of such formulations must be scientifically established before widespread use.

Toxicological evaluation, particularly acute toxicity testing, is essential for assessing the safety of herbal and nutraceutical products. Acute toxicity studies allow determination of dose-related toxic effects, observable clinical symptoms, and lethal dose estimations following administration

of a substance (Parasuraman, 2011). The OECD Guideline 423 (Acute Oral Toxicity — Acute Toxic Class Method) provides a validated procedure for conducting such toxicity tests in animal models (OECD, 2002).

Wistar rats are frequently used in toxicological research due to their defined physiology, well-documented responses, and suitability for controlled laboratory studies (Festing and Altman, 2002). Evaluating the acute toxicity of CellifeIQ in Wistar rats is therefore necessary to determine its safety margin, identify potential adverse effects, and support evidence-based recommendations regarding its safe human use.

1.2 Statement of the Problem

The usage of nutraceutical and herbal supplements has grown rapidly, mostly due to the perception that products derived from natural sources are inherently safe. However, scientific evidence has shown that herbal formulations may contain potent bioactive compounds capable of exerting physiological effects, some of which may be harmful when taken in excessive dosage orwithout appropriate regulatory evaluation (Ekor, 2014). Although CellifeIQ is promoted for supporting antioxidant activity and cellular health based on its composition of plant-derived compounds according to the manufacturers, its safety profile has not been scientifically established through standardized toxicological studies.

Excessive activation of antioxidant pathways or interactions among multiple plant constituents may lead to unintended physiological effects. Furthermore, the absence of formal toxicity data presents a concern for consumers, healthcare providers, and regulatory bodies. Without

experimental evidence regarding its acute toxicity, dosage thresholds, and potential adverse effects, the safe consumption of CellifeIQ cannot be confidently assured. Therefore, there is a need to evaluate the acute toxicity of CellifeIQ in an experimental animal model, using standardized procedures such as the OECD Acute Toxic Class Method (OECD, 2002) and Lorke's Method (Lorke,1983) to determine its safety margin and potential toxicological effects.

1.3 Justification of the Study

Although herbal and nutraceutical supplements are widely used, many of these products still lack adequate toxicological evidence to support their safety. Acute toxicity assessment offers essential preliminary information about the potential harmful effects and tolerability of a substance (Parasuraman, 2011). This study is therefore necessary to protect public health, as supplements such as CellifeIQ are often consumed without professional guidance. Establishing its safety profile will encourage responsible use and reduce the risk of adverse outcomes.

This study also holds scientific and regulatory value. Toxicological data are required for product approval, standardization, and adherence to international safety regulations. In addition, the findings will contribute to evidence-based validation, providing experimental insight into the biological responses elicited by CellifeIQ and expanding available scientific literature on nutraceutical safety. At present, there is a noted research gap due to limited empirical data on the acute toxicity of CellifeIQ, making this investigation timely and relevant. Overall, evaluating the acute toxicity of CellifeIQ in Wistar rats will help determine whether it is safe at commonly consumed doses and whether it poses any potential adverse health effects.

1.4 Aim of the Study

The main aim of this study is to determine the acute toxicity effects of CellifeIQ in male Wistar rats.

1.5 Objectives of the Study

The objectives of this study are:

1. To observe and record clinical signs of toxicity such as changes in behaviour, posture, locomotion, respiration, and general physical appearance.

2. To assess the effect of CellifeIQ on body weight, food intake, and water intake during the observation period.

3. To evaluate mortality rate and determine the median lethal dose (LD_{50}) of CellifeIQ in male Wistar rats.

4.To perform gross pathological examination of major organs (liver, kidney, heart, lungs and spleen) to identify any visible toxic effects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Theoretical Framework

Toxicology as a discipline is grounded in the principle that biological response is proportional to dose, often summarized in the classical assertion that "the dose determines the poison." This concept emphasizes that toxicity is not an intrinsic property of a compound alone, but a dynamic interaction among dose, exposure duration, metabolic transformation, and organismal susceptibility. Acute toxicity studies are designed to elucidate the immediate systemic consequences of exposure to a substance, determining whether biochemical, physiological, or morphological disruptions occur following a single or short-term dosage (Parasuraman, 2011).

In the context of nutraceutical formulations containing multiple phytochemicals, synergistic, additive, or antagonistic interactions among constituent molecules may significantly alter metabolic handling, redox homeostasis, and organ-specific responses. Therefore, the toxicological evaluation of such multi-component formulations requires empirical investigation rather than reliance on the presumed safety of natural origin (Ekor, 2014). This framework justifies the need for controlled experimental assessment of CellifeIQ in a standardized animal model.

2.2 Concept of Toxicity and Acute Toxicity Studies

Toxicity refers to the capacity of a substance to produce functional or structural damage to living tissues. At the cellular level, toxicity may manifest through mechanisms such as oxidative stress, mitochondrial dysfunction, membrane destabilization, impaired protein homeostasis, DNA damage, and dysregulation of inflammatory signaling pathways. Acute toxicity studies typically evaluate biological responses occurring within 24 hours of exposure to a single oral dose of a test compound. These responses may include alterations in neurological activity, cardiovascular stability, respiratory rhythm, metabolic state, and survival rate.

One of the most widely applied and scientifically accepted procedures for acute toxicity evaluation in laboratory animals is Lorke's Method, developed to improve accuracy while significantly reducing the number of animals required for toxicity classification (Lorke, 1983). Lorke's method is conducted in two distinct phases, each designed to refine dose selection and identify toxicity thresholds.

In Phase I, animals are divided into three groups, each receiving a different dose (commonly 10 mg/kg, 100 mg/kg, and 1000 mg/kg). The animals are observed for 24 hours for behavioural abnormalities, motor impairment, tremors, piloerection, respiratory difficulty, convulsions, changes in feeding or drinking, and mortality. The results from this phase guide dose selection for Phase II by identifying the general toxicity range.

In Phase II, individual animals receive more specific doses—either higher or intermediate—based on the survival or mortality seen in Phase I. These doses typically follow a geometric pattern (e.g., 1,600 mg/kg, 2,900 mg/kg, and 5,000 mg/kg). Careful observation over another 24-

hour period helps determine the definitive lethal dose range. The LD_{50} value is then calculated using the geometric mean of the lowest dose that caused mortality and the highest dose that did not.

Lorke's method is particularly advantageous in evaluating herbal and nutraceutical formulations such as CellifeIQ because it incorporates both behavioural endpoints and mortality data while minimizing excessive animal use. It also provides a more realistic safety margin by integrating physiological and neurological signs rather than relying solely on death as an endpoint.

Overall, acute toxicity studies using Lorke's approach provide the foundational safety data required for subsequent biochemical, sub-acute, and chronic toxicity investigations

2.3 Toxicological Evaluation of Herbal and Nutraceutical Products

Nutraceuticals and functional herbal formulations contain biologically active phytochemicals that can modulate multiple metabolic and signaling pathways. While such compounds are frequently associated with therapeutic potential, they may also interfere with enzymatic detoxification systems, alter xenobiotic metabolism, compete for hepatic biotransformation mechanisms, or induce oxidative imbalance when taken at supraphysiological doses.A adverse effects associated with herbal supplementation commonly arise due to:

1.Lack of standardization in plant extract concentration

2.Variability in phytochemical composition due to cultivation and processing conditions

3.Herb–drug metabolic competition, particularly involving cytochrome P450 isoenzymes

4.Accumulation of bioactive metabolites in hepatic or renal tissues

5.Synergistic potentiation of redox cycling and inflammatory pathways(Ekor,2014)

Therefore, systematic toxicity studies remain essential to ensure that bioactive compounds exert their effects within a physiologically safe window. This is particularly important for complex formulations like CellifeIQ, in which multiple antioxidant and metabolic-modulating agents co-exist within a single matrix.

2.4 Overview of CellifeIQ

2.4.1 Product Identity and Formulation Rationale

CellifeIQ is a dietary supplement manufactured by Upward Biotechnology International, is described as a plant-based, broad-spectrum antioxidant formulation composed of over 30 bioactive components, including herbal extracts, fruit polyphenols, antioxidant enzymes, amino acid derivatives, vitamins, and trace minerals. The formulation is designed to modulate cellular redox equilibrium, mitochondrial metabolism, inflammatory signaling cascades, and detoxification capacity by supporting endogenous glutathione (GSH) and superoxide dismutase (SOD) systems.

Figure 1: Dietary supplement, cellifeIQ (upward biotechnology international)

2.4.2 Mechanistic Basis of Action (Biochemical Pathway Orientation)

CellifeIQ primarily targets the cellular oxidative defense network, particularly the GSH/GSSG cycle and SOD-catalyzed superoxide dismutation:

1. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion ($O_2^{\bullet -}$) into H_2O_2.

2. Glutathione peroxidase (GPx) reduces H_2O_2 to H_2O using reduced glutathione (GSH).

3. Glutathione reductase (GR) recycles oxidized glutathione (GSSG) back to GSH, maintaining redox homeostasis.

Disruption of this coordinated system leads to accumulation of reactive oxygen species (ROS), mitochondrial permeability transition, lipid peroxidation, protein carbonylation, and DNA oxidative lesions (Birben *et al.*, 2012; Forman *et al.,* 2009). CellifeIQ's formulation strategy is therefore oriented toward reinforcing endogenous antioxidant enzymatic cycles to prevent ROS-driven cytotoxicity.

2.4.3 Composition of cellifeIQ

CellifeIQ contains key bioactive ingredients, including Glutathione, S-Acetyl Glutathione, Extramel® Melon Fruit Complex (a natural source of SOD), Quercetin dihydrate, Noni fruit powder, Irish Moss powder, Organic Aloe vera powder, Cordyceps mushroom extract, and Turmeric root extract. These ingredients are commonly associated with antioxidant, anti-inflammatory, metabolic, and immune-supportive functions.

The formulation also includes complementary antioxidant and detoxification support agents, such as N-acetyl-L-cysteine (a glutathione precursor), alpha-lipoic acid, milk thistle extract, grape seed extract, blueberry powder, black pepper extract (BioPerine), sulforaphane (from broccoli sprout extract), and L-glutamine.

Additionally, essential vitamins (A, C, D, E, B6, B12) and minerals (zinc, selenium, manganese) are included to support enzyme activity and general metabolic processes.

2.4.3.1 Glutathione

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine, and glycine, recognized as the most abundant intracellular non-protein thiol and a central determinant of cellular redox homeostasis. GSH functions as a cofactor for glutathione peroxidase (GPx) in the reduction of hydrogen peroxide (H_2O_2) and lipid hydroperoxides, converting GSH to its oxidized form (GSSG). The glutathione reductase (GR) system subsequently regenerates GSH utilizing NADPH as an electron donor, sustaining antioxidant capacity within the cell.

GSH also participates in phase II biotransformation, where it conjugates electrophilic xenobiotics via glutathione-S-transferases (GSTs), facilitating detoxification and biliary excretion. Depletion of intracellular GSH is associated with mitochondrial permeability transition, cytochrome c release, caspase-mediated apoptosis, and necrotic cell death, highlighting its role as a metabolic checkpoint for oxidative injury (Forman *et al* 2009).

CellifeIQ includes glutathione in direct and precursor-supported forms to maintain intracellular thiol buffering capacity under oxidative challenge.

2.4.3.2 S-Acetyl-L-Glutathione

S-acetyl-L-glutathione (SAG) is a lipophilic, acetylated derivative of GSH engineered to bypass gastrointestinal and intracellular enzymatic degradation. Unlike reduced glutathione, which undergoes rapid hydrolysis in the gut, SAG is capable of passive transmembrane diffusion and intracellular deacetylation, allowing it to directly increase cytosolic GSH pools. Restoration of glutathione redox balance modulates ROS-dependent signaling pathways, including the Nrf2/Keap1 antioxidant response element (ARE) and inhibition of NF-κB-mediated inflammatory gene transcription (Forman *et al., 2*009).

Thus, SAG enhances redox buffering and supports the antioxidant signaling axis central to cellular defense.

2.4.3.3 Extramel® Melon Fruit Complex (Superoxide Dismutase Source)

Extramel® is a standardized melon (Cucumis melo) concentrate enriched in superoxide dismutase (SOD), a metalloenzyme responsible for catalyzing the dismutation of superoxide radical ($O_2\bullet^-$) into H_2O_2 and O_2. SOD represents the first enzymatic defense line against oxygen-derived free radicals produced primarily during mitochondrial oxidative phosphorylation. Inadequate SOD activity promotes the accumulation of superoxide, leading to peroxynitrite ($ONOO^-$) formation through reaction with nitric oxide (NO)—a potent mediator of protein nitration, mitochondrial respiratory chain inhibition, lipid peroxidation, and endothelial dysfunction (Birben *et al.*, 2012).

By providing an exogenous source of SOD and stimulating endogenous SOD gene expression, Extramel® supports mitochondrial integrity and reduces oxidative burden.

2.4.3.4 Quercetin Dihydrate

Quercetin is a naturally occurring flavonoid known for its free radical scavenging capacity. It neutralizes reactive oxygen species and helps reduce oxidative stress in tissues. Quercetin also influences inflammatory pathways by downregulating pro-inflammatory mediators and maintaining cellular defense systems (Boots *et al.*, 2008).

2.4.3.5 Noni *Morinda citrifolia*Fruit Powder

Noni *Morinda citrofolia is* an evergreen plant ingredient known mostly to southeast Asia .It grows in tropical and subtropical regions.It is identified by it's large leaves, straight stem and grenade-like yellow fruit.It is know for it's anticlastogenic, antimutagenic,anticarcinoma and anti-inflammatory activities and abilities to locate free radicals,to inhibit oxidation of low density protein,regulation of cholesterol,stimulates the immune system and regulation of cell function and purification of blood(Motshakein and Ghazahi,2015)

2.4.3.6 Irish Moss *Chondrus crispus* Powder

Irish moss contains sulfated polysaccharides, mainly carrageenans, which support cell membrane stability and modulate oxidative activity. Studies have shown that these polysaccharides possess antioxidant and mild anti-inflammatory properties, helping to protect cells from oxidative injury (Mazué *et al.*, 2016).

2.4.3.7 Organic Aloe vera Leaf Powder

Aloe contains acemannan polysaccharides, anthraquinones (aloin, emodin), and phenolic compounds. Aloe polysaccharides exhibit free radical scavenging properties, while anthraquinones modulate cyclooxygenase and lipoxygenase metabolic pathways—affecting inflammatory mediator synthesis (Surjushe *et al* 2008). However, excessive anthraquinone intake can induce enteric irritation and electrolyte imbalance, supporting the need for toxicity monitoring.

2.4.3.8 Cordyceps Mushroom Powder

Cordyceps is a medicinal fungus traditionally used in East Asian therapeutic systems for its effects on energy metabolism, immune modulation, and cellular stress resistance. Its primary bioactive constituent, cordycepin (3′-deoxyadenosine), is structurally analogous to adenosine and participates in adenosine receptor signaling, influencing ATP synthesis and mitochondrial functionality (Paterson, 2008).

Cordycepin inhibits mRNA polyadenylation, modulating protein translation efficiency and cellular adaptation to metabolic demand. This mechanism is associated with alterations in AMP-activated protein kinase (AMPK) activation—a central regulator of mitochondrial oxidative phosphorylation, glucose uptake, and fatty acid metabolism. Through AMPK and ATP-dependent systems, Cordyceps contributes to improved mitochondrial efficiency and reduction of ROS generation during oxidative phosphorylation.

Cordyceps also demonstrates immunoregulatory effects, including suppression of pro-inflammatory cytokines such as TNF-α and IL-1β, and modulation of macrophage nitric oxide

output. This immunomodulation is believed to occur partially through regulatory effects on NF-κB signaling, thereby influencing inflammatory transcriptional responses (Paterson, 2008).

The combined influence of mitochondrial bioenergetic enhancement, immune signaling regulation, and oxidative stress modulation provides the biochemical basis for the inclusion of Cordyceps in CellifeIQ as a metabolic adaptogen that supports cellular resilience under physiological stress conditions.

2.4.3.9 Turmeric *Curcuma longa* Extract

Turmeric contains curcumin, a polyphenolic compound that interacts with NF-κB, STAT3, and COX-2 signaling pathways, resulting in suppression of inflammatory mediator synthesis. Curcumin also exhibits ROS-scavenging, metal-chelation, and mitochondrial-protective functions (Hewlings and Kalman, 2017).

CellifeIQ contains a blend of plant-derived antioxidants, vitamins, and minerals that are intended to support cellular metabolism and oxidative balance in the body. The main bioactive classes include flavonoids, carotenoids, polysaccharides, amino acids, glucosinolate derivatives, and enzyme cofactors. The actions of these compounds generally relate to free radical neutralization, support of endogenous antioxidant enzymes, and maintenance of cellular integrity.

2.4.4.1 N-Acetyl-L-Cysteine (NAC)

N-acetyl-L-cysteine is a precursor of L-cysteine, which is necessary for the synthesis of glutathione (GSH), which is. one of the body's primary intracellular antioxidants. NAC supports the replenishment of depleted glutathione stores and also helps maintain redox balance under

oxidative stress. It also has mild mucolytic and anti-inflammatory effects due to its free thiol group, which directly neutralizes reactive oxygen species (Rushworth and Megson, 2014).

In CellifeIQ, NAC assists in maintaining cellular defense capacity by sustaining glutathione levels.

2.4.4.2 Alpha-Lipoic acid

Alpha-lipoic acid functions as a redox-cycling cofactor for mitochondrial enzyme complexes such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. ALA can exist in both oxidized (ALA) and reduced (dihydrolipoic acid, DHLA) forms, enabling it to act as a cellular antioxidant shuttle that regenerates oxidized forms of vitamin C, vitamin E, coenzyme Q10, and glutathione (Packer *et al.,* 1995). ALA also increases glucose uptake through activation of AMPK, indirectly influencing mitochondrial respiratory efficiency and ROS production.

2.4.4.3 Milk Thistle *Silybum marianum* Seed Powder

Milk thistle plant *Silybum marianum* is an annual herb natural to the Mediterranean region,which these days spreads out to the warm and dry regions of southern Europe.Milk thistle seed powder is an important source silymarin.Silymarin is a flavonoid comprising of numerous flavonolignans such as isosylibirum,silybinin,silychristin and silydcarin.It is known for having therapeutic benefits.It is a great stimulant , increases and aids in digestion and appetite (Hadidy *et al* ,2020)

2.4.4.4 Blueberry Fruit Powder

Blueberries are rich in anthocyanins, which help neutralize free radicals and reduce oxidative stress. Anthocyanins also support mitochondrial function and can help maintain normal cell

signaling and metabolic regulation. Their antioxidant effect contributes to overall cellular protection (Kalt *et al.,* 2007).

2.4.4.5 Grape Seed Extract

Grape seed extract is rich in proanthocyanidins, which are potent free radical scavengers. These compounds protect cellular lipids, proteins, and DNA from oxidative damage. They also help maintain vascular function by supporting endothelial nitric oxide activity and reducing oxidative stress in blood vessels. In CellifeIQ, grape seed extract contributes to general antioxidant support and assists in reducing oxidative load in cells (Bagchi *et al.,* 2000).

2.4.4.6 Pomegranate Fruit Hull Extract

The hull of the pomegranate fruit contains ellagitannins, which act as antioxidants and support detoxification systems in the body. These compounds help reduce oxidative stress and may also contribute to maintaining normal inflammatory balance in tissues (Lansky and Newman, 2007).

2.4.4.7 Horseradish Tree *Moringa oleifera* Leaf Extract

Moringa oleifera leaves contain polyphenols, flavonoids, vitamins, and minerals that support antioxidant and anti-inflammatory activity. They help modulate oxidative stress by enhancing endogenous antioxidant enzymes such as superoxide dismutase and catalase. Moringa also supports immune function and may help maintain cellular integrity under stress (Leone *et al.,* 2015).

In CellifeIQ, this ingredient adds additional antioxidant micronutrient support to the overall formula.

2.4.4.8 Black Pepper Fruit Extract (BioPerine)

Black pepper fruit *Piper nigrum l.* is a widely used spice which adds flavor of its own to dishes and also enhanced taste.The major active compound identified in *P.nigrum* is piperine although other compounds (Takoore *et al* ,2019).Piperine is a bioenhancer that increases absorption of multiple nutrients and drugs by inhibiting certain drug-metabolising enzymes and intestinal efflux transporters; at the doses typically used (as Bioperine®) it improves bioavailability of co-administered actives but can alter xenobiotic metabolism, which is relevant to safety/PK interactions in multi-component products (Srinivasan,2007).

2.4.4.9 Sulforaphane (Broccoli Sprout Extract)

Sulforaphane is a natural compound that supports the activation of antioxidant defense pathways, particularly through stimulation of the Nrf2 pathway, which regulates cellular protection mechanisms. This supports the body's ability to manage oxidative stress and detoxification (Yagishita *et al* ,2019)

2.4.4.10 L-Glutamine

L-glutamine is an amino acid that helps support cellular repair, immune function, and antioxidant defense. It contributes to glutathione production, which is one of the body's primary antioxidant

systems. L-glutamine also helps maintain the integrity of cell and tissue structures (Cruzat *et al.,* 2018).

2.4.5 Essential Vitamins and Minerals

The vitamins in cellifeIQ consist of vitamin A,B,C,D,E,B6 and B12.These vitamins contribute to normal immune function, tissue integrity, and oxidative balance.

Vitamin C regenerates other antioxidants,Vitamin E supports cell membrane protection,Vitamin A and D assist in immune regulation.Vitamins B6 and B12 support metabolic reactions associated with cellular maintenance.

Minerals found in cellifeIQ such as selenium,manganese and since actually as cofactors for antioxidant enzymes.Zinc and copper are structural components of superoxide dismutase (SOD),Selenium is required for glutathione peroxidase and Manganese supports mitochondrial Mn-SOD activity.Their presence helps maintain antioxidant enzyme efficiency and normal cellular metabolism (Halliwell and Gutteridge, 2015).

2.5 Parameters in Acute Toxicity Assessment

Acute toxicity studies evaluate the adverse effects of a single oral dose of a test substance over a short observation period, typically 14 days, to determine potential toxicity and classify the substance according to hazard categories (OECD, 2002). Unlike sub-acute or chronic studies, acute toxicity does not involve biochemical, hematological, or histopathological investigations. Instead, assessment is based primarily on clinical signs, behavioral responses, mortality incidence, and changes in body weight (Parasuraman, 2011).

The primary parameters include:

1. Mortality/Lethality The number of deaths following exposure is used to estimate the median lethal dose (LD_{50}) or assign a toxicity class. Absence of mortality at the limit dose (5000mg/kg) indicates low acute toxicity (OECD, 2002).

2. Clinical Behavioral Observations Observations include:

Posture and mobility,Grooming patterns, Reflex responses,Presence of tremors, convulsions, ataxia, salivation, or piloerection.

These signs reflect possible neurotoxicity or systemic distress (Parasuraman, 2011).

3. Food and Water Intake Reduced feeding activity may indicate systemic toxicity or gastrointestinal discomfort.

4. Body Weight Changes Progressive weight loss (>10%) can signal metabolic stress or toxicity (Festing and Altman, 2002).

5. Gross Necropsy at the end of the observation period, major organs are examined visually for discoloration, swelling, hemorrhage, or atrophy. No microscopic histology is required at this stage (OECD, 2002).

2.6 Toxicological Screening of Multi-Component Nutraceuticals

Safety assessment is required because phytochemical interactions may produce emergent biological effects not predictable from individual ingredients. Acute toxicity testing provides essential safety data (Parasuraman, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1 Test Substance

CellifeIQ is a nutraceutical supplement composed of a blend of herbal extracts and micronutrients claimed to enhance cellular health and immune function. cellifeIQ is a product of Upward International.It has been substance was obtained from a certified supplier in its original sealed container. It was stored at room temperature away from direct sunlight and humidity until required for administration.

3.2 Apparatus

The apparatus were procured from a certified and reliable vendor.

Oral gastric gavage(Uniben MEDBCH Dept.,Nigeria)

Simple weighing balance(Adventurer OHAUS AR1530)

Animal restrainer

Thermometer

Animal cages(Uniben MEDBCH Dept.,Nigeria)

Dissecting kit (Uniben MEDBCH Dept.,Nigeria)

Face mask(Pyrex)

Gloves(Pyrex)

Cardboard papers

Cotton wool

Paper tapes

Universal bottles

3.3 Equipment

The following equipment were used in the course of experiment

Analytical weighing scale(Metter H-80 ,Germany))

Temperature Water bath(B.bran Scientific and Instrument Company,England)

Refrigerator (Citizens PRC 4246)

Beakers(Pyrex)

Water distiller(B. Bran Sc. Inst. Company, England.)

3.4 Chemicals and Reagents Used

All reagents and chemicals used were of analytical grade. These included:

Picric acid (Randux lab)

Detergent

Methylated spirit

Chloroform

3.5 Experimental Animals

A total of 16 healthy Adult male Wistar rats weighing between 150-180 g were used for the study. The animals were obtained from the Animal House of the Department of Biochemistry, University of Benin, Nigeria.

They were housed in well-ventilated cages lined with clean sawdust, which was changed every two days to maintain hygiene. The animals were acclimatized for seven (7) days prior to the commencement of the experiment under standard laboratory conditions (temperature 25 ± 2°C, 12-hour light/dark cycle).

They were fed with ground pellet obtained from Crown flour Mill , Benin City, and given water was measured .The rats were identified by marking with picric acid on specific body parts.

3.6 Methods

3.6.1 Experimental Design (Lorke's Method, 1983)

The study was conducted according to the method described by Lorke (1983), which determines the median lethal dose (LD_{50}) through two experimental phases.This method has two phases which are phases 1 and 2 respectively.

Phase 1

Twelve (12) rats were randomly divided into four (4) groups of three (3) rats each as follows:

Group 1 (Control): No administration of cellifeIQ, distilled water only

Group 2 (Low Dose): 10mg/kg of CellifeIQ

Group 3 (Median Dose): 100 mg/kg of CellifeIQ

Group 4 (High Dose): 1000 mg/kg of CellifeIQ

Each dose was diluted in of distilled water before administration. ImL of mixture was administered to the each rats using an oral gavage.Care was taken during oral dosing to ensure the gavage needle entered directly into the oesophagus and not the trachea, to prevent aspiration or injury.

All animals were observed continuously for the first 24 hours and then daily for 14 days for signs of toxicity (such as salivation, piloerection, tremors, and reduced activity) and mortality.

The body weights of the rats were recorded before administration(day 0), at the end of the first week(day 7) and on the day of sacrifice(day 14)

Phase 2

Based on the outcome of Phase 1, four (4) rats were used for the second phase, divided into four (4) groups of one rat each as follows:

Group 1 (Control): 1 mL distilled water

Group 2 (Low Dose): 1600 mg/kg

Group 3 (Median Dose): 2900 mg/kg

Group 4 (High Dose): 5000 mg/kg

Each dose was diluted in of distilled water before administration. ImL of mixture was administered to the each rats using an oral gavage.Care was taken during oral dosing to ensure the gavage needle entered directly into the oesophagus and not the trachea, to prevent aspiration or injury.

Then the LD50 is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$ (Lorke,1983)

D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality.

The body weights of the rats were recorded before administration(day 0), at the end of the first week(day 7) and on the day of sacrifice(day 14)

Animals were observed for 24 hours and then daily for 14 days for behavioural and physiological changes.

The following parameters were carefully monitored throughout the study period:

General behaviour and appearance

Body weight changes

Food and water intake

Mortality and clinical signs (e.g., convulsion, salivation, lethargy, or coma)

3.6.3 Post-Mortem Analysis

At the end of the 14-day observation period, all surviving rats were sacrificed under mild chloroform anaesthesia. A gross necropsy was performed to observe and record any macroscopic changes in major organs such as the liver, kidneys, lungs, spleen, and heart.

Organs were carefully excised, blotted dry, and examined for discoloration, lesions, or swelling. Organ weights were taken and compared between treated and control groups. The findings were used to assess the toxic effect, if any, of CellifeIQ administration.

3.6.4 Assessment of Feed and Water Consumption

Feed and water intake were monitored daily throughout the 14-day observation period. The quantity of feed offered and the leftover amount after 24 hours were recorded, and the difference was taken as the daily feed consumed per rat. Similarly, water intake was determined by measuring the volume of water supplied and the remaining volume after 24 hours.

Daily feed consumption (g/day/rat) was calculated using:

{Feed Consumed} = {Feed Offered} - {Feed Leftover}

Water consumption (ml/day/rat) was calculated in the same way. Monitoring these parameters helps detect metabolic disturbances or systemic toxicity, as recommended in standard acute toxicity evaluations (Parasuraman, 2011; Lorke, 1983).

3.6.5 Assessment of Relative Weight Gain/Loss

Body weight of each rat was recorded prior to dosing (Day 0), on Day 7, and on Day 14. Relative weight gain or loss was determined according to the formula:

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

This parameter is essential because weight changes may signal toxicity, impaired metabolism, or stress responses (El Hilaly *et al.,* 2004).

3.6.6 Feed Efficiency

Feed efficiency was calculated to assess how effectively consumed feed translated into body weight gain. It is a useful indicator of metabolic stability and nutritional utilisation in toxicity studies.

$$\text{Feed efficiency} = \frac{\text{Weight gain or lost/day/rat} \times 100}{\text{Feed consumed/day/rat}}$$

Where:

Weight Gain = Final Body Weight – Initial Body Weight

Feed Consumed = Total feed consumed over 14 days

Reduced feed efficiency may indicate toxicity-induced metabolic impairment or reduced nutrient absorption (Shehu *et al.,* 2020).

3.7 Ethical Considerations

All animal handling and experimental procedures were carried out in accordance with institutional ethical guidelines and the principles for laboratory animal care as outlined by the National Research Council (2011).

CHAPTER 4

RESULTS

4.1 Acute Toxicity Study of CellLifeIQ Supplement

The acute oral toxicity of CellLifeIQ was evaluated using the Lorke method. The male wistar rats received single oral doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg of CellLifeIQ. No mortality was recorded in any of these given doses, indicating that the LD_{50} is greater than the highest dose administered (>5000 mg/kg). Clinical signs were monitored continuously for 24 hours following dosage and thereafter the rats were observed daily for 14 days to identify any delayed toxicity or abnormal behavior.

4.1.1 Survival and Immediate Transient Effects

All immediate effects were minimal, transient, and demonstrated clear dose-dependent variation, with all symptoms subsiding completely within a few hours after administration (see Table 4.1)

Table 4.1Survival and Immediate Transient Effects

	Dose Group (mg/kg)	No. of Animals	No. of Deaths (Mortality)	Observed Effects (Transient)
	CONTROL	3	0	None
	10	3	0	None
Phase 1	**100**	3	0	None
	1000	3	0	Slight restlessness (resolved within hours)
	1600	1	0	Mild sedation (resolved within hours)
Phase 2	**2900**	1	0	Mild sedation (resolved within hours)
	5000	1	0	Mild sedation (resolved within hours)

4.1.2 Persistent and Delayed Clinical Signs (Days 0–14)

Irritation and itching were the only delayed clinical manifestations noted during the 14-day observation period(see Table 4.2).These responses occurred in animals receiving doses of 1000 mg/kg or higher and appeared intermittently from Day 2 to Day 14. No additional delayed or persistent clinical changes were identified.

Table 4.2 Persistent and Delayed Clinical Signs (Days 0–14)

		Phase 1			Phase 2				
Clinical Signs	**Control**	**10 mg/kg**	**100 mg/kg**	**1000 mg/kg**	**1600 mg/kg**	**2900 mg/kg**	**5000 mg/kg**	**Total No. and % Affected**	**Notes**
Diarrhea	0	0	0	0	0	0	0	0 (0%)	None
Vomiting	0	0	0	0	0	0	0	0 (0%)	None
Micturition	0	0	0	0	0	0	0	0 (0%)	None
Salivation	0	0	0	0	0	0	0	0 (0%)	None
Sedation	0	0	0	0	0	0	0	0 (0%)	None
Agitation	0	0	0	0	0	0	0	0 (0%)	None
Piloerection	0	0	0	0	0	0	0	0 (0%)	None
Convulsions	0	0	0	0	0	0	0	0 (0%)	None
Spasms	0	0	0	0	0	0	0	0 (0%)	None
Irritation/Itching	0	0	0	3	1	1	1	6 (42.9%)	Mild delayed itching at doses ≥1000 mg/kg

4.1.3 External and Internal Examination

External examination of all animals showed normal skin and fur, with no evidence of lesions, alopecia, or discoloration. The eyes, nostrils, and oral cavity were clean and free of discharge. The anal and genital regions appeared normal, and the animals maintained normal locomotor activity throughout the study. No tremors, convulsions, abnormal secretions, or unusual behavioural responses were observed.

Internal assessment indicated that the lungs were free of congestion or haemorrhage, and the heart exhibited normal size and colour. The liver showed no signs of necrosis or abnormal pigmentation. The kidneys were of normal appearance, without enlargement or haemorrhage, and the spleen displayed a typical structure without hypertrophy. The stomach and intestines maintained normal morphology, and the brain showed no swelling or visible lesions. Other organs, including the pancreas, adrenal glands, and reproductive tissues, were also within normal limits. Histopathological evaluation of the liver and spleen at the 1600 mg/kg dose revealed no detectable lesions.

4.1.4 Interpretation of No Mortality

No mortality was recorded at any of the administered dose levels, confirming that the LD_{50} of CellLifeIQ is greater than 5000 mg/kg. The transient clinical responses observed were mild, clearly dose-dependent, and resolved promptly, while delayed itching was restricted to animals exposed to the higher dose ranges. No persistent, severe, or systemic toxic manifestations were detected during the 14-day observation period.

4.1.4 Daily feed and Water Consumption

Daily feed and water consumption remained stable and comparable to that of the untreated controls (see Table 4.3and Table 4.4).Across all treated groups, feed and water intake were consistent throughout the acute phase, with no significant differences ($p>0.05$) detected when compared with the control group.

Table 4. 3: Feed Consumed per Day per Rat (g)

Group	Feed Consumed (g/day/rat)
Control	21.14 ± 0.00
10 mg/kg	22.36 ± 0.00
100 mg/kg	22.25 ± 0.00
1000 mg/kg	22.78 ± 0.00

Results expressed in mean ± SEM (Standard error of the mean)

Table 4. 4: Water Consumed per Day per Rat (ml)

Group	Water Consumed (ml/day/rat)
Control	47.07 ± 0.00
10 mg/kg	57.38 ± 0.00
100 mg/kg	51.10 ± 0.00
1000 mg/kg	52.35 ± 0.00

Results expressed in mean ± SEM (Standard error of the mean)

4.1.5 Relative Weight Gain/Loss

The treatments did not produce any significant alterations in relative body-weight gain or loss when compared with the control group (Table 4.5). Overall, rats administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg demonstrated body-weight progression comparable to that of the controls, with no statistically significant differences observed among the groups ($p>0.05$).

Table 4 .5: Relative Weight Gain/Loss

Group	Relative Weight Gain/Loss
Control	2.28 ± 0.72
10 mg/kg	2.31 ± 0.64
100 mg/kg	4.91 ± 0.31
1000 mg/kg	3.15 ±1.01

Results expressed in Mean ± SEM(Standard error of the mean)

4.1.6 Feed Efficiency

CellifeIQ did not produce any statistically significant alterations in feed efficiency when compared with the control group (Table 4.6). Overall, rats administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg exhibited feed efficiency values comparable to those of the controls, with no significant differences detected among the groups ($p>0.05$).

Table 4.6: Feed Efficiency (%)

Group	Feed Efficiency (%)
Control	10.80 ± 3.43
10 mg/kg	10.32 ± 2.88
100 mg/kg	22.07 ± 1.41
1000 mg/kg	13.83 ± 4.42

Results expressed in percentage (%), Mean ± SEM (Standard error of the mean)

4.1.7 Body Weight

The initial body weights of the rats on Day 0 showed no significant differences among the experimental groups ($p>0.05$) (see Table 4.7). Throughout the study period, weekly assessments indicated that body weights remained statistically comparable across all groups at Day 7 (Week 1) and Day 14 (Week 2) ($p>0.05$) (Table 4.5). Overall, the 10 mg/kg, 100 mg/kg, and 1000 mg/kg treatment groups maintained body-weight patterns consistent with those of the control group.

Table 4.7: Body Weight (g) (Mean ± SEM)

Group	Day 0 (Start)	Day 7 (Week 1)	Day 14 (Week 2)
Control	166.68 ± 12.86	188.68 ± 7.72	198.64 ± 2.74
10 mg/kg	169.02 ± 8.66	195.96 ± 8.03	201.32 ± 0.60
100 mg/kg	132.19 ± 4.47	197.53 ± 8.35	200.95 ± 1.45
1000 mg/kg	155.41 ± 15.03	186.85 ± 2.68	199.51 ± 0.97

Results expressed in Mean ± SEM (Standard error of the mean)

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Discussion

The aim of the study was to evaluate the acute oral toxicity of CellifeIQ in male Wistar rats using the Lorke method. Results demonstrated that CellifeIQ is non-toxic, as no mortality was recorded even at the limit dose of 5000 mg/kg.The mild sedation and restlessness observed at higher doses were transient, dose-dependent, and resolved within hours, indicating low neurobehavioral toxicity.

The delayed itching observed between Days 2–14 in rats exposed to ≥1000 mg/kg may suggest a mild cutaneous or metabolic response, though non-life-threatening.Body weight changes, feed intake, water intake, and feed efficiency all remained statistically similar to the control, indicating no interference with metabolism or appetite.This profile is comparable to the report of Parasuraman (2011), who noted that herbal preparations with wide safety margins often produce minimal or self-limiting clinical signs during acute toxicity evaluation.

Gross post-mortem examination revealed no structural or morphological abnormalities in major organs. This indicates that acute exposure to CellifeIQ does not induce hepatotoxicity, nephrotoxicity, cardiotoxicity, or gastrointestinal toxicity. .The outcomes also correspond with the findings of El Hilaly *et al.* (2004), who observed that *Ajuga iva* extract produced no mortality in rodents at doses as high as 12 g/kg, with normal behaviour and physiological appearance maintained throughout the study. This similarity suggests that acute exposure to CellifeIQ, like non-toxic herbal preparations, does not induce hepatotoxicity, nephrotoxicity, cardiotoxicity, or gastrointestinal toxicity to the rats .

In the same line, Shehu *et al.* (2020) reported no mortality at doses up to 5000 mg/kg during their acute toxicity assessment of Pavonia senegalensis. Their study also indicated stable body weight and normal physical appearance during the observation period. The current findings closely reflect this pattern since CellifeIQ did not cause significant differences in body weight, feed intake, or water consumption compared with controls. These physiological parameters remained within normal ranges throughout the 14-day observation period, suggesting that metabolic disruption did not occur.

Furthermore, Dibua *et al. (*2022) observed no mortality during acute toxicity testing of *Nigella sativa and Moringa oleifera* seed extracts at doses up to 5000 mg/kg but reported the presence of histopathological liver changes at the highest dose. In contrast, the present study recorded no visible organ abnormalities during gross necropsy of major organs including the liver, kidney, spleen, heart, lungs, and gastrointestinal tract. This distinction may indicate a comparatively milder acute toxicological profile for CellifeIQ relative to some other plant-based extracts.

A report from Pharmaceutical Sciences Asia (2022) also classified a tested herbal preparation as practically non-toxic after establishing an LD_{50} greater than 5000 mg/kg. The consistency of these findings with the present study reinforces the position that CellifeIQ demonstrates a very wide safety margin in acute oral exposure.

Altogether, the results indicate that CellifeIQ exhibits very low acute toxicity, with no mortality, no major behavioural alteration, no impairment of feeding or hydration patterns, and no gross organ damage following single-dose administration.

5.2 Conclusion

CellifeIQ was observed to be non toxic, with an estimated LD_{50} greater than 5000 mg/kg. No mortality, significant organ damage, behavioural disturbances, or metabolic alterations were observed. Therefore, CellifeIQ can be considered safe under acute exposure conditions in male Wistar rats.

However, while the lack of acute toxicity is reassuring, further work is recommended: sub-acute and chronic toxicity studies should be conducted to assess the effects of prolonged or repeated administration. Additionally, more detailed histopathological and biochemical analyses (e.g., liver enzymes, kidney function, hematology) would be valuable to confirm that no subtle organ damage arises over time. Investigations into genotoxicity or reproductive toxicity would also help to fully establish the long-term safety profile of CellLifeIQ.

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