ACUTE TOXICITY STUDIES OF MAX GLUCAGON LIKE PEPTIDE (MAX GLP-1) ON MALE WISTAR RATS

BY

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CERTIFICATION

We the undersigned hereby certify that OSADOLOR DIVINE OSAWESE (BMS2201865) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

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DEDICATION

To the Hands that Shaped and raised Me

With deepest gratitude and love, I dedicate this project to those who have profoundly impacted my life and academic journey. To my loving parents, Mr. and Mrs. OSADOLOR, whose love, guidance, and sacrifices have been my greatest motivation. I also extend heartfelt gratitude to my friends, colleagues, and everyone who has supported and encouraged me throughout this journey. Your belief in me made this achievement possible. May this project be a testament to the love, hard work, and dedication that have brought me this far.

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) is an incretin hormone with potential therapeutic applications in metabolic disorders, including type 2 diabetes mellitus. While its pharmacological effects have been extensively studied, data on its acute toxicity profile remain limited. This study aimed to evaluate the acute oral toxicity of a MaxGLP-1 supplement in male Wistar rats using the Lorke method. Experimental animals were administered single oral doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg and monitored continuously for 24 hours and subsequently for 14 days to detect immediate, persistent, or delayed toxic effects. Observations included clinical signs, mortality, feed and water consumption, body weight changes, and external and internal organ examinations, supplemented by histopathological evaluation of the liver and spleen. No mortality occurred at any dose, establishing an LD_{50} greater than 5000 mg/kg. Immediate effects were mild and transient, including slight restlessness at 1000 mg/kg and mild sedation at higher doses, which resolved within hours. Delayed adverse effects were limited to intermittent mild irritation or itching in animals exposed to doses ≥1000 mg/kg. Feed and water intake, relative weight gain, feed efficiency, and body weight progression were not significantly altered ($p>0.05$) across all groups. External and internal examinations revealed no gross pathological changes, and histopathological analysis of liver and spleen at 1600 mg/kg showed no lesions. These findings indicate that MaxGLP-1 possesses low acute oral toxicity, with a conservative No Observed Adverse Effect Level (NOAEL) of 100 mg/kg in male Wistar rats. This study provides foundational safety data supporting the further preclinical development of MaxGLP-1 and shows the need for subsequent subacute and chronic toxicity evaluations to establish long-term safety profiles.

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CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Max GLP-1 is a novel dietary supplement formulated to enhance metabolic balance and promote glucose regulation by mimicking or stimulating the physiological effects of the endogenous incretin hormone, glucagon-like peptide-1 (GLP-1). GLP-1, naturally secreted from intestinal L-cells in response to nutrient intake, plays a critical role in maintaining glucose homeostasis through stimulation of insulin secretion, inhibition of glucagon release, and delayed gastric emptying (Jamali & Soni, 2023). Max GLP-1 supplement has been developed as a nutraceutical blend containing bioactive compounds intended to modulate similar pathways, providing non-pharmacological support for metabolic health, weight management, and oxidative balance.

Recent research has shown that GLP-1 and its receptor agonists exert pleiotropic physiological effects across multiple organ systems, including cardiovascular, renal, and central nervous systems (Abood *et al.,* 2021). Drawing from this foundation, supplements such as Max GLP-1 have been formulated to harness comparable mechanisms using natural or semi-synthetic bioactive ingredients that influence GLP-1 signaling, antioxidant defenses, and energy metabolism. For example, phytoconstituents capable of enhancing incretin activity, reducing oxidative stress, or modulating insulin sensitivity have been incorporated to achieve systemic metabolic improvement.

Preclinical findings have demonstrated that GLP-1 mimetics or enhancers may possess cytoprotective, anti-inflammatory, and antioxidant properties (Abood *et al.,* 2021; El Amin Ali *et al.,* 2022). These benefits are particularly relevant to supplements like Max GLP-1, which aim to deliver such physiological effects through safe, non-drug formulations. However, despite its potential advantages, the biological activity of Max GLP-1—mediated through complex biochemical and cellular pathways—necessitates thorough toxicological evaluation to confirm its safety profile, especially during acute exposure.

Pharmacokinetic and toxicological investigations of GLP-1-based compounds have revealed diverse absorption and systemic effects (Jamali & Soni, 2023). Similarly, supplements containing bioactive GLP-1 modulators could exhibit dose-dependent biological variability, making it essential to evaluate their safety margins. While many GLP-1 analogues and mimetics demonstrate mitochondrial protection and anti-apoptotic benefits (Atef *et al.,* 2023), supra-physiological or excessive exposure to bioactive components may induce oxidative imbalance or organ stress.

Given this background, it is scientifically relevant to assess the acute toxicity of Max GLP-1 in male Wistar rats. This will help determine whether its constituent bioactives pose any toxicological risks at single or varying doses. Male Wistar rats are a standard preclinical model for such studies due to their genetic stability, physiological resemblance to humans, and well-characterized toxicological responses. Therefore, this study aims to evaluate the acute toxicity of Max GLP-1 supplement in male Wistar rats, providing foundational evidence for its safety and dosage optimization in nutraceutical and therapeutic applications.

1.1 STATEMENT OF PROBLEM

In recent years, GLP-1–based supplements such as Max GLP-1 have gained widespread popularity for weight management and metabolic support. Although these products are marketed as natural and safe, there is limited scientific evidence validating their safety profiles, particularly regarding acute toxicity. Most available information comes from manufacturers ’claims rather than independent laboratory studies.

This lack of verified data poses a potential public health concern, as consumers may be exposed to unknown risks when using these supplements. Therefore, there is a need to conduct a controlled acute toxicity study using animal models to determine the safety margin of Max GLP-1 and establish whether its consumption poses any immediate adverse effects.

1.2 JUSTIFICATION OF THE STUDY

The rising use of GLP-1-based nutraceuticals such as Max GLP-1 underscores the need for systematic toxicological assessment to establish their safety margins. Although several studies have confirmed the beneficial metabolic, antioxidant, and cardiovascular roles of GLP-1 receptor agonists, limited data exist regarding the potential adverse effects of GLP-1-inspired supplements following acute exposure (Jamali & Soni, 2023; El Amin Ali *et al.,* 2022). Because Max GLP-1 is designed to interact with key metabolic and signaling pathways—including those regulating glucose metabolism, mitochondrial activity, and oxidative stress—its biological potency warrants rigorous evaluation.

Additionally, compounds with strong antioxidant and metabolic-modulating properties can exhibit paradoxical pro-oxidant or cytotoxic effects at high doses (Abood *et al.,* 2021; Atef *et al.,* 2023). Hence, assessing acute toxicity is crucial for determining safe dosage limits, identifying sensitive organs, and preventing potential adverse events in future clinical or consumer use.

Male Wistar rats serve as an ideal model for this evaluation, given their stable metabolic profiles and established baseline data for biochemical and histopathological analysis. Furthermore, the growing popularity of Max GLP-1 as a metabolic supplement for weight management and glycemic regulation justifies the need to verify its short-term safety. Therefore, this study is justified as a foundational preclinical step toward confirming the safety margin, tolerability, and acute toxicological profile of Max GLP-1.

1.3 AIM OF THE STUDY

The main aim of this study is to evaluate the acute toxicity profile of Max GLP-1 supplement in male Wistar rats.

1.4 OBJECTIVES OF THE STUDY

1. Assess the behavioral and clinical signs of toxicity including changes in behavior, locomotion, respiration, posture and physical appearance in wistar rats following acute administration of Max GLP-1.

2. To evaluate the effects of Max GLP-1 on body weight, as well as food and water consumption, during the observation period.

3. To determine the mortality rate and estimate the median lethal dose (LD_{50}) of Max GLP-1 in male Wistar rats.

4. To conduct gross pathological examinations of key organs (liver, kidneys, heart, lungs, and spleen) to detect any visible toxic effects.

1.5 RESEARCH QUESTIONS

1. Does acute administration of Max GLP-1 produce observable changes in behaviour, locomotion, respiration, posture or physical appearance in male Wistar rats?
2. What effect does acute Max GLP-1 have on body weight, daily food intake and water consumption during the observation period?
3. What is the mortality rate and estimated median lethal dose (LD_{50}) of Max GLP-1 in male Wistar rats after a single acute dose?
4. Do gross pathological examinations of liver, kidneys, heart, lungs and spleen reveal visible toxic effects after acute Max GLP-1 exposure?

1.6 SCOPE AND LIMITATION OF THE STUDY

This research focuses exclusively on the acute toxicity evaluation of Max GLP-1 supplement in male Wistar rats. The scope includes behavioral observation within a short-term exposure period following single-dose administration. This study does not encompass chronic, sub-chronic, reproductive, or genotoxic investigations, as these would require prolonged exposure and more extensive experimental setups.

The findings are limited by interspecies differences in metabolism and bioavailability, which may affect the direct extrapolation of results to human use. Variations in gastrointestinal absorption, peptide metabolism, and receptor distribution between rats and humans could influence toxicity thresholds. Moreover, due to financial and logistical constraints, the experiment may employ a restricted dose range, potentially affecting the precision of LD_{50} estimation.

Despite these limitations, this study remains an essential step in establishing the preclinical safety and toxicological baseline of Max GLP-1 supplement, ensuring scientific validation before human consumption or further pharmacological testing.

CHAPTER TWO

LITERATURE REVIEW

2.1 CONCEPT OF TOXICITY AND ITS TYPES

Toxicity refers to the degree to which a substance can cause harm to a living organism. It is a measure of the adverse effects produced when a chemical, drug, or compound interacts with biological systems. In toxicology, the harmful response of an organism depends on the dose, duration of exposure, route of administration, and the susceptibility of the test subject. Toxicity studies are therefore essential in assessing the safety of new drugs, food additives, herbal preparations, and environmental chemicals.Toxicity can be classified based on the duration and nature of exposure as follows:

2.1.1 Acute Toxicity

Acute toxicity refers to the harmful effects that occur after a single dose of a substance, or multiple doses given within a short time, usually within 24 hours. It is the very first stage of toxicity testing done in animals before moving to long-term studies. The main purpose is to find out the approximate dose that can cause visible signs of illness, behavioral changes, or death in test animals.

In most cases, the results from acute toxicity studies are used to estimate the median lethal dose (LD_{50}) — which is the dose expected to kill 50% of the test animals. This helps researchers to classify how toxic a substance is and to decide what dosage is safe to use in future experiments. Apart from determining LD_{50}, acute toxicity studies also help identify the target organs that may be affected first when a substance enters the body.

Observations are usually made for 14 days, and during this period, the animals are closely monitored for any signs of toxicity such as restlessness, tremors, convulsions, weakness, changes in

feeding or movement, and even mortality. Afterward, the organs are examined to check for internal damage or abnormalities.

Acute toxicity tests are usually done according to internationally accepted guidelines such as those of the Organisation for Economic Co-operation and Development (OECD). These guidelines provide standardized procedures to ensure the results are reliable and comparable. (Anionye *et al.,* 2017)

2.1.2 Sub-Acute Toxicity

Sub-acute toxicity means the harmful effects that can come from taking a substance repeatedly over a short period, usually about two to four weeks. This kind of study helps to check if the substance can cause any damage when used every day for some time.

During sub-acute studies, animals are given the test substance daily at different doses. Their body weight, food and water intake, behaviour, and general health are monitored. After the study period, their organs are examined to check for any internal changes or damage.

The results help to find out the safe dose range of the substance and whether it causes any early toxic effects that may not show up in single-dose studies.

2.1.3 Sub Chronic Toxicity

Sub-chronic toxicity involves giving a substance repeatedly for a longer period, usually between one to three months. The aim is to see how continuous exposure affects the body over time.

In this type of study, the animals are observed closely for any signs of organ damage, behavioural changes, or weight loss. Blood samples are taken to check for changes in biochemical and haematological parameters, which can reveal early signs of toxicity in organs like the liver, kidney, and heart.This study helps to identify the organs most affected by the compound and gives an idea

of what may happen if it is used for an extended period. It also helps to predict the possible long-term risks before testing in humans.

2.1.4 Chronic Toxicity

Chronic toxicity occurs when harmful effects develop after long-term or lifetime exposure to a substance. This type of study can last from six months to two years, depending on the animal species used.

It helps to show the cumulative effects of a substance and whether it can cause long-term problems like organ failure, reproductive issues, or cancer. The animals are given smaller doses over a long period, and their growth, behaviour, reproduction, and internal organs are regularly monitored.

Chronic studies are very important for drugs that may be used for a long time. For example, in GLP-1 and its analogues, chronic testing helps to confirm that continuous use will not cause serious side effects or damage to vital organs.

2.2 EXPERIMENTAL MODELS IN TOXICITY STUDIES

2.2.1 Characteristics of Wistar Rats

Wistar rats are widely used in biomedical and toxicological research due to their well-characterized physiology, docile nature, and ease of handling. They share several metabolic and physiological similarities with humans, making them a suitable model for studying the toxic effects of drugs and chemicals.

2.2.2 Rationale for Using Male Rats in Toxicity Studies

Male Wistar rats are often preferred in toxicity testing to minimize the influence of hormonal variations associated with the estrous cycle in females, which may affect metabolism and response

to toxic substances. Their use ensures more consistent and reliable results during dose-response assessments.

2.3 INTRODUCTION TO MAX GLP-1

Max GLP-1 is a nutraceutical formulation designed to support healthy glucose metabolism, appetite regulation, and metabolic balance through natural bioactive ingredients. Unlike pharmaceutical GLP-1 receptor agonists, Max GLP-1 does not act as a synthetic hormone. Instead, its ingredients — such as citrus bioflavonoids (Eriomin®), red sorghum polyphenols (ReDaxin™), postbiotics (PoZibio®), and trace minerals like chromium — work by enhancing the body's natural GLP-1 secretion and improving insulin sensitivity.

The product is commonly marketed for weight management, glucose control, reduced cravings, and overall metabolic wellness. Its multi-ingredient composition allows it to modulate various metabolic pathways without exerting strong pharmacologic effects, giving it a favourable safety profile.

2.4 PHYSIOLOGICAL ROLES OF MAX GLP-1

Since Max GLP-1 is a supplement and not a hormone, its "physiological roles" refer to the biological outcomes it promotes by stimulating natural GLP-1 activity and improving metabolic processes. These roles include:

1. Enhancement of Endogenous GLP-1 Secretion

Bioflavonoids and postbiotics from the formulation stimulate enteroendocrine L-cells in the intestine. This increases mild, physiological GLP-1 release after meals, aiding metabolic control.

2. Appetite and Satiety Regulation

By supporting GLP-1's natural signalling, the supplement reduces hunger signals, slows gastric emptying, and increases feelings of fullness. This contributes to decreased caloric intake and better weight regulation.

3. Improved Glucose Metabolism

Chromium picolinate enhances insulin receptor activity, while citrus and sorghum polyphenols promote better glucose uptake. Together, they support healthy fasting and post-prandial glucose levels.

4. Anti-Inflammatory and Antioxidant Effects

Ingredients such as ReDaxin™ activate antioxidant pathways like Nrf2, reducing oxidative stress and inflammation — both of which are linked to insulin resistance.

5. Gut Microbiota Support

Postbiotic compounds (PoZibio®) enhance microbial balance, increasing short-chain fatty acid production (e.g., butyrate), which can further stimulate GLP-1 release and improve gut integrity.

2.5 ORIGIN AND STRUCTURE OF MAX GLP-1

Origin

Max GLP-1 originates from the development of multi-nutrient metabolic supplements designed to emulate some benefits of GLP-1 physiology without synthetic pharmacotherapy. It is part of a broader trend in nutraceutical science where natural extracts (citrus, sorghum, probiotic/postbiotic derivatives, and trace minerals) are combined to support metabolic health.

The core ingredients are derived from:

- Citrus fruits (Eriomin® bioflavonoids)

- Red sorghum (ReDaxin™ 3-deoxyanthocyanidins)
- Fermented microbial cultures (postbiotics such as PoZibio®)
- Minerals (chromium picolinate)

Structure

Max GLP-1 does not have a defined chemical or peptide structure like liraglutide or semaglutide.

Instead, its "structure" refers to its formulation architecture, consisting of:

1. Polyphenolic complexes – anthocyanidins, flavanones, hesperidin derivatives
2. Postbiotic molecular fragments – microbial metabolites, short-chain fatty acids
3. Mineral chelates – chromium bound to picolinic acid
4. Antioxidant phytonutrients – luteolin, quercetin, naringin, ferulic acids

These components interact synergistically to produce mild GLP-1–enhancing and metabolic-supportive effects.

2.6 THERAPEUTIC IMPORTANCE OF MAX GLP-1

Max GLP-1 has gained attention in weight-management and metabolic-health research due to its multifaceted physiological benefits. Although not a medication, its therapeutic importance is supported by the documented effects of its individual ingredients.

1. Weight Management Support

By enhancing satiety, controlling appetite, and improving metabolic rate, Max GLP-1 can assist individuals dealing with overeating, cravings, and slow weight loss.

2. Glycemic Control

Ingredients like Eriomin® and chromium picolinate help reduce fasting glucose, improve insulin sensitivity, and support balanced post-meal glucose levels. This makes the supplement useful for prediabetes, insulin resistance, and metabolic syndrome.

3. Cardiometabolic Protection

Natural antioxidants from sorghum (ReDaxin™) and citrus flavonoids reduce oxidative stress and inflammation — key drivers of cardiovascular risk and chronic metabolic disease.

4. Gastrointestinal Health

Postbiotics improve gut integrity, reduce inflammation, and enhance beneficial gut–brain signalling pathways including GLP-1 and PYY, which influence appetite and glucose balance.

5. Non-Pharmacological Alternative

For individuals who cannot access or tolerate pharmaceutical GLP-1 analogues due to cost, side effects, or medical restrictions, Max GLP-1 provides a gentler, accessible, and safer alternative.

2.7 MAX GLP-1 SUPPLEMENTS: COMPOSITION AND PURPOSE

Max GLP-1 is a dietary supplement designed to "boost GLP-1 levels naturally" and promote appetite control, metabolic balance, and healthy weight management. It typically contains a blend of functional ingredients including Eriomin® (eriocitrin complex), chromium picolinate, postbiotic compounds (PoZibio®), and ReDaxin™ (red sorghum extract). Each of these components contributes distinct bioactive properties intended to support glucose regulation and metabolic health.

2.7.1 INGREDIENT-LEVEL EVIDENCE

2.7.2 Eriomin® / Eriocitrin complex

Eriomin® is a standardized lemon flavonoid complex rich in eriocitrin. Clinical and preclinical studies have shown that eriocitrin can modestly improve glycaemic control and increase endogenous GLP-1 levels. In a 12-week randomized placebo-controlled human study, supplementation with 200 mg/day Eriomin® significantly reduced fasting glucose and increased circulating GLP-1. Toxicological evidence indicates a low acute toxicity profile in rodent models, with no lethality or organ damage observed at doses several times higher than human exposure levels.

2.7.3 Chromium picolinate

Chromium is an essential trace element that potentiates insulin action and may aid glucose tolerance. Acute-toxicity studies reveal that chromium picolinate has a wide margin of safety; single oral doses up to 2000 mg/kg in rodents caused no mortality. Human trials report mild gastrointestinal effects at high doses but no acute toxicity at nutritional intake levels. Although its effect on GLP-1 secretion is indirect, chromium contributes to improved metabolic efficiency and energy balance in supplement formulations.

2.7.4 Postbiotics (PoZibio® and related compounds)

Postbiotics are non-viable microbial preparations or metabolic by-products that influence intestinal health. Studies show they can enhance gut barrier integrity, reduce inflammation, and indirectly support enteroendocrine function. While human data specific to PoZibio® are limited, available animal research on comparable postbiotics indicates no acute toxic effects at high oral doses. Postbiotics may support endogenous GLP-1 release through improved gut microbiota metabolism and short-chain fatty acid production.

2.7.5 ReDaxin™ (Red sorghum extract)

ReDaxin™ is a proprietary extract derived from a non-GMO red sorghum variety, standardized for its rich content of polyphenols — particularly 3-deoxyanthocyanidins, luteolin, and quercetin. These compounds possess strong antioxidant and anti-inflammatory properties that enhance cellular defense via the Nrf2 signalling pathway.

Recent clinical studies have shown that ReDaxin™ supplementation (250–500 mg/day) improves muscle recovery and reduces inflammation after exercise, confirming its bioactivity and tolerability. It is certified as GRAS (Generally Recognized As Safe) and non-toxic at standard doses.

Although no direct study links ReDaxin™ to GLP-1 elevation, its antioxidant and gut-modulating mechanisms may indirectly support GLP-1 pathways by reducing oxidative stress and improving metabolic balance. Importantly, no cases of acute toxicity or adverse reactions have been reported in either human or animal studies involving ReDaxin™, but formal acute-toxicity evaluation of the compound remains limited, warranting further investigation.

2.8 Previous studies on Max GLP-1 and its analogues (acute-toxicity focus)

Currently, no peer-reviewed acute-toxicity studies exist on the complete Max GLP-1 formulation. Data from its individual ingredients (eriocitrin, chromium picolinate, PoZibio®, and ReDaxin™) indicate low acute-toxicity profiles and wide safety margins at supplement-relevant doses.

In comparison, pharmacologic GLP-1 receptor agonists such as liraglutide and semaglutide have extensive acute- and repeat-dose toxicology data. These agents show predictable dose-dependent gastrointestinal effects (nausea, vomiting, reduced appetite) but no acute systemic toxicity at therapeutic doses. Although these drug analogues differ mechanistically from nutraceuticals, they highlight the importance of monitoring potential GLP-1-related physiological responses — such as decreased food intake, dehydration, and mild renal strain — even when evaluating natural GLP-1 stimulants.

Overall, the available literature underscores the need for formal acute-toxicity testing of Max GLP-1 to confirm safety and establish an evidence-based dose range for preclinical and future clinical applications.

2.8.1 Limitations in existing literature

- Limited number of independent toxicological studies on Max GLP-1 and its ingredient combinations.
- Absence of standardized dosing parameters across studies.
- Most available data come from manufacturer-sponsored or short-term trials.
- Lack of direct evaluation of GLP-1 response for ReDaxin™ and PoZibio®.

2.8.2 Knowledge gaps and rationale for the present study

The absence of comprehensive acute-toxicity data for the full Max GLP-1 formulation presents a major gap in safety documentation. While individual components have demonstrated tolerability, the interaction effects within the combined supplement remain unverified.

Therefore, this study is designed to assess the acute oral toxicity of Max GLP-1 in animal models, providing empirical evidence on its short-term safety, possible target-organ effects, and establishing its LD_{50} value in accordance with OECD guidelines.

2.9.1 Mechanisms contributing to safety

1. Natural bioactive modulation:

   Compounds such as Eriomin® and ReDaxin™ contain flavonoids and polyphenols that activate antioxidant pathways (notably Nrf2 and AMPK) and support normal glucose

metabolism without overstimulating the pancreas or liver. This ensures metabolic benefits without cellular stress or toxicity.

2. Controlled insulin sensitivity:

   Chromium picolinate enhances insulin receptor activity but does not directly induce insulin secretion. Its action is self-limited and dependent on glucose availability, which prevents hypoglycemia and maintains glucose balance.

3. Gut-protective postbiotic effects:

   PoZibio® and related postbiotic compounds support healthy gut microbiota, increase short-chain fatty acid production, and improve intestinal integrity. These effects reduce systemic inflammation and minimize the risk of gastrointestinal irritation or toxicity.

4. Absence of xenobiotic metabolism:

   Since Max GLP-1 components are largely dietary derivatives, they are metabolized through normal nutritional pathways and excreted efficiently. This limits accumulation in vital organs such as the liver and kidney, reducing the chance of organ-specific toxicity.

2.9.2 Potential mechanisms of toxicity

While Max GLP-1 is generally regarded as safe at recommended doses, potential toxicity may arise from excessive intake or ingredient interactions:

1. Oxidative or hepatic overload:

   Overconsumption of polyphenol-rich extracts (such as ReDaxin™) may increase hepatic metabolic load, potentially leading to mild liver enzyme elevation in sensitive individuals.

2. Electrolyte imbalance:

   High doses of chromium picolinate can interfere with iron or zinc absorption, and prolonged excessive intake could disturb electrolyte balance, although this is uncommon.

3. Gastrointestinal irritation:

   Rapid changes in gut microbiota from concentrated postbiotic ingestion may cause transient bloating, loose stools, or mild discomfort — effects typically reversible upon dose adjustment.

4. Hypoglycemic tendency:

   Although mild, the supplement's glucose-lowering effect could potentiate low blood sugar levels when combined with diabetic medications or fasting.

Figure 1: Image of Max GLP-1. **Source:** *max international.com*

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS:

3.1. Test substance

MaxGLP-1 is a dietary supplement made from a blend of medicinal plants, minerals, and microbial components sourced from a licensed pharmacy. It is registered with Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC), and each batch carries confirmed production and expiration dates along with the manufacturer's intact seals.

3.2 Apparatus

Apparatus and Equipment 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:

- Beakers (50, 150, 250 ml) (Pyrex, England)
- Face mask
- Cuvettes (Pyrex, England)
- Universal bottles
- Test tubes (UNIBEN MEDBCH Dept., Nigeria)
- Gloves
- Dissecting kit (UNIBEN MEDBCH Dept., Nigeria)
- Paper tapes
- cardboard papers and pins
- Animal cages (UNIBEN MEDBCH Dept., Nigeria)

- Animal restrainers
- Oro-gastric Gavage (UNIBEN MEDBCH Dept., Nigeria)

3.3 Equipments

The following equipments were used in the course of this study

- Needles and syringes (1 ml, 2 ml, 5 ml, 10 ml)
- Cotton wool and methylated spirit
- Test tube racks (UNIBEN MEDBCH Dept., Nigeria)
- Refrigerator (Citizens PRC4246)
- 80-2 model Electric Centrifuge (B. Bran Scientific and Instrument Company, England)
- HH-W Constant Temperature Water Bath (B. Bran Sc. Inst. Company, England)

3.4 Chemical/Reagents

All chemicals and reagents used in this study were of high purity and obtained from accredited suppliers or their official representatives in Nigeria.

Distilled water

Methanol

Chloroform

Picric acid

3.5 Experimental Animals and Housing

Male Wistar rats weighing between 150 and 180 g were sourced from the Anatomy Department of the University of Benin. The animals were kept in standard polypropylene cages under controlled laboratory conditions, including a temperature range of 25–29°C, relative humidity of 50–70%, and a 12-hour light/dark cycle. They had free access to clean water and standard rat feed. All animals were allowed a 7-day acclimatization period before the experiment began. The handling and procedures followed the ethical standards outlined by the NRC (2011) and OECD Good Laboratory Practice guidelines

3.6: METHODS

3.6.1 Experimental Design(Lorke's method 1983)

This research was carried out as an in vivo study to evaluate the acute toxicity profile of MaxGLP-1 in male Wistar rats. The experimental procedure was adapted from the Lorke's standard method for acute toxicity testing. The study assessed the response of the animals to single oral doses of the supplement over the observation period. The acute toxicity assessment followed the two-phase approach described by Lorke (1983), using a total of fifteen healthy male Wistar rats observed for a 14-day period.

3.6.2 Phase one:Dose Range Finding

Twelve rats were assigned into four groups of three. Groups I–III received oral doses of 10, 100, and 1000 mg/kg of MaxGLP-1 respectively, while Group IV served as the control and received distilled water.

Each dose was calculated based on individual body weight and prepared by dissolving the required amount of the powdered supplement in 1 mL of distilled water immediately before use. Administration was done once via oral gavage.

Rats were monitored closely within the first 2 hours, intermittently during the first day, and daily thereafter for any behavioural changes, signs of toxicity, or death. Body weights were recorded on days 0, 7, and 14.

3.6.3 Phase Two: LD_{50} Determination

Based on Phase I results, three additional rats each received a single oral dose of 1600, 2900, or 5000 mg/kg. Individual doses were freshly weighed and dissolved in 1 mL of distilled water before administration. Care was taken not to exceed the recommended maximum oral volume of 2 mL per 100 g body weight.

Animals were continuously observed for the first 2 hours after dosing, monitored throughout the first 24 hours, and checked daily for 14 days. Observations included behaviour, posture, locomotor activity, feeding, water intake, and excretory patterns.

3.6.4 Body Weight Monitoring:

Weights were recorded on day 0, day 7, and day 14. Any significant reduction or stagnation in weight was considered a possible indicator of systemic toxicity.

LD_{50} Calculation:

The LD_{50} value was estimated using the arithmetic method:

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

where D_0 is the highest non-lethal dose and D_{100} is the lowest dose producing mortality.

3.7 Post-mortem Examination:

At the end of the 14-day observation period, surviving animals were euthanized under anesthesia. A gross necropsy was performed, and major organs (liver, kidney, heart, and lungs) were excised, examined, and weighed. Organ-to-body weight ratios were calculated and compared with control values to identify any organ-specific toxic effects.

3.8 Ethical Considerations

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

CHAPTER FOUR

RESULTS

4.1 Acute Toxicity Study of MaxGLP-1 Supplement

The acute oral toxicity of MaxGLP-1 was evaluated using the Lorke method. Experimental animals received single oral doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg of MaxGLP-1. No mortality occurred at any of these 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 dosing, and thereafter animals were observed daily for 14 days to identify any delayed toxicity or abnormal behavior.

Table 4.1.1 Survival and Immediate Transient Effects

Dose Group (mg/kg)	No. of Animals	No. of Deaths (Mortality)	Observed effects (Transients)

CONTROL	3	0	None
10	3	0	None
Phase1 100	3	0	None
1000	3	0	Slight restless (resolved within hours)
1600	1	0	Mild Sedation (resolved within hours)
Phase2 2900	1	0	Mild Sedation (resolved within hours)
5000	1	0	Mild Sedation (resolved within hours)

All observed immediate effects were mild, transient, and dose dependent. Symptoms resolved within a few hours after administration.

Table 4.1.2 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. & % 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	3	1	1	1	6 (42.9%)	Mild delayed itching at doses ≥1000 mg/kg
Irritation/Itching									

Irritation and itching were the only delayed clinical signs observed throughout the 14-day period. This effect appeared in animals exposed to doses at or above 1000 mg/kg, occurring intermittently between Day 2 and Day 14. No other delayed or persistent clinical abnormalities were recorded.

4.2 External and Internal Examination

The external examination of all animals showed that the skin and fur were normal, without lesions, alopecia, or discoloration. The eyes, nostrils, and oral cavity appeared clean and free from any discharge. The anus and genital region were normal, and the animals displayed normal locomotor activity throughout the observation period. No tremors, convulsions, abnormal secretions, or unusual behavioral patterns were recorded.

Internal examination revealed that the lungs were free from congestion or hemorrhage, and the heart maintained a normal size and coloration. The liver appeared normal with no evidence of necrosis or discoloration. The kidneys showed no signs of enlargement or hemorrhage, while the spleen exhibited a normal structure without enlargement. The stomach and intestines displayed healthy morphology, and the brain appeared normal without swelling or lesions. Other organs, including the pancreas, adrenal glands, and reproductive tissues, were also found to be normal. Histopathological examination of the liver and spleen at 1600 mg/kg showed no observable lesions.

4.3 Interpretation of No Mortality

No mortality occurred at any dose administered, establishing that the LD_{50} of MaxGLP-1 is greater than 5000 mg/kg. The transient effects observed were mild, dose dependent, and resolved quickly, while delayed itching occurred only in animals exposed to higher doses. No permanent, severe, or systemic toxic manifestations were identified during the entire 14-day observation period.

4.4 Effect of Acute toxicity of Max GLP-1 on feed and water consumption

The different single doses of the MaxGLP-1 caused no significant difference ($p>0.05$) in daily feed and water consumption of the rats when compared with the control group (Table 4.1 and 4.2). Similarly, the doses did not significantly alter feed or water consumption ($p>0.05$) across the groups (Table 4.1)

Table 4.1.3 Feed Consumed per Day per Rat (g)

Group	Feed Consumed
Control	23.56 ±0.00
10 mg/kg	27.06 ±0.00
100 mg/kg	25.38 ±0.00
1000 mg/kg	24.60 ±0.00

Table 4.1.4 Water Consumed per Day per Rat (ml)

Group	Water Consumed
Control	48.59 ±0.00
10 g/kg	52.06 ±0.00
100 mg/kg	54.38 ±0.00
1000 mg/kg	58.19 ±0.00

4.5 Effect of Acute toxicity of MaxGLP-1 on Relative weight gain/Loss per Rat per day

Relative weight gain per day per rat as well as feed efficiency was not significantly affected ($p>0.05$) by any of the treatments when compared with the control rats (Table 4.3 and 4.4). Similarly, there was also no significant difference ($p>0.05$) among the groups (Table 4.3).

Table 4.1.5 Relative Weight Gain/Loss per Rat per Day

Group	Weight Gain/Loss/day

Control	1.32 ±0.36
10 mg/kg	2.68 ±0.73
100 mg/kg	3.08 ±1.46
1000 mg/kg	1.20 ±0.37

Table 4.1.6 Feed Efficiency (%)

Group	Feed Efficiency (%)
Control	5.69 ±1.49
10 mg/kg	8.92 ±3.68
100 mg/kg	12.15 ±5.75
1000 mg/kg	4.87 ±1.48

4.6 Effect of Acute toxicity of MaxGLP-1 on Body weight (g)

Furthermore, the start body weights of the rats were comparable across all groups ($p>0.05$) at the beginning of the study (Table 4.5). No significant differences ($p>0.05$) were observed in body weight progression after the first and second weeks of treatment (Table 4.5)

Table 4.1.7 Weekly Body Weights (g)

Group	Day 0	Day 7	Day 14

Control	186.07 ±0.92	191.12 ±26.62	204.84 ±4.48
10 mg/kg	160.99 ±6.40	206.38 ±12.51	198.49 ±3.91
100 mg/kg	153.91 ±17.78	189.99 ±12.33	197.08 ±5.09
1000 mg/kg	183.54 ±3.61	202.30 ±18.73	200.33 ±1.55

Overall, administration of the test compound at 10, 100, and 1000 mg/kg did not significantly influence feed intake, Feed efficiency, relative weight gain, water consumption, or body weight of male Wistar rats during the study period.

CHAPTER FIVE

5.0 DISCUSSION

The acute toxicity assessment of MaxGLP-1 in male Wistar rats revealed a generally favourable safety profile across all administered doses, ranging from 10 to 5000 mg/kg. Using the Lorke method, no mortality was recorded at any dose level, indicating that the LD_{50} of MaxGLP-1 exceeds 5000 mg/kg. Substances with LD_{50} values above this threshold are typically considered to possess low acute toxicity potential, aligning with previous findings that GLP-1–related compounds often demonstrate wide safety margins and minimal lethality following single-dose exposure (Abood *et al.*, 2021; Bhat *et al.*, 2018). The complete absence of mortality therefore suggests that MaxGLP-1, like other GLP-1–based peptides and analogues, does not elicit severe systemic toxic effects when administered orally as a single high dose.

Clinical observations within the first 24 hours showed only mild and transient behavioural effects. Slight restlessness was noted at 1000 mg/kg, while mild sedation became apparent at higher doses (1600–5000 mg/kg). These transient behavioural changes resolved spontaneously within hours. Such temporary signs at extremely high doses are not uncommon in acute toxicity studies and may reflect nonspecific stress responses or short-lived central nervous system depression due to high oral loading. Importantly, these effects did not progress into sustained neurological deficits, as none of the animals exhibited convulsions, tremors, persistent agitation, abnormal gait, or any behavioural abnormalities during the full 14-day observation period. The lack of prolonged neurotoxicity is consistent with earlier toxicity profiles reported for GLP-1 analogues, which rarely produce lasting neurobehavioral disturbances after single administrations (Bhat *et al.,* 2018).

Throughout the 14-day monitoring phase, the only persistent or delayed effect observed was intermittent mild irritation or itching, which appeared in animals exposed to doses of 1000 mg/kg and above. No such effect occurred at 10 or 100 mg/kg, suggesting a clear dose threshold for this

response. The emergence of itching may reflect a high-dose inflammatory or immunological reaction, possibly involving histamine release or local hypersensitivity. Although the effect was mild and did not indicate systemic toxicity, its consistent appearance at high doses suggests that it should be regarded as a treatment-related adverse effect. For this reason, and in line with toxicological practice, the dose of 100 mg/kg, which produced no adverse signs, can be regarded as the conservative No Observed Adverse Effect Level (NOAEL) for acute exposure (HamaSalih *et al.,* 2024).

Both external examination and internal necropsy findings further reinforce the safety of MaxGLP-1. Externally, all animals maintained normal skin, fur, mucous membranes, and locomotor behaviour, with no abnormalities such as piloerection, salivation, ocular discharge, or motor impairment. Internally, all examined organs—including the heart, lungs, liver, kidneys, spleen, stomach, intestines, brain, pancreas, and reproductive tissues—appeared normal in size, texture, and coloration, with no signs of congestion, necrosis, hemorrhage, or other pathological lesions. Histological examination of the liver and spleen at 1600 mg/kg also showed no microscopic abnormalities. These findings strongly suggest that MaxGLP-1 does not produce structural organ damage at the tested doses, supporting previous observations that GLP-1–related molecules generally lack strong organotoxic effects in acute studies (Abood *et al.,* 2021).

In addition to behavioural and pathological assessments, physiological parameters such as feed intake, water consumption, relative weight gain, feed efficiency, and progressive body weight measurements were evaluated. Across all dose groups, none of these parameters differed significantly from the control. Stability of these indicators implies the absence of systemic metabolic distress, gastrointestinal toxicity, or impaired nutrient utilization. This stability also reflects the overall well-being of the animals despite exposure to high doses of MaxGLP-1. The maintenance of consistent body weight trajectories further supports the interpretation that MaxGLP-1 lacked acute metabolic or appetite-modulating toxicity in this experimental context.

While the results clearly indicate that MaxGLP-1 is safe in terms of acute toxicity, the study does carry inherent limitations typical of the Lorke method. Higher-dose groups used small sample sizes, which may reduce the ability to detect rare adverse outcomes. Additionally, histopathology was limited to the liver and spleen, meaning subtle lesions in other organs may have remained undetected. The absence of clinical chemistry and hematological analyses also limits the ability to rule out subclinical hepatic, renal, or hematological changes. Moreover, acute toxicity studies only reflect the effects of a single dose; thus, they do not predict the potential for cumulative toxicity, immunological effects, or organ-specific alterations that may appear following repeated dosing. For these reasons, further subacute and chronic toxicity studies remain essential to establish long-term safety.

This study shows that MaxGLP-1 exhibits low acute oral toxicity in male Wistar rats, with an LD_{50} greater than 5000 mg/kg and only mild, reversible behavioural effects at very high doses. The only notable adverse effect was delayed mild itching at doses of 1000 mg/kg and above, suggesting that a conservative acute NOAEL is 100 mg/kg. No significant alterations in physiological parameters, no mortality, and no gross or microscopic organ damage were observed, supporting the conclusion that MaxGLP-1 is safe under acute exposure conditions. These findings, consistent with previous reports on GLP-1–related compounds (Abood *et al.,* 2021; Bhat *et al.,* 2018; HamaSalih *et al.,* 2024), provide a strong foundation for advancing to repeated-dose toxicity studies required for further toxicological characterization and eventual therapeutic development.

5.1 CONCLUSION

The findings of this study demonstrate that MaxGLP-1 exhibits a wide margin of safety in male Wistar rats, with no mortality recorded at doses up to 5000 mg/kg and only mild, transient clinical signs observed at higher dose levels. Physiological parameters—including feed and water intake, weight gain, and feed efficiency—remained unaffected, while both gross and histopathological examinations showed no treatment-related organ damage. The only notable delayed effect was mild itching at doses ≥1000 mg/kg, establishing 100 mg/kg as the conservative acute NOAEL. Overall, MaxGLP-1 can be considered non-toxic under acute exposure conditions, providing strong preliminary evidence to support its further investigation in subacute and chronic toxicity studies to fully characterize its long-term safety profile.

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