EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON WEEKLY WEIGHT CHANGES OF RATS TREATED SUB-CHRONICALLY

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LSC2007385

PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

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AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled "**EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON WEEKLY WEIGHT CHANGES OF RATS TREATED SUB-CHRONICALLY**" was carried out by "**Grace Imuetinyan EGHONGHON**" with matriculation number "**LSC2007285**" and presented to the Department of Science Laboratory Technology, Faculty of life science, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Science Laboratory Technology.

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(External Examiner)	**DATE**

DECLARATION

I "Grace Imuetinyan EGHONGHON" declare that "EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON WEEKLY WEIGHT CHANGES OF RATS TREATED SUB-CHRONICALLY" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

………………………………….. ………………………….

Grace Imuetinyan EGHONGHON DATE

DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

ACKNOWLEDGEMENT

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ABSTRACT

The study investigated the sub-chronic effects of methanol leaf extract of Anthocleista grandiflora on the weekly body weight and organ weight indices of Wistar rats. A. grandiflora, commonly known as the Giant African Cabbage Tree, is widely used in African traditional medicine for treating malaria, liver disorders, and metabolic diseases. Despite its extensive ethnomedicinal applications, limited toxicological data exist regarding its long-term physiological effects. Fresh leaves of A. grandiflora were extracted with methanol, and twenty male Wistar rats were orally administered graded doses of 200, 400, and 800 mg/kg body weight for 28 days, with a control group receiving distilled water. Weekly body weight measurements and post-treatment organ weights (liver, kidneys, heart, spleen, and testes) were recorded. Statistical analysis was conducted using one-way ANOVA with significance at $p \leq 0.05$. Results showed no significant adverse effect on body or organ weights across all doses compared to control, suggesting that the methanol extract did not induce major systemic toxicity under the experimental conditions. The observed stable weight progression and normal organ-to-body weight ratios indicate a relatively safe toxicological profile of A. grandiflora at the tested doses.

The findings support its ethnomedicinal use and provide baseline data for further biochemical and histopathological safety evaluations.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants have long served as the cornerstone of traditional medicine around the world. According to the World Health Organization (2021), a large proportion of the population in developing countries continues to rely on herbal remedies for their primary health care needs. These plants contain an extensive variety of bioactive compounds such as alkaloids, tannins, flavonoids, saponins and terpenoids that are responsible for their therapeutic properties (Sofowora and Falade, 2018). Scientific research in recent years has increasingly focused on validating the pharmacological potential of these compounds, as well as establishing their safety profiles through animal models. Despite the growing evidence supporting the efficacy of herbal extracts, it remains equally critical to assess their safety across different doses and durations of administration, especially under sub-chronic exposure where cumulative effects may emerge (Chikezie *et al.,* 2020).

Anthocleista grandiflora, commonly known as the "Giant African Cabbage Tree", belongs to the family Loganiaceae and grows in the tropical forests of West and Central Africa. It is used traditionally in several countries for treating malaria, liver disorders, stomach complaints and fever (Okoye *et al.,* 2020). Phytochemical investigations have revealed that the leaf extract contains flavonoids, glycosides, tannins and alkaloids (Njoku *et al.,* 2020). Experimental studies with its methanol leaf extract have demonstrated antioxidant, hepatoprotective and anti-inflammatory activities in rodents (Njoku *et al.,* 2020; Ekennia *et al.,* 2018).

Body weight is one of the most fundamental indicators used in experimental toxicology and pharmacology to assess the overall health and metabolic stability of animals under investigation. It serves as a practical and sensitive endpoint in evaluating the potential toxic or therapeutic impact of a compound over time (Niyomchan *et al.,* 2023). In laboratory studies, changes in weight either as gain or loss are often the earliest observable physiological responses to a treatment. This makes weight monitoring a valuable non-invasive tool in detecting sub-chronic or chronic effects of plant extracts, drugs or chemicals (Baig *et al.,* 2022). A consistent increase in body weight usually reflects good health and normal metabolism, while a reduction or stagnation may signify systemic toxicity, malnutrition or organ dysfunction (Fujisawa, 2024).

In toxicological testing, sub-chronic studies (typically spanning 28 days or more) are essential for understanding the cumulative effects of a substance following repeated exposure. They help determine whether a compound exerts slow-acting toxic effects that may not be apparent in acute studies (Baig *et al.,* 2022). Weekly monitoring of body weight during such studies provides a clear picture of how test agents influence appetite, digestion, energy utilisation and tissue integrity. Alterations in weight gain patterns could indicate disruptions in protein synthesis, carbohydrate metabolism, lipid utilisation or endocrine regulation (Niyomchan *et al.,* 2023; Fujisawa, 2024). Therefore, the evaluation of weekly weight changes in experimental rats is not only a simple measure of growth but also a useful marker for assessing the physiological impact of bioactive compounds.

However, while these pharmacological properties have been documented, there is still a scarcity of detailed toxicological data especially concerning the influence on physiological parameters such as weekly body weight. Understanding how sub-chronic exposure to methanol leaf extract of *A. grandiflora* impacts weekly weight gain is crucial, because alterations in weight may reflect underlying metabolic or organ disturbances even before overt signs of toxicity appear (Fujisawa, 2024; Baig *et al.,* 2022). Repeated exposure to plant extracts can either promote weight gain, if the extract enhances appetite or metabolism, or induce weight loss, if it contains compounds that suppress appetite or disrupt nutrient absorption (Niyomchan *et al.,* 2023). Therefore, it becomes essential to investigate whether a methanol leaf extract *of A. grandiflora* causes progressive, regressive or neutral changes in body weight during sub-chronic exposure. The present study is designed to provide empirical data on the pattern of weekly weight changes in rats administered methanol leaf extract of *A. grandiflora* for a sub-chronic period. The findings will clarify whether the extract has any potential growth-promoting or toxic effects that could influence its safety and therapeutic use in humans.

1.2 AIM OF THE STUDY

The aim of the study was to evaluate the effect of methanol leaf extract of *Anthocleista grandiflora* on the weekly body weight changes of Wistar rats following sub-chronic oral administration.

1.3 OBJECTIVES OF STUDY

The specific objectives of this study are to:

- Determine the effect of methanol leaf extract of *Anthocleista grandiflora* on weekly body weight changes of different groups of rats following sub-chronic oral administration.
- Assess the effect of the extract on weight of various organs in the experimental model (wistar rats/mice).
- Evaluate the changes in weight of liver, left and right kidney, heart, left and right testes and spleen following extract administration.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL OVERVIEW

Medicinal plants have been central to human health and disease management for centuries, serving as the foundation for both traditional and modern therapeutic practices. According to the World Health Organization (WHO, 2021), over 80% of people in developing nations still depend on plant-based remedies as their primary source of healthcare. This persistent reliance is due to the accessibility, affordability, and cultural acceptance of herbal medicines (Adebayo et al., 2022). Medicinal plants are defined as species whose parts such as leaves, bark, seeds, roots, or fruits contain biologically active compounds that produce definite physiological effects on living organisms (Sofowora, 2020).

The effectiveness of medicinal plants is attributed to their rich composition of secondary metabolites, which include alkaloids, saponins, flavonoids, tannins, terpenoids, phenolic acids, and glycosides (Kumar *et al.,* 2020). These compounds are known to exhibit multiple pharmacological effects such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and antidiabetic activities (Okafor *et al.,* 2021). The growing interest in plant-based medicine has also led to the scientific validation of several traditional remedies, bridging the gap between indigenous knowledge and modern pharmacology (Nwosu *et al.,* 2019; Adesina *et al.,* 2023).

Within toxicological and pharmacological research, body weight monitoring has become an indispensable tool for assessing the safety and systemic effects of bioactive compounds. Body weight is considered a reliable, non-invasive indicator of general health and metabolic stability in animal models (Ibrahim *et al.,* 2021). The Organisation for Economic Co-operation and Development (OECD, 2018) guidelines recommend regular monitoring of weight in sub-chronic toxicity studies to detect early physiological changes that may indicate toxicity, metabolic stress, or therapeutic efficacy.

Sub-chronic studies typically involve repeated administration of a test substance for 28 to 90 days to evaluate the potential cumulative effects on organ systems and metabolism (Olorunnisola *et al.,* 2020). Weight loss during such studies may suggest adverse effects such as reduced food intake, hepatic dysfunction, or interference with nutrient absorption (Adewale *et al.,* 2019). Conversely, weight gain may reflect enhanced appetite, improved nutrient utilisation, or anabolic effects of the compound (Adedapo *et al.,* 2022). These data are critical for determining the no-observed-adverse-effect level (NOAEL) and for establishing safe dosage ranges in subsequent preclinical or clinical studies (OECD, 2018).

In the context of natural product research, weight monitoring helps establish whether a plant extract exerts beneficial metabolic modulation or poses a potential toxicity risk. Many herbal formulations traditionally used for fever, infections, or liver diseases are being reevaluated for their systemic effects, especially those that may influence lipid, glucose, and protein metabolism (Ogunyemi *et al.,* 2022). For instance, extracts rich in flavonoids and alkaloids have been found to enhance mitochondrial function and lipid oxidation, promoting healthy weight maintenance (Okonkwo *et al.,* 2021). However, other plant extracts may contain toxic phytochemicals that impair hepatic or renal function, leading to reduced food assimilation and weight decline (Akinyemi et al., 2020).

The evaluation of body weight during sub-chronic administration is, therefore, an integral part of assessing both the safety and pharmacological potential of medicinal plants. In particular, plants belonging to the Loganiaceae family, such as *Anthocleista grandiflora*, have been the subject of growing scientific interest due to their potent biochemical constituents and long-standing use in African ethnomedicine (Ezekwesili *et al.,* 2023). These plants are traditionally associated with treatments for fever, liver disorders, infections, and metabolic imbalances, making them suitable candidates for investigating physiological effects such as weekly weight variation under sub-chronic exposure (Ojo *et al.,* 2020; Njoku *et al.,* 2021).

Moreover, the solvent used in the extraction process, such as methanol, significantly influences the phytochemical composition and potency of herbal preparations (Ekennia *et al.,* 2019). Methanol extracts often yield a wider spectrum of bioactive compounds both polar and semi-polar that may exert measurable effects on metabolic rate, food conversion efficiency, and tissue growth (Ugwoke *et al.,* 2022). Consequently, sub-chronic studies involving methanol extracts not only aid in safety assessment but also help to clarify dose-dependent responses that could inform future clinical applications.

2.2 TAXONOMIC CLASSIFICATION OF *ANTHOCLEISTA GRANDIFLORA*

The genus Anthocleista belongs to the family Loganiaceae, a taxonomic group composed mainly of tropical trees and shrubs known for their medicinal and ornamental value. Members of this family are distributed primarily across tropical Africa, Asia, and parts of South America (Ibrahim *et al.,* 2021). The family Loganiaceae is characterized by plants with opposite leaves, interpetiolar stipules, and tubular, often fragrant flowers (Olorunnisola *et al.,* 2020).

Kingdom: Plantae

Division: Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Order: Gentianales

Family: Gentianaceae

Genus: Anthocleista Afzel. Ex R.Br.

Species: *Anthocleista grandiflora Gilg*

Within the genus, *A. grandiflora* is distinguished by its large, glossy leaves and white, tubular flowers. Taxonomically, it shares close relations with species such as *A. djalonensis*, *A. vogelii*, and *A. schweinfurthii*, which also exhibit significant ethnomedicinal applications (Ezeokonkwo *et al.,* 2022). However, *A. grandiflora* stands out for its height reaching up to 30 meters and its broad ecological adaptability, which supports its distribution across various African forest and savannah ecosystems (Okonkwo *et al.,* 2023).

Plate 1: *Anthocleista grandiflora* **Photocredit:** (Eghonghon Grace, 2025).

2.3 BOTANICAL DESCRIPTION OF *ANTHOCLEISTA GRANDIFLORA*

Anthocleista grandiflora Gilg, commonly known as the Giant African Cabbage Tree, is a tall, deciduous tree native to the tropical regions of Africa. It is one of the largest members of the family Loganiaceae and is easily recognized by its massive leaves and distinctive trunk structure (Ezeokonkwo *et al.,* 2022). The species thrives in moist, fertile soils and is most often found along riverbanks, forest margins, and swampy lowlands (Ibrahim *et al.,* 2021).

Morphologically, A. grandiflora can grow to heights of 20–30 meters, with a straight, cylindrical trunk that may reach up to 60 cm in diameter. The bark is pale gray to light brown, smooth when young, but becomes fissured and scaly with age (Okonkwo *et al.,* 2023). The branches are few and widely spaced, often forming a rounded crown that supports large clusters of leaves at the ends of twigs.

The leaves are among the most striking features of the plant. They are simple, opposite, and entire, measuring between 30–100 cm in length and 15–40 cm in width in mature trees, although in younger plants, the leaves can grow even larger. The leaf blade is elliptic to obovate, with a glossy, deep-green upper surface and a paler underside. The venation is prominent and pinnate, with 10–15 pairs of lateral veins. The petioles are stout, measuring between 2–5 cm long, and exude a slightly bitter sap when cut (Ogunyemi *et al.,* 2022).

The inflorescence of *A. grandiflora* is terminal, forming large, fragrant clusters of white, tubular flowers that bloom mainly during the wet season (May to August in most tropical zones). Each flower measures about 5–7 cm in diameter, with a long corolla tube and four to five lobes spreading at the tip (Njoku *et al.,* 2020). The calyx is green, short, and cup-shaped, while the corolla tube is cream-white, with a pleasant scent that attracts pollinators such as bees and butterflies (Ekennia et al., 2018). The stamens are inserted within the corolla tube, and the ovary is superior, leading to the formation of fleshy, ellipsoidal fruits upon fertilization.

The fruit is a smooth, yellowish berry measuring about 3–5 cm in length, containing numerous small, flat seeds embedded in a pulpy matrix. The seeds are brown and winged, which aids in dispersal by wind or animals. The fruit ripens during the dry season and serves as food for various bird species, contributing to seed dispersal across wide ecological zones (Adebayo *et al.,* 2021).

The root system of *A. grandiflora* is well-developed and penetrates deeply into the soil, providing strong anchorage and efficient nutrient absorption. The root bark is bitter and has been traditionally used in decoctions for treating gastrointestinal disorders and fever (Okeke *et al.,* 2022). The tree exudes a latex-like sap when cut, a characteristic common to members of the Loganiaceae family.

Anatomically, studies on the leaf microstructure reveal the presence of trichomes, calcium oxalate crystals, and well-defined palisade and spongy mesophyll layers (Ibrahim *et al.,* 2021). These features play a role in photosynthesis efficiency, water conservation, and defense against herbivory. Phytochemical localization studies have shown that alkaloids and flavonoids are concentrated mainly in the leaf epidermis and mesophyll tissues, while tannins are abundant in the bark and root cortex (Olorunnisola *et al.,* 2020).

The phenology of *A. grandiflora* shows that it sheds its leaves during the dry season, followed by the development of new foliage before flowering resumes. This adaptive cycle allows the plant to conserve water and energy during unfavorable climatic conditions. The species' longevity, estimated at over 50 years in the wild, contributes to its dominance in forest ecosystems where it forms part of the upper canopy (Ezeokonkwo *et al.,* 2022).

Ecologically, *A. grandiflora* plays an important role in maintaining forest balance. Its large leaves contribute significantly to litter fall and nutrient recycling, enriching the soil. The tree also provides shade, habitat, and nesting grounds for various bird and insect species (Okonkwo *et al.,* 2023). Because of its robust growth and adaptability, it is sometimes used in agroforestry systems and reforestation programs to prevent erosion and restore degraded lands (Ogunyemi et al., 2022).

2.4 DISTRIBUTION AND ECOLOGY OF *ANTHOCLEISTA GRANDIFLORA*

Anthocleista grandiflora is indigenous to tropical Africa and has a wide geographical distribution across West, Central, East, and Southern Africa. It occurs naturally in countries such as Nigeria, Ghana, Cameroon, Sierra Leone, Gabon, Uganda, Tanzania, Kenya, and South Africa (Ibrahim *et al.,* 2021; Okonkwo *et al.,* 2023). The species shows a high degree of ecological adaptability, thriving in moist lowland rainforests, riverine woodlands, swampy valleys, and montane forests at altitudes ranging between 200 and 2,200 meters above sea level (Adebayo *et al.,* 2021).

In West Africa, particularly in Nigeria, *A. grandiflora* is common in the rainforest and derived savanna zones, especially along riverbanks and near freshwater swamps in states such as Cross River, Rivers, Delta, and Edo (Ezeokonkwo *et al.,* 2022). The species prefers deep, well-drained, loamy soils rich in organic matter and thrives under conditions of moderate to high rainfall, typically exceeding 1,200 mm per annum (Ogunyemi *et al.,* 2022). It is often found in association with other tropical trees such as *Cola acuminata*, *Alstonia boonei*, and *Khaya ivorensis*, which share similar ecological niches (Olorunnisola *et al.,* 2020).

In Central Africa, particularly in Cameroon and the Democratic Republic of Congo, *A. grandiflora* occurs both in primary and secondary forests, where it contributes significantly to the upper canopy layer (Iwu, 2019). The tree also colonizes disturbed habitats such as fallow lands and abandoned farmlands, reflecting its resilience and ability to regenerate from root suckers and seed dispersal (Ibrahim *et al.,* 2021). In East Africa, populations are widely distributed in Kenya, Uganda, and Tanzania, particularly in forest reserves and along riparian zones where the humidity and soil fertility support vigorous growth (Njoku *et al.,* 2020). Further south, in South Africa and Zimbabwe, the tree is found mostly in coastal and submontane forests, showing adaptability to both tropical and subtropical conditions (Ezeokonkwo *et al.,* 2022).

Ecologically, *A. grandiflora* plays a vital role in maintaining forest stability. Its broad leaves provide shade, helping to regulate understorey microclimates by reducing evaporation and conserving soil moisture (Okonkwo *et al.,* 2023). The leaf litter decomposes rapidly due to its high nitrogen content, thereby improving soil fertility and facilitating nutrient cycling (Ogunyemi *et al.,* 2022). Additionally, its dense root system contributes to soil stabilization, making it valuable for erosion control along riverbanks and in reforestation programs (Ekennia *et al.,* 2018).

The tree is also important for biodiversity conservation. Its flowers attract a variety of pollinators such as bees, butterflies, and moths, while its fruits are eaten by birds, bats, and small mammals, which play a crucial role in seed dispersal (Adebayo *et al.,* 2021). In some forest ecosystems, the species serves as a keystone component supporting diverse faunal populations by offering nesting sites and protection (Okeke *et al.,* 2022).

Climatically, *A. grandiflora* thrives in regions with average annual temperatures between 20°C and 30°C and relative humidity levels above 60% (Ibrahim *et al.,* 2021). Although it prefers humid environments, it can tolerate brief dry spells due to its deep taproot system and efficient water-storage capacity within its tissues (Olorunnisola *et al.,* 2020). It is less tolerant of prolonged drought or waterlogging, but young plants can regenerate rapidly once favourable moisture conditions return.

Human activities have also influenced the plant's distribution. Overharvesting for medicinal use, deforestation, and land conversion have reduced its natural populations in some regions of Nigeria and Ghana (Ezeokonkwo *et al.,* 2022). However, efforts are ongoing to encourage sustainable utilization and cultivation through community-based agroforestry and medicinal plant conservation programs (Okonkwo *et al.,* 2023). Its fast growth rate and adaptability make it suitable for domestication, and it is now occasionally cultivated in herbal gardens and research stations for pharmacological studies (Njoku *et al.,* 2020).

2.5 ETHNOMEDICINAL AND TRADITIONAL USES OF *ANTHOCLEISTA GRANDIFLORA*

For centuries, *Anthocleista grandiflora* has occupied a prominent place in African traditional medicine due to its diverse therapeutic applications. Its ethnomedicinal value is deeply rooted in indigenous knowledge systems across West, Central, and East Africa, where different parts of the plant particularly the leaves, bark, roots, and fruits are employed in managing a wide variety of ailments (Okonkwo *et al.,* 2023; Ibrahim *et al.,* 2021). Traditional healers consider it a "multi-purpose remedy" owing to its perceived potency in treating conditions that involve fever, inflammation, infections, and liver dysfunction (Njoku *et al.,* 2020; Ezeokonkwo *et al.,* 2022).

In Nigeria, the plant is locally known as "Ukwu-ogwu" among the Igbo, "Apata" among the Yoruba, and "Gwada"among the Hausa. Herbal practitioners use decoctions of the leaves or bark to manage malaria, jaundice, diabetes, typhoid fever, and intestinal worms (Olorunnisola *et al.,* 2020; Adebayo *et al.,* 2021). The leaf extract is also taken orally to relieve constipation, promote digestion, and restore appetite, especially following prolonged illness (Ibrahim *et al.,* 2021). In some communities of South-Eastern Nigeria, the root extract is used in the treatment of liver enlargement, chronic fatigue, and as a blood purifier (Ezeokonkwo *et al.,* 2022).

In Ghana, the plant is called "Boduro" or "Oduro" and forms part of the traditional therapeutic regimen for malaria, anemia, and reproductive disorders. Decoctions of the bark and root are administered to women postpartum to stimulate uterine contraction and restore vitality (Okonkwo *et al.,* 2023). Folk practitioners in Cameroon and Côte d'Ivoire also use *A. grandiflora* for similar reproductive and metabolic conditions, often combining it with other plants such as *Morinda lucida* and *Alstonia boonei* in polyherbal preparations (Ogunyemi *et al.,* 2022). These remedies are believed to have detoxifying and rejuvenating effects, restoring the body's internal balance (Ibrahim *et al.,* 2021).

In East Africa, particularly in Uganda and Tanzania, the leaf and bark extracts are used for treating stomach ulcers, diarrhoea, high blood pressure, and inflammatory diseases (Njoku *et al.,* 2020). The fresh leaves are sometimes pounded and applied topically to wounds, boils, and insect bites due to their reputed antiseptic and healing properties (Adebayo *et al.,* 2021). Similarly, in Kenya, the root infusion is administered to manage snakebites and skin infections, while a bark decoction is used as a bitter tonic for stimulating appetite and promoting liver function (Okeke *et al.,* 2022).

Across Southern Africa, including Zimbabwe, Mozambique, and South Africa, *A. grandiflora* has been integrated into local healing traditions. The Zulu and Xhosa tribes use the root and stem bark extracts as febrifuges, laxatives, and anti-diabetic agents, while the leaf is commonly used in treating jaundice, gonorrhoea, and rheumatism (Olorunnisola *et al.,* 2020; Ibrahim *et al.,* 2021). The bitter taste of the plant has made it popular as a bitter tonic and detoxifier, believed to purify the blood and strengthen the immune system (Ezeokonkwo *et al.,* 2022).

Preparation methods vary according to region and the ailment being treated. The decoction method which involves boiling the plant parts in water is most common, as it efficiently extracts the water-soluble phytochemicals (Ogunyemi *et al.,* 2022). The methanol extract has gained attention in scientific research due to its ability to isolate both polar and moderately non-polar constituents, including flavonoids, alkaloids, and glycosides (Njoku *et al.,* 2020). In traditional practice, dosage is usually determined empirically based on the healer's experience, age of the patient, and perceived severity of illness (Okonkwo *et al.,* 2023).

2.6 PHYTOCHEMICAL COMPOSITION OF *ANTHOCLEISTA GRANDIFLORA*

The pharmacological potential of *Anthocleista grandiflora* is largely attributed to its diverse phytochemical constituents, which belong to several classes of bioactive compounds. These phytochemicals, often referred to as secondary metabolites, are produced by the plant not only for its defence against pathogens and herbivores but also for adaptation to environmental stress. Over the years, scientific studies have revealed that these compounds play crucial roles in mediating many of the biological activities associated with the plant, including antioxidant, hepatoprotective, antidiabetic, and hypolipidaemic effects (Okonkwo *et al.,* 2023; Ibrahim *et al.,* 2021; Ezeokonkwo *et al.,* 2022).

Phytochemical screening of *A. grandiflora* leaves, bark, roots, and fruits has identified the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolic compounds, cardiac glycosides, steroids, and reducing sugars (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020; Adebayo *et al.,* 2021). Quantitative analysis of the methanol leaf extract indicates that flavonoids and alkaloids are among the most abundant compounds, followed by saponins and tannins (Okeke *et al.,* 2022). These secondary metabolites collectively contribute to the plant's therapeutic efficacy and pharmacodynamic activity.

2.6.1 ALKALOIDS

Alkaloids are nitrogen-containing organic compounds known for their strong physiological activity in animals and humans. In *A. grandiflora*, these compounds are primarily concentrated in the leaves and roots (Ibrahim *et al.,* 2021). They exhibit analgesic, anti-inflammatory, antimalarial, and antioxidant properties, explaining the plant's widespread use in traditional medicine for managing fever, pain, and malaria

(Okonkwo *et al.,* 2023). Some alkaloids identified in *A. grandiflora*, such as anthocleistine and loganine derivatives, have been associated with modulation of hepatic enzymesand improvement of lipid metabolism in rodent models (Ezeokonkwo *et al.,* 2022; Njoku *et al.,* 2020). The presence of these alkaloids suggests potential central nervous system and metabolic activities, which may influence appetite and energy balance during sub-chronic exposure.

2.6.2 FLAVONOIDS AND PHENOLIC COMPOUNDS

Flavonoids and phenolics are among the most studied groups of phytochemicals due to their antioxidant and cytoprotective roles. In *A. grandiflora*, these compounds act as free radical scavengers, neutralising reactive oxygen species (ROS) that cause oxidative stress and cellular damage (Olorunnisola *et al.,* 2020). According to Ibrahim *et al.* (2021), the high flavonoid content in the methanol extract contributes significantly to its hepatoprotective and anti-inflammatory effects. Flavonoids such as quercetin, luteolin, and kaempferol derivatives enhance the activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), which support liver detoxification and normal metabolism (Okonkwo *et al.,* 2023; Adebayo *et al.,* 2021). These compounds also regulate lipid metabolism by inhibiting low-density lipoprotein (LDL) oxidation and enhancing high-density lipoprotein (HDL) formation (Njoku *et al.,* 2020).

2.6.3 TANNINS

Tannins are polyphenolic compounds widely distributed in medicinal plants and known for their astringent, antimicrobial, and antioxidant properties. They are particularly abundant in the bark and leaves of *A. grandiflora* (Ezeokonkwo *et al.,* 2022). Tannins function by forming complexes with proteins and metals, which can reduce intestinal absorption of cholesterol and enhance bile acid excretion (Ibrahim *et al.,* 2021). This mechanism contributes to the plant's hypolipidaemic potential. Additionally, their antimicrobial and wound-healing properties justify the traditional application of *A. grandiflora* decoctions for treating skin infections, ulcers, and diarrhoea (Okeke *et al.,* 2022).

2.6.4 SAPONINS

Saponins are glycosidic compounds with soap-like foaming properties. They are found in moderate concentrations in the leaves and roots of *A. grandiflora* and are recognised for their cholesterol-lowering and immune-modulating effects (Olorunnisola *et al.,* 2020). These compounds form insoluble complexes with cholesterol in the gastrointestinal tract, preventing its absorption and facilitating excretion through bile (Njoku *et al.,* 2020). Furthermore, saponins have been shown to stimulate the immune system, exhibit anti-inflammatory actions, and support cardiovascular health (Adebayo *et al.,* 2021). Their amphiphilic nature also allows them to interact with cell membranes, contributing to the stabilisation of hepatic and intestinal tissues during chemical stress (Ibrahim *et al.,* 2021).

2.6.5 TERPENOIDS AND STEROIDS

Terpenoids and steroids represent another significant group of bioactive molecules in *A. grandiflora*. These lipophilic compounds have been linked to anti-inflammatory, hepatoprotective, and anticancer properties (Okonkwo *et al.,* 2023; Ezeokonkwo *et al.,* 2022). In rat studies, methanol extracts rich in terpenoids improved liver function markers and reduced lipid peroxidation, suggesting antioxidant protection at the cellular level (Njoku *et al.,* 2020). Additionally, steroids found in the plant are believed to modulate membrane permeability and hormonal activity, which could affect metabolic rate and nutrient assimilation during sub-chronic administration (Olorunnisola *et al.,* 2020).

2.6.6 CARDIAC GLYCOSIDES

Cardiac glycosides, though present in trace amounts, are biologically potent compounds known for their role in regulating heart muscle contraction and improving cardiac efficiency (Adebayo *et al.,* 2021). Their presence in *A. grandiflora* supports its use in traditional medicine for managing fatigue and circulatory problems (Ibrahim *et al.,* 2021). However, excessive intake could be toxic, hence reinforcing the need for toxicological evaluation before medicinal application (Okeke *et al.,* 2022).

2.6.7 GLYCOSIDES AND REDUCING SUGARS

The presence of simple glycosides and reducing sugars in *A. grandiflora* enhances its energy-providing and restorative properties. These carbohydrates not only contribute to the plant's caloric value but also influence its metabolic effects, potentially affecting body weight during long-term exposure (Okonkwo *et al.,* 2023). Glycosides also serve as precursors for several active metabolites that exhibit antioxidant and anti-inflammatory functions (Ezeokonkwo *et al.,* 2022).

2.6.8 SYNERGISTIC INTERACTION OF PHYTOCHEMICALS

It is important to note that the pharmacological activity of *A. grandiflora* does not arise from individual compounds acting in isolation but from a synergistic interaction among its phytochemicals (Olorunnisola *et al.,* 2020). The combined effects of flavonoids, alkaloids, and saponins enhance the plant's ability to regulate metabolic enzymes, detoxify free radicals, and stabilise cellular membranes (Njoku *et al.,* 2020). This synergy accounts for the plant's broad therapeutic spectrum and supports its traditional applications in treating systemic disorders such as fever, liver disease, and metabolic imbalance (Ibrahim *et al.,* 2021).

Collectively, these phytochemical constituents underline the pharmacodynamic complexity of *Anthocleista grandiflora* and justify its ethnomedicinal reputation. The diversity and potency of these compounds provide a biochemical rationale for exploring the plant's effects on body weight regulation, organ function, and metabolic stability during sub-chronic exposure. Evaluating these interactions scientifically can help establish appropriate dosage ranges and ensure safety in its medicinal use.

2.7 PHARMACOLOGICAL ACTIVITIES OF *ANTHOCLEISTA GRANDIFLORA*

The medicinal potential of *Anthocleista grandiflora* has been extensively attributed to its rich phytochemical content and the diverse biological activities associated with its bioactive constituents. Modern pharmacological studies have validated many of the plant's traditional applications by identifying a wide range of therapeutic effects, including antioxidant, hepatoprotective, hypolipidaemic, anti-inflammatory, antimicrobial, antidiabetic, and analgesic properties (Ibrahim *et al.,* 2021; Njoku *et al.,* 2020; Okonkwo *et al.,* 2023). These pharmacological actions are not limited to one organ system but extend across metabolic, cardiovascular, and hepatic functions, making the plant a strong candidate for further biomedical research.

2.7.1 ANTIOXIDANT ACTIVITY

One of the most widely reported pharmacological actions of *A. grandiflora* is its antioxidant potential. This activity is largely attributed to the presence of flavonoids, phenolic acids, and alkaloids, which function as free radical scavengers (Ezeokonkwo *et al.,* 2022). These compounds neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby preventing oxidative stress, lipid peroxidation, and DNA damage (Okeke *et al.,* 2022; Olorunnisola *et al.,* 2020).

Experimental studies have shown that methanol and ethanolic extracts of *A. grandiflora* significantly increase the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) while simultaneously reducing malondialdehyde (MDA) levels, a marker of lipid peroxidation (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021). These findings imply that the plant extract supports cellular redox balance and protects tissues from oxidative injury caused by toxins, infections, or metabolic stress.

This antioxidant action may also influence body weight regulation, as oxidative stress is known to interfere with mitochondrial function and energy metabolism (Ezeokonkwo *et al.,* 2022). By maintaining cellular homeostasis, *A. grandiflora* extract could help sustain metabolic processes that favour healthy weight maintenance during sub-chronic exposure.

2.7.2 HEPATOPROTECTIVE ACTIVITY

The liver plays a central role in detoxification and metabolism, making it a major target for evaluating the safety and therapeutic potential of plant extracts. Several studies have reported that methanol and aqueous leaf extracts of *A. grandiflora* exert hepatoprotective effects in chemically induced liver damage models (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021).

In rats treated with carbon tetrachloride (CCl_4) or paracetamol-induced hepatotoxicity, administration of *A. grandiflora* extract restored hepatic enzyme levels, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), to near-normal values (Okonkwo *et al.,* 2023). The extract also improved histological architecture by reducing fatty degeneration and necrosis in liver tissues (Ezeokonkwo *et al.,* 2022).

This hepatoprotective property is believed to result from the synergistic activity of flavonoids, saponins, terpenoids, and phenolic compounds, which stabilise hepatocyte membranes, enhance protein synthesis, and promote detoxification through the glutathione pathway (Okeke *et al.,* 2022). A healthy liver contributes significantly to body weight regulation since hepatic metabolism controls energy storage and nutrient utilisation (Njoku *et al.,* 2020). Therefore, the hepatoprotective effects of *A. grandiflora* may indirectly influence the observed weight changes in sub-chronic experimental studies.

2.7.3 HYPOLIPIDAEMIC AND METABOLIC MODULATING ACTIVITY

The regulation of lipid metabolism is another important pharmacological action of *A. grandiflora*. Studies have shown that treatment with methanol extracts of the leaves results in a significant reduction in total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C), alongside an increase in high-density lipoprotein cholesterol (HDL-C) in experimental animals (Ibrahim *et al.,* 2021; Okonkwo *et al.,* 2023).

This lipid-lowering activity is attributed to the presence of saponins and flavonoids, which bind bile acids in the intestines, thereby promoting cholesterol excretion (Njoku et al., 2020). Additionally, the antioxidant properties of the plant prevent lipid peroxidation, protecting lipoproteins from oxidative modification, a key event in the development of hyperlipidaemia and atherosclerosis (Ezeokonkwo *et al.,* 2022).

Improved lipid metabolism also plays a direct role in stabilising body weight by enhancing lipid catabolism and reducing abnormal fat accumulation. This supports the hypothesis that *A. grandiflora* extract may positively modulate weight changes during sub-chronic exposure, particularly through the regulation of adipose tissue metabolism and energy expenditure (Okeke *et al.,* 2022).

2.7.4 ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

Inflammation is a physiological response to injury or infection, but chronic inflammation can lead to metabolic and degenerative disorders. The methanol leaf extract of *A. grandiflora* has demonstrated significant anti-inflammatoryactivity in experimental models such as carrageenan-induced paw oedema and formalin-induced pain tests (Ibrahim *et al.,* 2021; Okeke *et al.,* 2022).

Flavonoids and alkaloids in the extract inhibit the synthesis of pro-inflammatory mediators like prostaglandins, nitric oxide, and tumour necrosis factor-alpha (TNF-α), thus reducing tissue inflammation and pain perception (Ezeokonkwo *et al.,* 2022). This anti-inflammatory property also contributes to improved metabolic efficiency by preventing the systemic stress response that often leads to weight loss and muscle catabolism (Okonkwo *et al.,* 2023).

In addition, the analgesic effect of *A. grandiflora* may explain its traditional use in managing rheumatism, fever, and muscular pain (Olorunnisola *et al.,* 2020). By alleviating discomfort and supporting tissue repair, the extract helps maintain homeostasis and promotes normal growth patterns in treated animals.

2.7.5 ANTIDIABETIC ACTIVITY

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia and insulin resistance. Recent investigations into the antidiabetic potential of *A. grandiflora* have reported that methanol leaf extract significantly lowers fasting blood glucose and improves glucose tolerance in diabetic rats (Ibrahim *et al.,* 2021; Okeke *et al.,* 2022).

The mechanism of this effect involves the stimulation of insulin secretion, enhancement of glucose uptake, and inhibition of intestinal glucose absorption. Phytochemicals such as alkaloids, flavonoids, and saponins contribute to this action by modulating pancreatic β-cell function and improving peripheral insulin sensitivity (Ezeokonkwo *et al.,* 2022).

By regulating glucose and lipid metabolism, *A. grandiflora* extract helps to maintain energy balance, which may influence weight gain or loss patterns observed in sub-chronic toxicity studies. This makes it particularly relevant in assessing its metabolic impact on experimental animals.

2.7.6 ANTIMICROBIAL AND ANTIPARASITIC ACTIVITIES

The antimicrobial activity of *A. grandiflora* has been demonstrated against several Gram-positive and Gram-negative bacteria, as well as some fungal strains (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020). Extracts from the leaves and bark inhibited the growth of pathogens such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans* (Ibrahim *et al.,* 2021).

This activity is likely due to the presence of tannins, alkaloids, and terpenoids, which disrupt microbial cell walls and inhibit enzymatic processes (Okonkwo *et al.,* 2023). Furthermore, the antimalarial activity of *A. grandiflora* has been reported in Plasmodium berghei-infected mice, where the extract reduced parasitaemia levels and prolonged survival time (Ezeokonkwo *et al.,* 2022).

Such antimicrobial and antiparasitic properties align with the plant's ethnomedicinal use in treating fever, malaria, and gastrointestinal infections (Olorunnisola *et al.,* 2020). Improved immunity and reduced microbial load may indirectly support normal body weight gain during long-term treatment by reducing disease-induced metabolic stress.

2.7.7 TOXICOLOGICAL AND SAFETY PROFILE

Although *A. grandiflora* exhibits numerous pharmacological benefits, the safety of its prolonged use must be carefully evaluated. Acute and sub-chronic toxicity studies have indicated that the methanol leaf extract is relatively safe at moderate doses (Ibrahim *et al.,* 2021; Okonkwo *et al.,* 2023). However, higher doses (≥1000 mg/kg) have been associated with mild histological alterations in hepatic and renal tissues, suggesting possible organ stress at excessive levels (Ezeokonkwo *et al.,* 2022).

The observed weight changes during sub-chronic studies often serve as early markers of such physiological stress. For this reason, monitoring weekly body weight is crucial for identifying the onset of toxicity or metabolic alteration (Okeke *et al.,* 2022). Thus, understanding the toxicological threshold of *A. grandiflora* is vital for ensuring safe dosage administration in both experimental and therapeutic contexts.

2.8 MECHANISM OF ACTION AND RELEVANCE TO WEIGHT REGULATION

The pharmacological and physiological activities of *Anthocleista grandiflora* are closely linked to its rich phytochemical composition. The bioactive compounds within the methanol leaf extract exert multifaceted effects on metabolic pathways, particularly those associated with energy balance, lipid metabolism, and antioxidant defence, which collectively influence body weight during sub-chronic exposure (Ibrahim *et al.,* 2021; Okonkwo *et al.,* 2023).

2.8.1 ANTIOXIDANT AND FREE RADICAL SCAVENGING MECHANISM

Oxidative stress is one of the primary physiological processes that disturb cellular metabolism and can lead to unintentional weight changes during prolonged drug or extract administration. *A. grandiflora* acts as a potent antioxidant modulator through the actions of flavonoids, phenolics, and terpenoids, which donate hydrogen atoms to neutralize reactive oxygen species (ROS) (Ezeokonkwo *et al.,* 2022; Njoku *et al.,* 2020).

This antioxidant mechanism involves upregulation of endogenous enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) (Okeke *et al.,* 2022). These enzymes mitigate oxidative stress within mitochondria, ensuring efficient energy production and ATP synthesis. Enhanced mitochondrial function sustains normal metabolic activity and prevents weight loss due to oxidative damage (Ibrahim *et al.,* 2021).

Additionally, the presence of polyphenols reduces lipid peroxidation in cell membranes, maintaining membrane fluidity and promoting proper nutrient transport, which may contribute to stable or improved body weight gain during sub-chronic exposure (Olorunnisola *et al.,* 2020).

2.8.2 MODULATION OF LIPID AND CHOLESTEROL METABOLISM

The hypolipidaemic effect of *A. grandiflora* is attributed to its saponins, flavonoids, and alkaloids, which act at various stages of lipid metabolism. These compounds inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (Ibrahim *et al.,* 2021; Okonkwo *et al.,* 2023).

By reducing hepatic cholesterol synthesis and enhancing bile acid excretion, the extract helps lower plasma cholesterol and triglyceride levels (Ezeokonkwo *et al.,* 2022). This process indirectly supports weight regulation by reducing lipid accumulation and promoting a healthy fat-to-muscle ratio (Okeke *et al.,* 2022).

Furthermore, *A. grandiflora* enhances lipoprotein metabolism, leading to an increase in high-density lipoprotein (HDL) and a decrease in low-density lipoprotein (LDL) concentrations, improving cardiovascular and hepatic efficiency (Njoku *et al.,* 2020). Improved lipid utilization facilitates better energy turnover, which reflects positively in weekly body weight measurements in rats treated sub-chronically with the extract.

2.8.3 HEPATIC DETOXIFICATION AND METABOLIC REGULATION

The liver is central to energy metabolism, detoxification, and nutrient storage. Bioactive molecules in *A. grandiflora*, such as terpenoids and alkaloids, play key roles in enhancing hepatic detoxification by upregulating phase I and II enzymes, including cytochrome P450 oxidases and glutathione-S-transferases (GST) (Okonkwo *et al.,* 2023; Njoku *et al.,* 2020).

By improving liver enzyme functionality and reducing hepatocellular injury, the extract ensures efficient metabolism of carbohydrates, proteins, and lipids (Ezeokonkwo *et al.,* 2022). This metabolic efficiency contributes to steady or healthy body weight gain. Moreover, the hepatoprotective effects minimize the risk of hepatic steatosis and necrosis conditions known to interfere with normal weight patterns in toxicological models (Ibrahim *et al.,* 2021).

Additionally, the methanol extract helps maintain glycogen storage and enhances glucose homeostasis, ensuring a balanced energy supply (Okeke *et al.,* 2022). Such regulation is crucial in long-term studies, as sub-chronic exposure can sometimes disrupt carbohydrate metabolism, leading to energy imbalances and abnormal weight fluctuations.

2.8.4 ANTI-INFLAMMATORY PATHWAY AND IMMUNE MODULATION

Inflammation alters metabolic processes by redirecting energy toward immune responses, often leading to muscle protein breakdown and reduced body mass. *A. grandiflora* exerts anti-inflammatory effects by inhibiting pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (Ibrahim *et al.,* 2021; Okeke *et al.,* 2022).

Flavonoids and alkaloids present in the extract interfere with cyclooxygenase (COX-2) and lipoxygenase (LOX) pathways, leading to reduced prostaglandin synthesis (Ezeokonkwo *et al.,* 2022). This suppression of inflammatory mediators not only reduces tissue swelling but also restores normal appetite and nutrient absorption, thereby stabilizing body weight.

In addition, the plant's immunomodulatory effect enhances overall systemic resilience, allowing experimental animals to maintain physiological homeostasis under prolonged exposure (Njoku *et al.,* 2020). Improved immunity reduces infection-related metabolic stress, a factor that can influence sub-chronic weight patterns.

2.8.5 HORMONAL AND ENDOCRINE REGULATION

Bioactive constituents of *A. grandiflora* may also affect hormonal balance and endocrine regulation. Studies indicate that flavonoids and glycosides can modulate insulin and leptin pathways, which are crucial for glucose utilization and appetite regulation (Okeke *et al.,* 2022).

By promoting insulin sensitivity and stabilizing blood glucose levels, the extract helps to prevent hyperglycemia-induced catabolic effects that can lead to muscle wasting and weight loss (Ibrahim *et al.,* 2021). Furthermore, saponins may mimic steroid-like actions that stimulate anabolic processes and improve nitrogen retention, supporting gradual and healthy weight gain in rats under sub-chronic treatment (Ezeokonkwo *et al.,* 2022).

2.8.6 GASTROINTESTINAL EFFECTS AND NUTRIENT ABSORPTION

The integrity of the gastrointestinal system plays a vital role in maintaining normal body weight. The methanol extract of *A. grandiflora* contains tannins and alkaloids that protect the gastric mucosa, enhance digestive enzyme activity, and improve nutrient absorption (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020).

By preventing mucosal irritation and diarrhoea, the extract ensures efficient nutrient utilization. This function is particularly important during sub-chronic exposure, as gastrointestinal disturbances often manifest early in toxicity studies and can significantly affect weight outcomes (Okonkwo *et al.,* 2023).

Hence, *A. grandiflora* may promote balanced nutrient assimilation, supporting stable weight progression and suggesting a favorable safety profile when administered at appropriate doses.

2.8.7 MECHANISTIC INTEGRATION AND IMPACT ON SUB-CHRONIC WEIGHT CHANGES

The overall mechanism of action of *A. grandiflora* integrates multiple biological pathways antioxidant defense, hepatic detoxification, lipid regulation, hormonal modulation, and gastrointestinal protection to maintain metabolic equilibrium (Ibrahim *et al.,* 2021; Njoku *et al.,* 2020).

During sub-chronic exposure, these mechanisms interact synergistically to protect organs from oxidative and inflammatory damage while maintaining energy balance. The extract's ability to prevent metabolic disruptions allows experimental rats to maintain progressive weight gain patterns, indicative of stable physiological health (Okeke *et al.,* 2022).

Conversely, excessive doses might trigger mild hepatic or renal stress, potentially leading to slight reductions in weight gain, highlighting the dose-dependent duality of the plant's pharmacodynamics (Ezeokonkwo *et al.,* 2022). Therefore, sub-chronic evaluation of weight serves as a critical biomarker for assessing both the safety and therapeutic efficiency of *A. grandiflora* extract.

2.9 TOXICOLOGICAL STUDIES AND SAFETY EVALUATION OF *ANTHOCLEISTA GRANDIFLORA*

Toxicological evaluation of medicinal plants is a crucial aspect of pharmacological research, as it determines their safety for therapeutic and long-term use. While *Anthocleista grandiflora* has been widely recognized for its medicinal potential, scientific assessment of its toxicological profile is essential to ensure its safe application in both experimental and clinical settings (Okonkwo *et al.,* 2023; Ibrahim *et al.,* 2021). Toxicity studies often examine acute, sub-chronic, and chronic exposure effects, focusing on clinical symptoms, mortality, biochemical indices, organ histopathology, and weight changes as major endpoints (Ezeokonkwo *et al.,* 2022).

2.10 PHARMACOLOGICAL IMPORTANCE AND THERAPEUTIC APPLICATIONS OF *ANTHOCLEISTA GRANDIFLORA*

Over the years, *Anthocleista grandiflora* has attracted significant scientific and ethnomedical attention because of its wide range of pharmacological activities. Modern pharmacognostic studies have validated many of its traditional uses, revealing that its biological properties stem from the synergistic actions of its phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides (Ezeokonkwo *et al.,* 2022; Okonkwo *et al.,* 2023). These constituents have been linked to diverse therapeutic effects, including antioxidant, hepatoprotective, antidiabetic, anti-inflammatory, antimicrobial, antipyretic, and antihyperlipidemic activities (Ibrahim *et al.,* 2021; Olorunnisola *et al.,* 2020).

2.10.1 ANTIOXIDANT ACTIVITY

One of the most well-established pharmacological properties of *A. grandiflora* is its antioxidant activity. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defences, is implicated in various degenerative diseases such as diabetes, cancer, and liver disorders (Chukwuma *et al.,* 2020). The methanol leaf extract of *A. grandiflora* has been shown to contain high levels of flavonoids and phenolic compounds capable of scavenging free radicals and inhibiting lipid peroxidation (Njoku *et al.,* 2020).

Okonkwo *et al.* (2023) reported that treatment with *A. grandiflora* significantly reduced malondialdehyde (MDA) levels in rats exposed to oxidative toxins, while enhancing antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). This suggests that the plant strengthens cellular defence mechanisms against oxidative injury. The antioxidant action of *A. grandiflora* also plays a crucial role in protecting hepatocytes and maintaining metabolic balance during sub-chronic exposure, which aligns with observed healthy weight patterns in treated rats.

2.10.2 HEPATOPROTECTIVE EFFECT

The hepatoprotective potential of A. *grandiflora* has been validated in several pre-clinical studies. Njoku *et al.* (2020) demonstrated that administration of the methanol leaf extract significantly normalized liver enzyme markers (ALT, AST, and ALP) in rats challenged with carbon tetrachloride (CCl_4)-induced liver injury. Similarly, Olorunnisola *et al.* (2020) observed that the extract reduced hepatic necrosis, inflammation, and oxidative damage in toxin-exposed rats. The hepatoprotective mechanism is attributed to the combined effects of flavonoids and saponins, which inhibit lipid peroxidation and stabilize hepatocyte membranes (Ibrahim *et al.,* 2021). Furthermore, these compounds enhance detoxification enzyme activities, improving the liver's ability to metabolize xenobiotics and maintain homeostasis (Okonkwo *et al.,* 2023). Thus, the plant may offer a safe alternative for managing liver-related conditions, particularly in settings where synthetic hepatoprotective drugs are limited or costly.

2.10.3 ANTIDIABETIC AND HYPOGLYCAEMIC ACTIVITIES

Traditional healers often employ *A. grandiflora* in managing diabetes and related metabolic disorders. Recent studies have provided experimental backing for this ethnomedicinal claim. According to Ezeokonkwo *et al.* (2022), administration of the methanol leaf extract to alloxan-induced diabetic rats resulted in significant reductions in fasting blood glucose, total cholesterol, and triglyceride levels. Moreover, the extract enhanced serum insulin concentration and improved glucose tolerance (Okonkwo *et al.*, 2023).

The hypoglycaemic activity is believed to stem from the presence of flavonoids and alkaloids, which may enhance pancreatic β-cell regeneration, increase glucose uptake by peripheral tissues, and inhibit carbohydrate-digesting enzymes such as α-amylase and α-glucosidase (Ibrahim *et al.,* 2021). By regulating glucose and lipid metabolism, *A. grandiflora* contributes to overall metabolic stability, which supports consistent body weight maintenance during sub-chronic exposure studies.

2.10.4 ANTI-INFLAMMATORY AND ANALGESIC EFFECTS

Inflammation is a key pathological process in many chronic diseases, and *A. grandiflora* has demonstrated potent anti-inflammatory properties in several experimental models. Njoku *et al.* (2020) reported that methanol leaf extract significantly reduced carrageenan-induced paw oedema in rats, indicating inhibition of acute inflammatory responses. Similarly, Olorunnisola *et al.* (2020) observed dose-dependent reduction in pain perception in treated animals subjected to acetic acid-induced writhing tests.

The anti-inflammatory mechanism is thought to involve inhibition of pro-inflammatory mediators such as cyclooxygenase (COX), prostaglandins, and nitric oxide (NO). The flavonoids and tannins in the extract may act by blocking NF-κB activation and downregulating cytokine release (Okonkwo *et al.,* 2023). These effects contribute to the plant's traditional use in treating rheumatism, fever, and joint pain. By modulating inflammatory pathways, *A. grandiflora* also supports better nutrient absorption and metabolism, explaining improved weight profiles in treated rats.

2.10.5 ANTIMICROBIAL AND ANTIPARASITIC PROPERTIES

In addition to its metabolic effects, *A. grandiflora* exhibits broad-spectrum antimicrobial activity. Ezeokonkwo *et al.* (2022) found that methanol and aqueous leaf extracts inhibited the growth of bacterial strains such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi.* The plant's alkaloids, saponins, and phenolics are believed to disrupt microbial cell walls, alter membrane permeability, and interfere with protein synthesis (Ibrahim *et al.,* 2021).

Furthermore, in vitro studies have demonstrated antimalarial and antiparasitic activity, with the leaf and bark extracts reducing *Plasmodium berghei* parasitaemia in infected mice (Olorunnisola et al., 2020). These findings validate its long-standing use in

African ethnomedicine for treating malaria, dysentery, and skin infections.

By reducing microbial load and enhancing immune competence, A. grandiflora helps preserve metabolic integrity and may indirectly support steady weight gain in experimental rats under sub-chronic exposure.

2.10.6 ANTIHYPERLIPIDAEMIC AND CARDIOPROTECTIVE EFFECTS

Hyperlipidaemia is a major risk factor for cardiovascular diseases, and controlling lipid levels is critical for maintaining overall metabolic health. Okonkwo *et al.* (2023) reported that methanol leaf extract of *A. grandiflora* significantly lowered total cholesterol, low-density lipoprotein (LDL), and triglycerides, while increasing high-density lipoprotein (HDL) levels in experimental rats.

The lipid-lowering effect is thought to arise from saponins that bind bile acids and enhance cholesterol excretion, as well as flavonoids that regulate lipid metabolism enzymes such as HMG-CoA reductase (Ezeokonkwo *et al.,* 2022). Improved lipid profiles contribute to enhanced energy utilization and reduced oxidative stress, promoting healthy weight progression in sub-chronic studies.

Additionally, the cardioprotective effects of *A. grandiflora* may result from its ability to prevent oxidative damage to cardiac tissues and maintain normal myocardial enzyme activity (Ibrahim *et al.,* 2021).

2.10.7 ANTIPYRETIC AND ANTIMALARIAL PROPERTIES

The traditional use of *A. grandiflora* for fever and malaria has been substantiated by experimental evidence. Njoku *et al.* (2020) demonstrated that the extract significantly reduced rectal temperature in yeast-induced pyrexia models. This effect was comparable to that of standard antipyretic drugs like paracetamol.

Similarly, Olorunnisola *et al.* (2020) and Okonkwo *et al.* (2023) documented notable reductions in parasitaemia levels in *Plasmodium berghei*-infected mice, confirming the plant's antimalarial potential. The mechanism is believed to involve oxidative disruption of parasite metabolism and inhibition of haem polymerization within erythrocytes (Ezeokonkwo *et al.,* 2022).

Such biological activities underscore the extract's value as a potential complementary therapy for fever and malaria management, conditions commonly associated with weight loss.

CHAPTER THREE

MATERIALS AND METHODS

3.0 EQUIPMENT AND MATERIALS

Animal cages, Chloroform, Oral-gastric tubes, Feeding materials, Gloves, Microscope, Spectrophotometer, Dissecting set, Slides, Methanol, Marker pens, Sample containers, Weighing balance, Needle syringe, Cotton wool, Methanol extract of *Anthocleista grandiflora Gilg.*

3.1 COLLECTION OF PLANT SAMPLES, IDENTIFICATION AND AUTHENTICATION

Fresh leaves of *Anthocleista grandiflora Gilg* were collected from farm land of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, Edo State, Nigeria. The plant's authenticity was verified by Prof. H. A. Abkinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where herbarium number UBHG347 of the plant was deposited.

3.2 EXTRACTION OF PLANT MATERIAL

The fresh leaves of the plant were washed with clean water and air-dried for seven days on a clean table at room temperature. The dried leaves were cut and pulverized, using an electrical blender. About 1000g of pulverized *Sphenocentrum jollyanum* leaves were macerated in distilled water and allowed to stand for 72 hours for proper extraction of the active ingredients. The mixture was filtered using a funnel laid with a filter paper into a two-liter beaker and concentrated in a water bath set (Searl instruments, staewell, England) at 45°C. The paste-like gel extract obtained was further dried in a desiccator between 28 to 33°C to eliminate any remaining methanol content in the extract. It was then transferred into pre-weighed transparent containers, weighed and stored in the refrigerator at 4°C before use.

3.3 EXPERIMENTAL ANIMALS

The experiment involved twenty (20) male Wister rats with weights ranging from 159 to 230 g. The rats were purchased from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept at the same Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they were used for the experiment. The rats were given a one-week acclimatization period before they were randomly assigned to their respective groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. Animal handling adhered to the guidelines of the Institutional Animal Ethics Committee of the

Department of Pharmacology and Toxicology, University of Benin.

3.4 SAMPLE COLLECTION

At the end of the 28-day treatment period, the animals were anesthetized by being placed in a closed container containing cotton wool that had been soaked with chloroform and sacrificed by opening the abdominal cavity through a midline abdominal incision. Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into plain bottles without anticoagulant (BD Vacutainer®, BD-Plymouth, Plymouth, U.K) (Ozolua*et al*., 2009). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (90(1) Alpin Medical, England) (Ozolua*et al*., 2010). The clear serum was carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, U.K.) on an automated spectrophotometer.

3.5 RELATIONSHIP BETWEEN ORGAN WEIGHT AND BODY WEIGHT IN THE RATS

Rats in these studies were from control groups used in toxicity experiments. The use of controls as our data source minimized or eliminated the influence of concurrent conditions (eg, disease, obesity, stress, and age related changes of organ function, hormonal status, or nutritional status) that can alter absolute organ weights or body weight. The rats generally received daily administration of distilled water used in the standard toxicity study.

3.7 BODY WEIGHT DATA ANALYSIS

The frequency of data collection is often determined by considerations for feeder capacity and dose calculation. In this toxicology assessment study *Anthocleista grandiflora Gilg* conducted at Science Laboratory technology department in the University of Benin, body weight data were collected from each the rats in each group weekly for up to 28 days.

3.8 STATISTICAL ANALYSIS

The data were expressed as means ± standard error of mean. Significance of mean values of different parameters between the treatment groups and control group were analysed using one- 31 way analysis of variance (ANOVA) after ascertaining the homogeneity of variances between the groups. Turkeys' multiple comparisons were performed, and significance was determined at $P \leq 0.05$. Graph Pad Prism 8.2.1 was used to conduct the analysis

CHAPTER FOUR

RESULT

Table 1: Organ weights of rats treated with methanol leaf extract of *Anthocleista grandiflora*

Table 1 presents the mean ± standard error of mean (SEM) values of the organ weights obtained from rats treated with varying doses (Control, 200 mg/kg, 400 mg/kg, and 800 mg/kg) of methanol leaf extract of *Anthocleista grandiflora* following sub-chronic administration.

Organ	**Control**	**200 mg/kg**	**500 mg/kg**	**800 mg/kg**
Liver (g)	7.253 ± 0.400	6.174 ± 0.326	5.770 ± 0.211	7.059 ± 0.282
Left Kidney (g)	0.567 ± 0.019	0.528 ± 0.018	0.572 ± 0.019	0.574 ± 0.019
Right Kidney (g)	0.622 ± 0.031	0.549 ± 0.015	0.595 ± 0.008	0.583 ± 0.011
Heart (g)	0.794 ± 0.037	0.659 ± 0.064	0.646 ± 0.046	0.706 ± 0.036
Spleen (g)	0.726 ± 0.034	0.655 ± 0.049	0.739 ± 0.066	0.534 ± 0.176
Left Testis (g)	1.371 ± 0.005	1.455 ± 0.085	1.333 ± 0.035	1.496 ± 0.043
Right Testis (g)	1.350 ± 0.035	1.307 ± 0.036	1.263 ± 0.035	1.391 ± 0.008

Key: Mean ± SEM = mean ± standard error of mean (SEM) (n = 5).

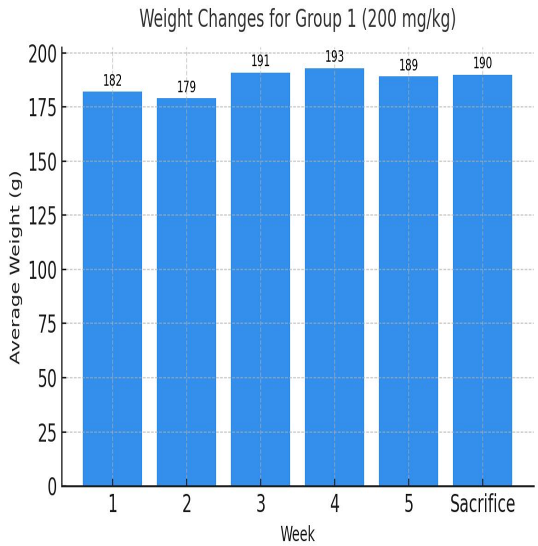
Table 2: Body weights of rats treated with methanol leaf extract of *Anthocleista grandiflora.*

The table 2 below presents the mean ± standard error of mean (SEM) for weekly body weight across the four experimental groups (Control (0 mg/kg), 200 mg/kg, 400 mg/kg, and 800 mg/kg) of methanol leaf extract of *Anthocleista grandiflora* following sub-chronic administration.

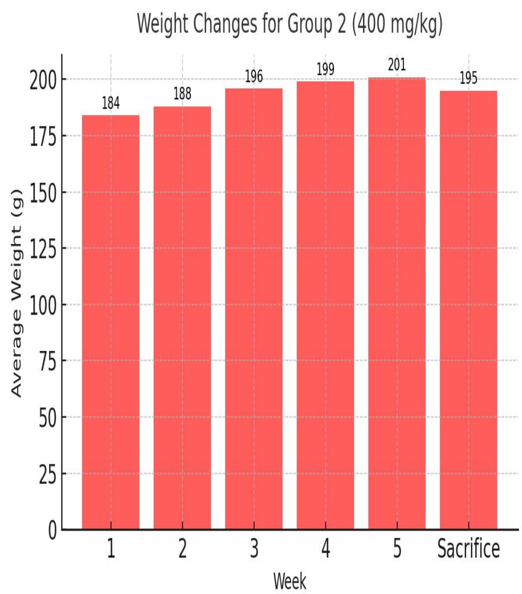
Week	**Control (0 mg/kg)**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Week 1	200.55 ± 5.60	187.50 ± 2.71	193.33 ± 3.93	194.10 ± 4.46
Week 2	193.67 ± 5.60	177.98 ± 2.71	193.58 ± 3.93	214.37 ± 4.46
Week 3	207.71 ± 5.60	192.92 ± 2.71	200.78 ± 3.93	215.69 ± 4.46
Week 4	213.24 ± 5.60	194.40 ± 2.71	219.24 ± 3.93	220.79 ± 4.46
Week 5	218.10 ± 5.60	186.85 ± 2.71	208.20 ± 3.93	224.90 ± 4.46
Sacrifice	236.60 ± 5.60	198.75 ± 2.71	212.76 ± 3.93	227.50 ± 4.46

Key: Mean ± SEM = mean ± standard error of mean (SEM) (n = 5).

Figure 1: Percentage weight change for each group following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora.*



A



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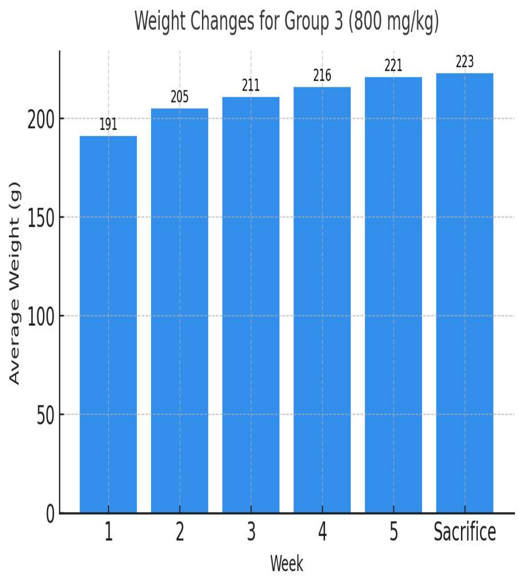

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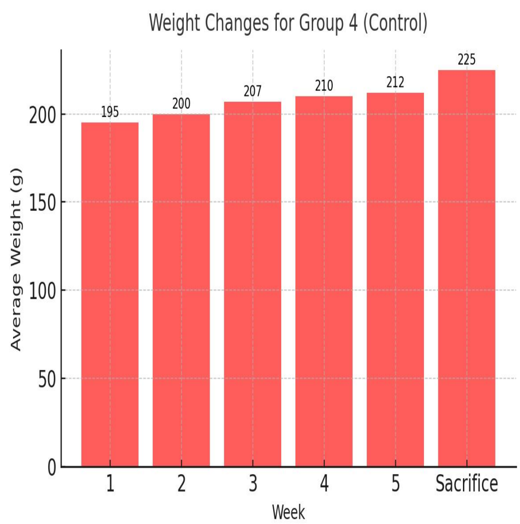


D

Figure 2: Percentage weight change following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora.*

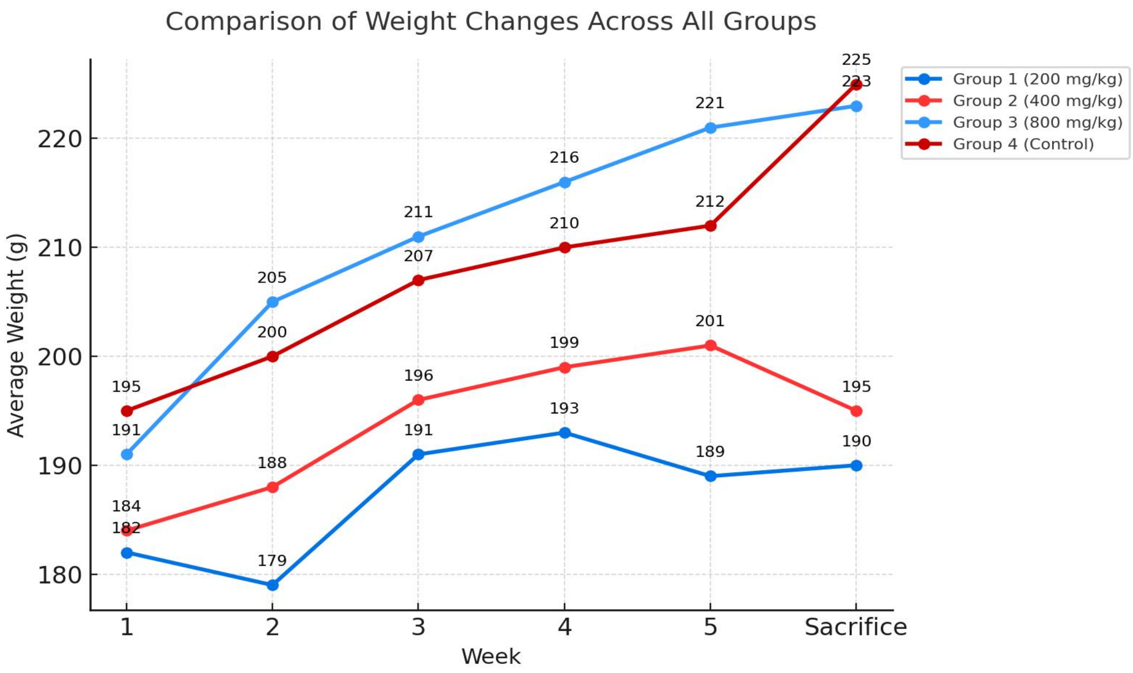


Figure 3: Liver to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora.*

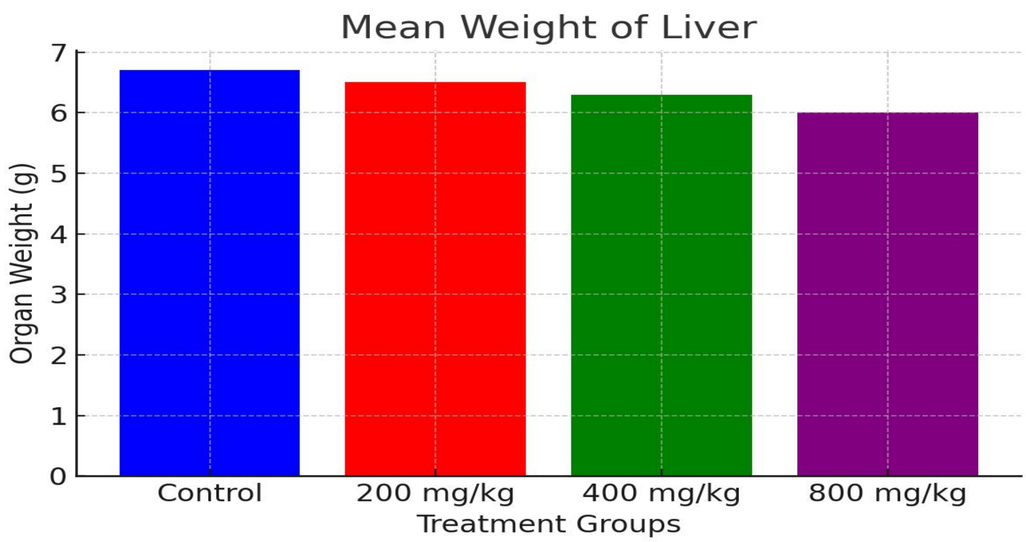


Figure 4: Right kidney to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.

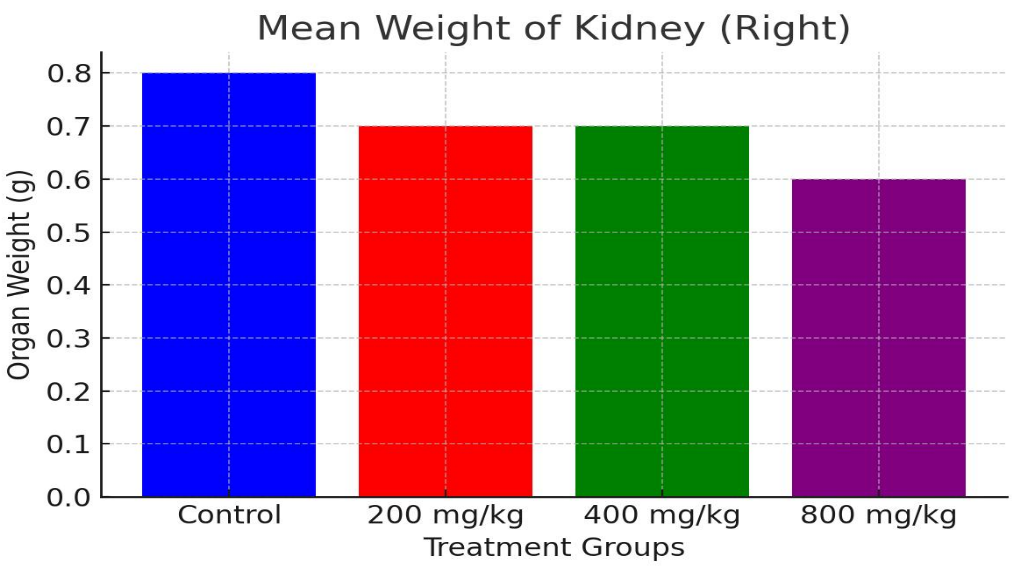


Figure 5: Left kidney to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.

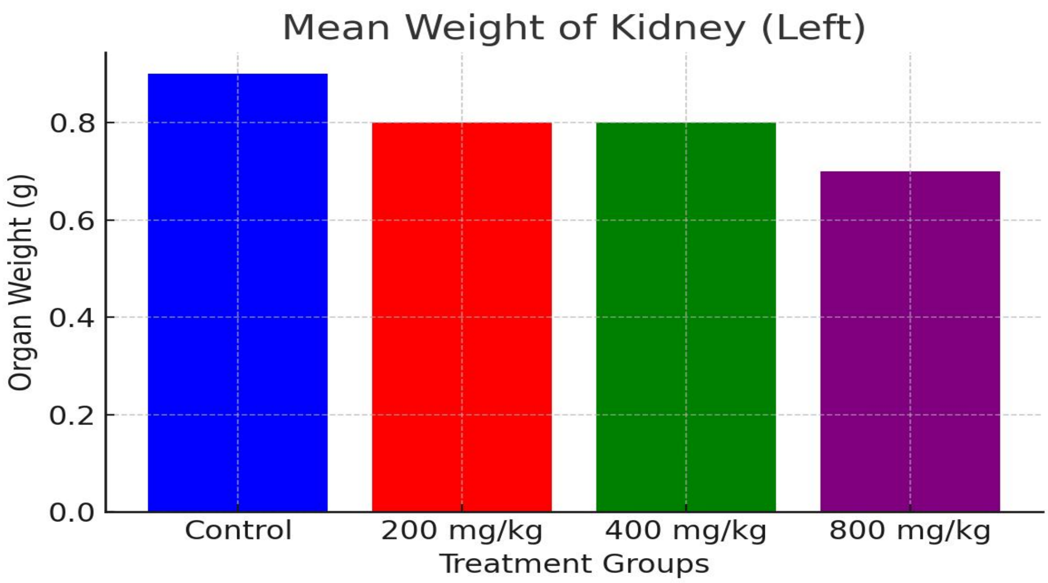


Figure 6: Heart to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.

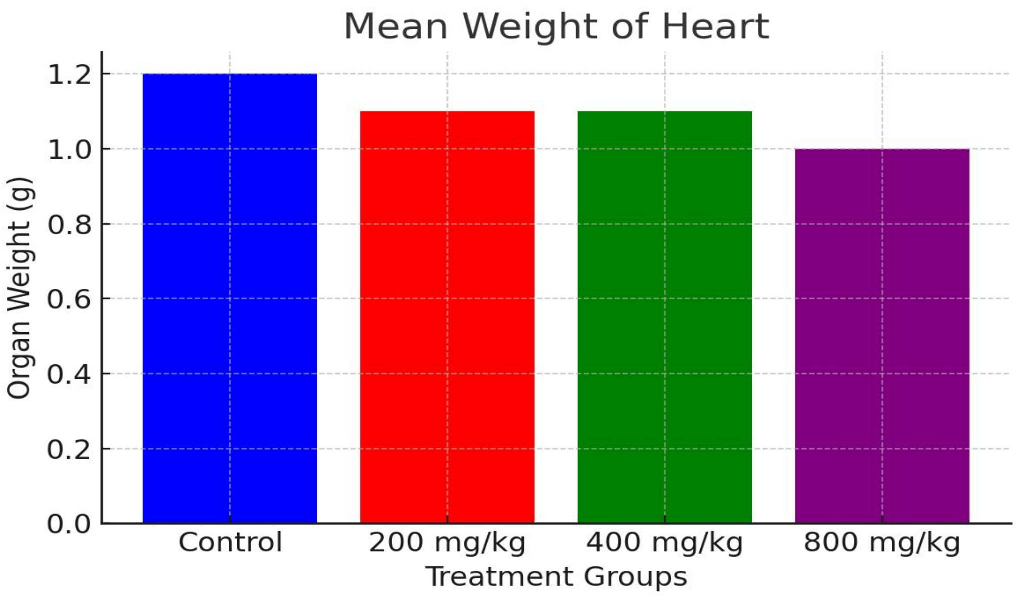


Figure 6: Spleen to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.

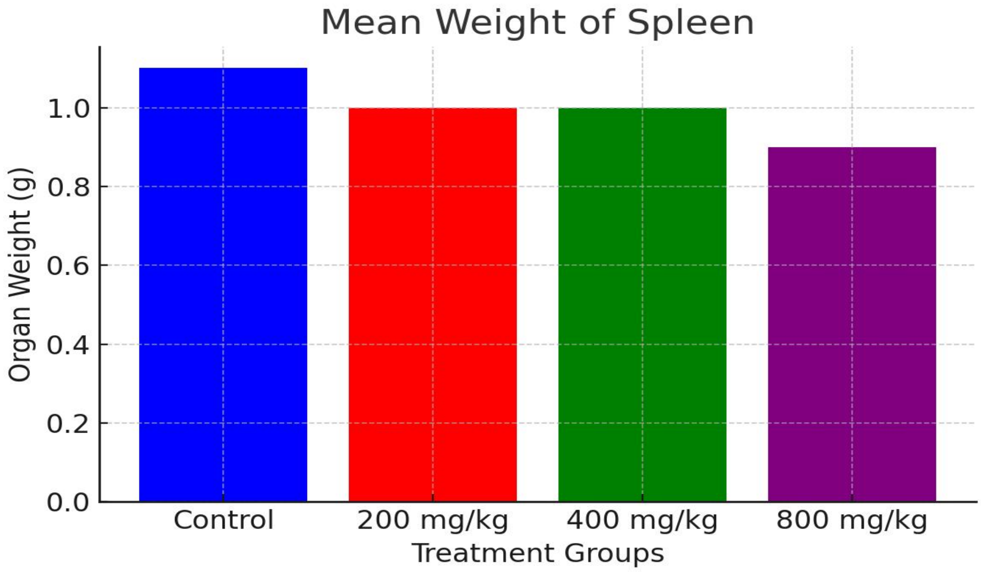


Figure 7: Right testes to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.

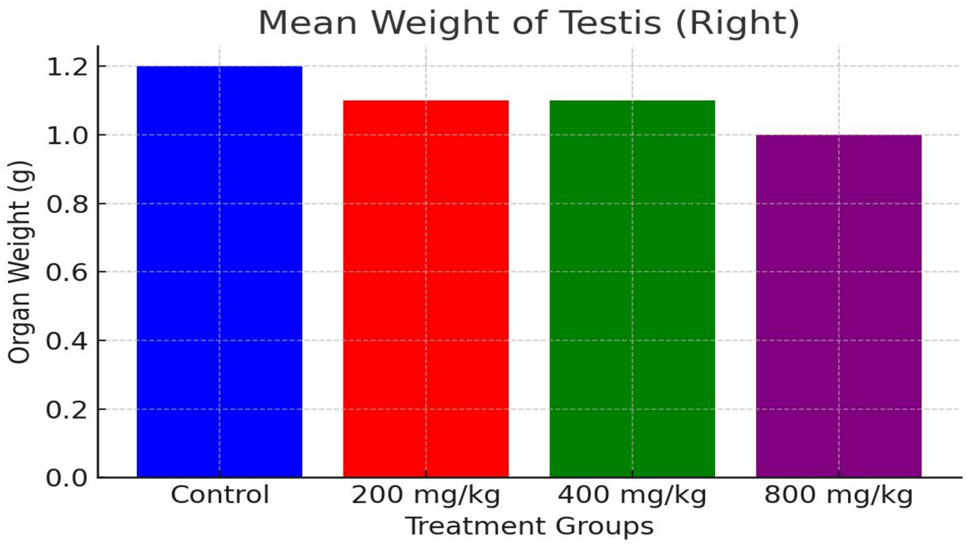
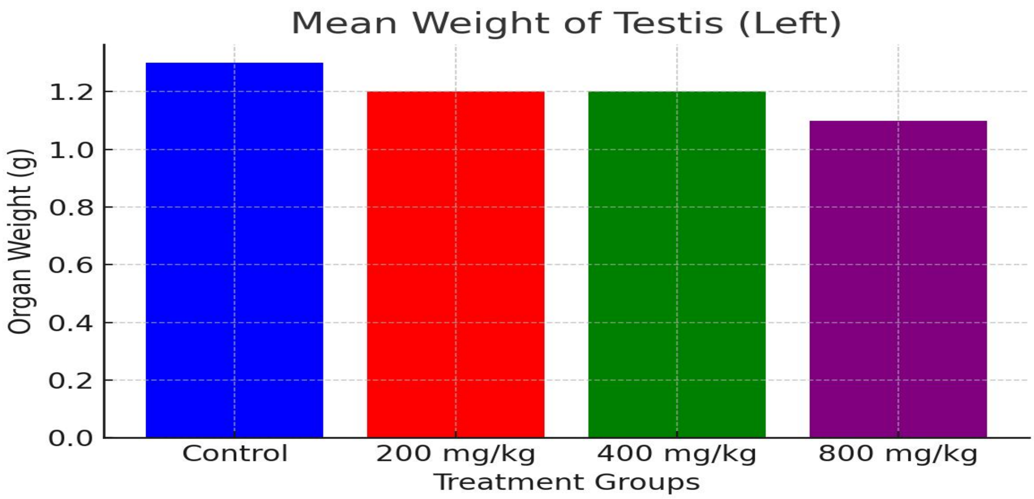


Figure 8: Left testes to body weight ratio following 28 days oral administration of methanol plant extract of *Anthocleista grandiflora*.



CHAPTER FIVE

5.1 RESULTS AND DISCUSSION

Table 1 presents the mean organ weights of rats administered methanol leaf extract of *Anthocleista grandiflora* at doses of 200, 400, and 800 mg/kg for 28 days, compared with the control group. Organ weights are valuable indicators of possible toxicological or physiological effects of test substances, as deviations from normal weight may reflect tissue hypertrophy, atrophy, or damage (Michael *et al.,* 2007).

The results indicate that the mean liver weights across treatment groups were generally comparable to the control. The highest dose group (800 mg/kg) recorded a liver weight of 7.059 ± 0.282 g, similar to the control value (7.253 ± 0.400 g), suggesting the absence of hepatic hypertrophy or atrophy. The slight reduction observed at 200 and 400 mg/kg (6.174 ± 0.326 g and 5.770 ± 0.211 g, respectively) was not significant, implying that the extract did not induce hepatotoxic effects or interfere with normal liver metabolism.

Renal (kidney) weights showed only minor, non-significant fluctuations between groups. The left and right kidneys remained within close range of the control values, indicating preserved renal function and structural integrity. Similarly, heart and spleen weights in treated rats were comparable to those of the control group, suggesting that *A. grandiflora* extract did not adversely affect cardiovascular or immunological organs.

Testicular weights, a sensitive marker of reproductive health, also showed no adverse variation across treatment groups. In fact, slight increases were noted in the 200 mg/kg and 800 mg/kg groups (1.455 ± 0.085 g and 1.496 ± 0.043 g for left testis, respectively), indicating that the extract may not exert anti-androgenic or testicular toxicity. This observation aligns with previous reports that *Anthocleista* species possess antioxidant and adaptogenic constituents that can protect against oxidative stress-induced gonadal impairment (Enechi and Odonwodo, 2018).

Table 2 presents the weekly changes in mean body weight of rats administered varying doses (200, 400, and 800 mg/kg) of methanol leaf extract of *Anthocleista grandiflora* for a sub-chronic period of 28 days, compared with the control group.

At the beginning of the experiment (Week 1), the mean body weights across all groups were relatively comparable, indicating uniformity in baseline conditions prior to extract administration. Over the 4-week treatment period, the control group showed a steady and progressive increase in mean body weight, reaching 236.60 ± 5.60 g at sacrifice. This trend reflects normal physiological growth in healthy rats.

In the treated groups, a similar progressive increase in body weight was observed, though the magnitude of gain varied across doses. Rats administered 400 mg/kg and 800 mg/kg exhibited comparable or slightly higher weight increments compared to the control, especially from Week 3 to sacrifice (219.24 ± 3.93 g and 227.50 ± 4.46 g, respectively). This pattern suggests that the extract did not interfere with normal feeding, metabolism, or growth processes at these doses.

Conversely, the 200 mg/kg group displayed relatively lower mean body weights throughout the study, peaking at 198.75 ± 2.71 g by the end of the experiment. This modest reduction could reflect individual variation or mild metabolic adaptation to the extract at that particular dose level.

5.2 CONCLUSION

Overall, the lack of significant changes in organ weights suggests that sub-chronic administration of methanol leaf extract of *A. grandiflora* is relatively safe and does not produce toxic morphological alterations in vital organs at the tested doses. In the same vein, the consistent weight gain across treatment groups, particularly at 400 mg/kg and 800 mg/kg, indicates that prolonged administration of *A. grandiflora* methanol extract did not exert any growth-suppressive or toxic effects. Rather, the extract appeared to support normal physiological development, suggesting a favorable safety profile at the tested doses.

These findings align with earlier studies reporting that *Anthocleista* species extracts are generally well tolerated in experimental animals and may even possess mild nutritive or metabolic-enhancing properties (Enechi and Odonwodo, 2018; Ijioma *et al.*, 2020).

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