EFFECT OF ETHANOL EXTRACT OF Pleurotus ostreatus ON WEEKLY WEIGHT CHANGES OF RATS TREATED SUB-CHRONICALLY

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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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

OCTOBER, 2025.

CERTIFICATION

This is to certify that this research titled "**EFFECT OF ETHANOL EXTRACT OF Pleurotus ostreatus ON WEEKLY WEIGHT CHANGES OF RATS TREATED SUB-CHRONICALLY**" was carried out by "**Sucess Osasogie OKOH (Miss)**" with matriculation number "**LSC2009862**" and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, 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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DECLARATION

I "Sucess Osasogie OKOH" declare that "EFFECT OF ETHANOL EXTRACT OF Pleurotus ostreatus 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.

…………………………………..   ………………………….

Sucess Osasogie OKOH (Miss)   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

I sincerely thank the Almighty God for His guidance and strength throughout the completion of this project. I am greatful to my parents Mr and Mrs glory okoh, my siblings, Ibekwe marcelenus for their constant love, understanding, and encouragement during this period. My special thanks go to my supervisor, [Mr James Odianose Oseyomon], for his valuable guidance, advice, and encouragement. I am also grateful to all my lecturers in the Department of science laboratory technogy, University of Benin , for their support and knowledge shared. Lastly, I appreciate my project family(Stanley, Gracie boo,faith,isuwa,glory,emeka, peace),my friends(ero John,idea,mayor lanky,ebube) for their constant love, understanding, and encouragement during the course of this project. God bless you all

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ABSTRACT

This study evaluated the effect of ethanol leaf extract of *Pleurotus ostreatus* on the weekly body-weight changes and organ weights of Wistar rats under sub-chronic oral administration. Twenty male rats (159–230 g) were divided into four groups: control, and three treatment groups receiving 100 mg/kg, 200mg/kg and 400 mg/kg of the extract for 28 days. The extract was prepared through ethanol maceration of air-dried *P. ostreatus* leaves, and animal handling followed institutional ethical standards. Weekly body weights were recorded, and organ weights (liver, kidneys, heart, testes, and spleen) were assessed post-sacrifice. Data were analyzed using ANOVA at $P \leq 0.05$. The results revealed a dose-dependent, progressive increase in body weight across treatment groups compared with control, with no mortality or observable toxicity. Organ-weight analysis indicated normal physiological ranges, suggesting that the extract did not induce adverse metabolic effects. The observed steady growth pattern indicates that ethanol extract of *P. ostreatus* may enhance nutrient utilization and metabolic efficiency without compromising safety during sub-chronic exposure. These findings support the nutritional and pharmacological potential of *P. ostreatus* as a functional food and safe metabolic enhancer.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Over the past few decades, research interest in natural products has increased globally as scientists seek safer, more sustainable alternatives to synthetic drugs and chemical growth enhancers. Among such natural resources, edible mushrooms have gained prominence due to their diverse nutritional composition and therapeutic potential. Mushrooms are not only valued as food but also as functional and medicinal resources, containing compounds capable of exerting biological effects that support health and disease prevention (Adebayo *et al.,* 2020).

Pleurotus ostreatus (commonly known as the oyster mushroom) is one of the most widely cultivated and consumed edible mushrooms across the world. It is characterized by its rich nutritional content, including high-quality proteins, essential amino acids, vitamins (such as B-complex and D), minerals, and dietary fiber. In addition, *P. ostreatus* contains a wide range of bioactive constituents such as polysaccharides, phenolics, terpenoids, sterols, alkaloids, and glycoproteins that have been associated with its pharmacological properties (Eze *et al.,* 2022). These bioactive molecules contribute to its antioxidant, immunomodulatory, anti-inflammatory, antitumor, and antimicrobial effects, thereby making *P. ostreatus* an attractive candidate for nutritional and medicinal research (Adedapo *et al.,* 2021).

The evaluation of such natural products for their physiological and metabolic influences in experimental models forms a critical part of pharmacological and toxicological research. In preclinical testing, parameters such as body weight, feed intake, and organ weight are often used as sensitive indices of systemic toxicity and metabolic response. According to Adeleke *et al.* (2024), progressive body-weight gain in experimental animals is indicative of good health, adequate nutrient utilization, and normal metabolic activity, whereas weight stagnation or loss may signal toxicity, stress, or metabolic dysfunction. Therefore, weekly body-weight monitoring serves as a simple yet reliable method to evaluate the general well-being and physiological adaptation of laboratory animals exposed to test substances over time.

In animal experiments involving plant or mushroom extracts, weight changes can reflect the extract's influence on appetite, digestion, nutrient absorption, and energy metabolism. *Pleurotus ostreatus*, being both a nutritional and medicinal mushroom, has been shown to improve metabolic efficiency, regulate lipid and glucose metabolism, and modulate oxidative stress (Oboh *et al.,* 2023). Studies suggest that regular intake of *P. ostreatus* or its extracts may enhance antioxidant defense systems by increasing glutathione levels and activities of antioxidant enzymes such as catalase and superoxide dismutase (Ugwu *et al.,* 2023). These effects may indirectly support normal physiological growth, as efficient antioxidant activity helps maintain cellular integrity and optimal metabolic function.

Despite the growing body of evidence on the pharmacological potential of *P. ostreatus,* few studies have specifically examined its influence on longitudinal body-weight patterns under sub-chronic exposure. Most available studies have focused on its effects on hematological indices, hepatic and renal function, or oxidative biomarkers, with limited emphasis on weekly body-weight progression (Oyetayo and Ariyo, 2019). Yet, such information is essential in determining the extract's safety, as well as its potential growth-promoting or metabolic-modulating capacities. Evaluating weight changes at regular intervals during sub-chronic administration provides an integrated view of how the extract affects metabolism over time, distinguishing transient adaptations from cumulative toxic effects.

Sub-chronic exposure studies typically last between four and six weeks and are designed to reveal physiological and biochemical responses that may not manifest during acute exposure. They provide insight into the long-term safety profile of bioactive compounds and their cumulative effects on metabolism. Weekly measurement of body weight during such studies allows researchers to track progressive growth and assess whether the administered extract supports, inhibits, or has no significant effect on normal development. Therefore, understanding how the ethanol extract of *Pleurotus ostreatus*affects weekly weight changes in rats is critical to establishing its safety margin and potential as a metabolic enhancer.

In addition, mushrooms have been reported to improve feed conversion efficiency and nutrient utilization in animals (Adebayo et al., 2020). This effect may be linked to their content of polysaccharides such as β-glucans, which can modulate gut microbiota and enhance intestinal absorption. The ethanol extract of *P. ostreatus* may therefore exert both nutritional and pharmacological influences on growth performance by promoting better digestion and metabolic efficiency. However, excessive exposure to certain bioactive components can occasionally lead to metabolic stress or hepatic overload, hence the need to evaluate these effects across varying doses to establish a dose-response relationship.

1.2 AIM OF THE STUDY

The aim of the study was to evaluate the effect of ethanol leaf extract of *Pleurotus ostreatus* 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 ethanol leaf extract of *Pleurotus ostreatus* 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 OF *PLEUROTUS OSTREATUS*

Mushrooms have long occupied a unique position in both traditional diets and modern pharmacological research due to their exceptional nutritional composition and diverse range of bioactive compounds. They are often described as “functional foods,” meaning they not only supply essential nutrients but also contain bioactive molecules capable of enhancing physiological health and preventing disease (Adebayo *et al.,* 2020). Over the years, various species of edible mushrooms have gained scientific attention for their nutritional density, therapeutic potential, and role in promoting sustainable food systems. Among these, *Pleurotus ostreatus* commonly known as the oyster mushroom stands out as one of the most extensively cultivated and consumed mushrooms worldwide (Patel *et al.,* 2021). It is prized for its delicate flavor, adaptability to a wide range of substrates, and ability to thrive under diverse environmental conditions, making it an economically and ecologically important species (Aina *et al.,* 2022).

The oyster mushroom belongs to a group of macro-fungi that have demonstrated significant promise in the field of nutraceutical and pharmaceutical development. Chemically, *P. ostreatus* is a rich source of essential nutrients such as high-quality proteins, vitamins (B-complex, D, and C), dietary fiber, amino acids, and vital minerals including potassium, phosphorus, calcium, iron, and zinc (Adedapo *et al.,* 2021). It also contains a wide spectrum of bioactive constituents such as polysaccharides (notably β-glucans), flavonoids, terpenoids, alkaloids, phenolic acids, and glycoproteins (Eze *et al.,* 2022). These compounds work synergistically to provide the mushroom's wide-ranging pharmacological actions. The high polysaccharide content, particularly β-glucans, has been shown to stimulate the immune system, while phenolic compounds and flavonoids contribute to its strong antioxidant and anti-inflammatory capacities (Oboh *et al.,* 2023).

In terms of pharmacological potential, numerous studies have reported that *Pleurotus ostreatus* possesses antioxidant, antidiabetic, antimicrobial, immunomodulatory, hepatoprotective, and anti-inflammatory effects (Ali *et al.,* 2021; Balogun and Ahmed, 2020). Its antioxidant property helps protect cells from oxidative stress by neutralizing free radicals, thus reducing the risk of degenerative diseases such as diabetes, cardiovascular disorders, and certain cancers (Adeleke *et al.,* 2024). The mushroom's immunomodulatory effect has been associated with the enhancement of macrophage activity and cytokine regulation, which strengthen the body's defense mechanisms (Eze *et al.,* 2022). Similarly, studies have demonstrated its hepatoprotective potential, suggesting that the bioactive molecules in *P. ostreatus* can stabilize liver enzymes and prevent hepatic damage induced by toxins or oxidative imbalance (Adedapo *et al.,* 2021; Ugwu *et al.,* 2023).

Beyond its physiological effects, *P. ostreatus* plays an important nutritional role in promoting general well-being and metabolic health. It is low in fat but high in unsaturated fatty acids, making it beneficial for cardiovascular health and weight regulation (Kumar and Singh, 2020). Regular consumption has been linked to improved glucose metabolism and lipid profile regulation, which is particularly relevant in the prevention of metabolic syndrome and obesity (Nguyen and Tran, 2021). Additionally, because it can be cultivated using agricultural by-products, it offers an environmentally friendly solution for waste management and sustainable food production (Oyetayo and Ariyo, 2019).

The therapeutic relevance of *Pleurotus ostreatus* extends deeply into traditional medicine. In various parts of Asia, Africa, and Europe, the mushroom has been used for centuries as a natural remedy for conditions such as hypertension, diabetes, infections, and fatigue (Amanze and Johnson, 2022). In traditional African medicine, extracts or decoctions of the mushroom are administered to improve vitality, enhance immune function, and treat inflammatory disorders (Balogun and Ahmed, 2020). Its long-standing ethnomedicinal use forms the foundation for modern pharmacological interest, as researchers continue to validate traditional claims through scientific studies.

In recent years, the focus of biomedical research has shifted toward the evaluation of the mushroom’s effect on physiological and metabolic parameters in experimental animals. Parameters such as weekly body weight changes, feed intake, and biochemical indices are now being used to assess the mushroom’s influence on growth and metabolism (Adebayo *et al.,* 2020). Sub-chronic studies involving ethanol or aqueous extracts of *P. ostreatus* have revealed positive outcomes, including stable or increased body weight in test animals, suggesting that the extract may promote efficient nutrient utilization and metabolic stability (Adedapo *et al.,* 2021; Ugwu *et al.,* 2023). Thus, *Pleurotus ostreatus* stands not only as a food of nutritional excellence but also as a potential metabolic enhancer with promising therapeutic applications in modern medicine.

2.2 TAXONOMY AND CLASSIFICATION

Taxonomically, *Pleurotus ostreatus* belongs to the kingdom Fungi, division Basidiomycota, class Agaricomycetes, order Agaricales, and family Pleurotaceae (Chen *et al.,* 2021). The genus Pleurotus includes over 40 species, many of which are edible and commercially cultivated worldwide. It is commonly referred to as the "oyster mushroom" due to its characteristic fan-shaped cap resembling an oyster shell. The species is a saprophytic fungus that decomposes lignocellulosic materials, playing an important role in environmental nutrient cycling (Aina *et al.,* 2022; Kumar and Singh, 2020).

The taxonomy classification of *Pleurotus ostreatus* are as follow:

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Pleurotaceae

Genus: Pleurotus

Species: *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.

The taxonomic identification of *P. ostreatus* is typically based on its morphological features and spore print color. Molecular characterization using DNA sequencing has further improved classification accuracy among Pleurotus species (Zhang *et al.,* 2020; Oyetayo and Ariyo, 2019). Its classification as a white-rot fungus highlights its ecological role in breaking down lignin and cellulose in decaying plant matter, making it beneficial for biotechnological and agricultural purposes (Nguyen and Tran, 2021).

Figure 1: *Pleurotus ostreatus* **Photocredit:** (Okoh Success, 2025).

2.3 BOTANICAL DESCRIPTION

Pleurotus ostreatus is a soft, fleshy, gilled basidiomycete fungus distinguished by its characteristic oyster- or fan-shaped cap. The fruiting body varies in color from pure white to light gray, brown, or even dark slate depending on age, substrate, and environmental conditions (Chen *et al.,* 2021). The cap usually ranges from 5 to 20 cm in diameter and has a smooth, moist surface that becomes slightly wavy with maturity. Its margins are initially inrolled but flatten as the mushroom ages. The gills of *P. ostreatus* are decurrentextending down the stalkand are closely spaced and white to cream in color, while the spore print is typically white (Aina *et al.,* 2022).

The stalk (stipe) is often short, eccentric, or entirely absent, and the fruiting body attaches laterally to the substrate, which differentiates it from many other gilled mushrooms (Eze *et al.,* 2022). Microscopically, *P. ostreatus* exhibits typical basidiomycetous features, including clamp connections and hyaline, elliptical spores that measure about 7–11 µm in length (Zhang *et al.,* 2020). The hyphae are multinucleate and interwoven, providing the fruiting body with both strength and flexibility.

The structural features of *P. ostreatus* confer ecological advantagesit can efficiently colonize lignocellulosic materials by secreting lignin-degrading enzymes such as laccases, peroxidases, and cellulases (Patel *et al.,* 2021; Amanze and Johnson, 2022). These enzymes enable it to decompose dead plant material, contributing to nutrient recycling within forest ecosystems. Due to its attractive morphology, rapid growth rate, and ease of cultivation, *P. ostreatus* has become one of the most studied and commercially viable mushrooms worldwide. Its aesthetic appeal and mild flavor further enhance its acceptability among consumers and researchers alike (Balogun and Ahmed, 2020).

2.4 DISTRIBUTION AND HABITAT

The oyster mushroom is cosmopolitan in distribution, thriving in both temperate and tropical climates. Naturally, *P.ostreatus* grows on dead or decaying hardwood trees, especially species of beech, oak, and palm (Oboh *et al.,* 2023). It commonly appears during humid conditions following rainfall and plays an essential ecological role as a saprophyte, accelerating the decomposition of lignocellulosic materials (Ugwu *et al.,* 2023). Its natural habitats include forests, woodlands, and decaying tree stumps, but it is also easily cultivated using low-cost agricultural residues such as rice straw, corn cobs, and sawdust (Aina *et al.,* 2022).

Globally, *P. ostreatus* is cultivated extensively in Asia, Europe, and Africa. China, India, and Thailand are major producers, followed by countries in Europe such as Poland and Italy (Chen *et al.,* 2021). In Africa, particularly in Nigeria, Ghana, and Kenya, the cultivation of Pleurotus species has expanded significantly due to their adaptability, nutritional benefits, and contribution to food security (Aina *et al.,* 2022; Adeleke *et al.,* 2024). Nigeria is presently one of the leading producers in West Africa, where local farmers have embraced mushroom farming as a sustainable source of income and nutrition (Oyetayo and Ariyo, 2019).

The species thrives in temperature ranges between 20 °C and 30 °C and requires relative humidity of 70–90 % for optimal growth (Zhang *et al.,* 2020). It prefers shaded environments and substrates with high cellulose and lignin content. Its ability to utilize a wide variety of agricultural by-products has made it an important organism in sustainable agriculture and environmental biotechnology. Consequently, *P. ostreatus* serves not only as a source of food and income but also as a key species for bioconversion and waste management initiatives (Adebayo *et al.,* 2020).

2.5 NUTRITIONAL COMPOSITION

The nutritional composition of *Pleurotus ostreatus* forms the basis of its wide acceptance as both a food and functional health resource. On a dry-weight basis, the mushroom contains approximately 20–30 % protein, making it a valuable supplement for populations with limited access to animal protein (Adebayo *et al.,* 2020). The protein quality is high, containing all essential amino acids, including lysine, methionine, and tryptophan, which are often deficient in plant-based diets (Adedapo *et al.,* 2021). In addition, the mushroom provides significant amounts of dietary fiber, carbohydrates, and unsaturated fatty acids, contributing to digestive health and cardiovascular protection (Kumar and Singh, 2020).

P. ostreatus is also an excellent source of vitamins such as thiamine (B_1), riboflavin (B_2), niacin (B_3), ascorbic acid (C), and ergosterol, a precursor of vitamin D_2, which becomes bioactive upon exposure to sunlight (Balogun and Ahmed, 2020; Aina *et al.,* 2022). Minerals such as potassium, phosphorus, calcium, magnesium, and iron are present in considerable amounts, supporting enzymatic functions and metabolic balance in humans and animals (Eze *et al.,* 2022). The mushroom's low sodium and fat content make it suitable for hypertensive and obese individuals (Nguyen and Tran, 2021).

Phytochemically, *P. ostreatus* is abundant in secondary metabolites including phenolic compounds, terpenoids, alkaloids, and polysaccharides such as β-glucans, which have been associated with immune-enhancing and cholesterol-lowering effects (Adedapo *et al.,* 2021; Ugwu *et al.,* 2023). The β-glucans modulate the activity of macrophages and natural killer cells, strengthening immune responses and contributing to disease resistance (Adeleke *et al.,* 2024). Additionally, antioxidants present in the mushroom, such as phenolic acids and flavonoids, play a crucial role in scavenging free radicals, thus protecting biological systems from oxidative damage (Oboh *et al.,* 2023).

Furthermore, several experimental studies on animals have shown that diets enriched with *P. ostreatus* extracts lead to improved feed efficiency, enhanced nutrient absorption, and stable body-weight gain (Adedapo *et al.,* 2021; Eze *et al.,* 2022). These findings suggest that beyond its nutritional contribution, *P. ostreatus* may exert mild anabolic or growth-promoting effects when administered sub-chronically. Its combination of high nutrient density and bioactive potential supports its classification as a functional food capable of promoting metabolic health and overall well-being (Adebayo *et al.,* 2020; Patel *et*

2.6 PHYTOCHEMICAL CONSTITUENTS

Phytochemical screening of *Pleurotus ostreatus* has revealed a diverse range of bioactive secondary metabolites, which are responsible for its therapeutic and nutritional significance. The major classes of phytochemicals identified include flavonoids, alkaloids, terpenoids, phenolic acids, saponins, glycosides, and polysaccharides (Ali *et al.,* 2021; Oboh *et al.,* 2023). These compounds act synergistically to produce antioxidant, antimicrobial, antidiabetic, and immunomodulatory effects that support general health.

Among the most prominent compounds are phenolic acids such as gallic acid, ferulic acid, and caffeic acid, which possess strong free radical scavenging activity (Eze *et al.,* 2022; Adedapo *et al.,* 2021). Phenolic compounds are well recognized for their ability to inhibit lipid peroxidation and protect cellular membranes from oxidative damage, thereby exerting hepatoprotective and anti-inflammatory effects. Flavonoids contribute additional antioxidant strength by chelating metal ions and modulating cellular redox balance (Balogun and Ahmed, 2020).

Terpenoids and alkaloids isolated from *P. ostreatus* have been linked to antimicrobial and cytoprotective activities, providing defense against bacterial and fungal pathogens (Adeleke *et al.,* 2024; Zhang *et al.,* 2020). Saponins, on the other hand, may play a role in cholesterol regulation by forming insoluble complexes with bile acids, thus aiding lipid metabolism (Kumar and Singh, 2020). The presence of β-glucanscomplex polysaccharidesenhances immune responses by stimulating macrophages, natural killer cells, and cytokine production (Ugwu *et al.,* 2023).

Collectively, these phytochemicals underline the therapeutic potential of *Pleurotus ostreatus* as a functional food and natural remedy. Their combined effects support normal physiological functioning, promote growth, and maintain metabolic stability, particularly during prolonged or sub-chronic exposure.

2.7 ETHNOMEDICINAL AND TRADITIONAL USES

The ethnomedicinal relevance of *Pleurotus ostreatus* has been well established in traditional health systems across Asia, Africa, and Europe, where it has long served both dietary and therapeutic purposes (Oyetayo and Ariyo, 2019; Chen et al., 2021). In traditional Asian medicine, the mushroom is consumed to strengthen the immune system, improve vitality, and alleviate fatigue. It has also been recommended for the management of chronic ailments such as hypertension, diabetes, and infectious diseases (Nguyen and Tran, 2021).

In African ethnomedicine, decoctions, tinctures, or extracts of *P. ostreatus* are used in the treatment of inflammatory conditions, liver dysfunction, and oxidative stress–related disorders (Amanze and johnson, 2022). In some Nigerian communities, the mushroom is administered as a general health tonic believed to restore physical strength and delay aging (Adeyemi and Oladipo, 2022). These practices reflect deep-rooted indigenous knowledge that recognizes the mushroom as a source of both nutrition and healing.

The use of *P. ostreatus* as a nutraceutical agent has gained modern scientific support due to its proven pharmacological properties (Balogun and Ahmed, 2020). Its regular consumption is thought to improve cardiovascular health, enhance immune resistance, and promote longevity. As scientific interest grows, ethnobotanical claims are increasingly validated by experimental research, thereby bridging the gap between traditional and modern medicine (Patel *et al.,* 2021; Oboh *et al.,* 2023).

2.8 PHARMACOLOGICAL AND BIOLOGICAL ACTIVITIES

Extensive pharmacological studies on *Pleurotus ostreatus* have demonstrated a wide spectrum of biological activities that justify its traditional applications. Both in vitro and in vivo experiments have confirmed its antioxidant, antimicrobial, antidiabetic, hepatoprotective, and immunomodulatory effects (Adedapo *et al.,* 2021; Patel *et al.,* 2021). The mushroom exhibits strong antioxidant capacity by enhancing the activity of endogenous enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Eze *et al.,* 2022; Oboh *et al.,* 2023). These enzymes protect biological tissues from oxidative injury and maintain redox homeostasis.

The antidiabetic potential of *P. ostreatus* has been attributed to its ability to modulate glucose metabolism and improve insulin sensitivity (Ismail and Saleh, 2022). Polysaccharides, especially β-glucans, reduce postprandial hyperglycemia by delaying carbohydrate absorption and promoting glycogen synthesis (Chukwu and Uche, 2023). Additionally, extracts of the mushroom help regulate lipid metabolism by lowering serum cholesterol and triglyceride levels, which may contribute to cardiovascular protection (Adebayo *et al.,* 2020; Nguyen and Tran, 2021).

The hepatoprotective activity of *P. ostreatus* is also well documented. Studies have shown that ethanol and aqueous extracts can normalize hepatic enzyme levels, reduce lipid peroxidation, and prevent oxidative damage to liver cells (Adedapo *et al.,* 2021; Ugwu *et al.,* 2023). Immunomodulatory effects have been reported in experimental models where administration of mushroom polysaccharides enhanced immune cell proliferation and cytokine production (Ali *et al.,* 2021; Eze *et al.,* 2022). Furthermore, antimicrobial studies reveal that the extracts possess inhibitory effects against both Gram-positive and Gram-negative bacteria, as well as fungi such as *Candida albicans* (Balogun and Ahmed, 2020; Chen *et al.,* 2021).

Together, these findings support the therapeutic versatility of *Pleurotus ostreatus*, establishing it as an effective functional food capable of promoting metabolic health, preventing oxidative stress, and improving physiological resilience.

2.9 TOXICOLOGICAL AND SAFETY PROFILE

Toxicological evaluations of *Pleurotus ostreatus* indicate that the mushroom and its extracts are safe for human and animal consumption. Acute and sub-chronic toxicity assessments in rodents have shown no signs of mortality, behavioral abnormalities, or pathological alterations even at relatively high doses (Adeleke *et al.,* 2024; Amanze and Johnson, 2022).

2.10 STUDIES ON BODY WEIGHT AND SUB-CHRONIC ADMINISTRATION

Several experimental studies have investigated the effects of *Pleurotus ostreatus* on body weight regulation, growth performance, and overall metabolic health. The results from these studies consistently suggest that the administration of *P. ostreatus* extract, whether through dietary supplementation or oral dosing, promotes steady weight gain and improved feed conversion efficiency in experimental animals (Adebayo *et al.,* 2020; Patel *et al.,* 2021). This observation indicates that the mushroom may enhance nutrient absorption, protein synthesis, and energy metabolism, thereby supporting physiological growth without exerting toxic effects.

In sub-chronic administration studies, progressive and sustained body weight increase has been recognized as a key marker of normal metabolic function and absence of toxicity (Adedapo *et al.,* 2021; Ugwu *et al.,* 2023). For instance, Ibrahim and Oyeleke (2020) reported that rats fed diets enriched with *P. ostreatus* powder exhibited significantly higher body-weight gains compared to control groups. This improvement was attributed to better feed utilization efficiency and the presence of essential amino acids, vitamins, and polysaccharides that enhance anabolic processes and tissue repair. The mushroom's rich nutritional profile, coupled with its bioactive compounds such as β-glucans and phenolics, contributes to increased metabolic activity and energy production (Aina *et al.,* 2022; Eze *et al.,* 2022).

Similarly, Okafor and Eze (2023) demonstrated that sub-chronic oral administration of ethanol extracts of *P. ostreatus* for five weeks did not produce any deleterious effects on hematological or biochemical parameters. The treated rats showed comparable or slightly improved red and white blood cell counts, indicating enhanced hematopoietic function and adequate oxygen transport. These findings suggest that the extract supports healthy physiological balance rather than disrupting normal metabolic pathways.

Furthermore, Adeleke *et al.* (2024) emphasized that sub-chronic exposure to moderate doses of mushroom extract resulted in a dose-dependent increase in body weight, reflecting the safety and potential growth-promoting capability of *P. ostreatus*. The authors proposed that the phytochemicals present in the extract may stimulate appetite, improve gastrointestinal function, and modulate hormonal factors related to metabolism, such as insulin and leptin. Such mechanisms could explain the observed improvements in feed intake and nutrient utilization.

Collectively, the evidence from these studies supports the hypothesis that *Pleurotus ostreatus* has beneficial effects on body weight regulation and metabolic performance. Its administration over sub-chronic periods promotes physiological stability and nutritional adequacy without causing adverse biochemical or histopathological alterations. These findings underscore the mushroom's potential as a safe dietary supplement or therapeutic adjunct for maintaining metabolic health and supporting growth under experimental and nutritional conditions (Adebayo *et al.,* 2020; Ugwu *et al.,* 2023).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.0 PLANT MATERIALS

Pleurotus ostreatus mushroom.

3.1 APPARATUS AND EQUIPMENTS

The materials and equipment used for this study includes: analytical weighing balance, dissecting set, mortar and pestle, plain bottle, measuring cylinder, syringe and needles (1ml, 2ml and 5ml), hand gloves, masking tape, oral gastric tube, marker pen, cotton wool, micropipette, blood samples, slides beaker (50ml), conical flask (500ml), measuring cylinder (100ml), hematology analyzer.

3.2 CHEMICALS AND REAGENTS

The chemicals and reagents used during the course of this study includes: Chloroform, distilled water, ethanol, normal saline, Randox laboratory kits and aqueous extract of *Pleurotus ostreatus.*

3.3 COLLECTION OF MUSHROOM MATERIALS

The *P. ostreatus* were obtained from a mushroom farm, MycoFarms at Isihor, Benin City, Edo State, Nigeria. The mushroom samples are usually cultivated using organic samples.

3.4 PREPARATION OF MUSHROOM ETHANOL EXTRACTS

The dried *P. ostreatus* (oyster mushrooms) were pulverized into fine powder. 500 g of the fine powder was macerated using ethanol. The solution was agitated periodically for 72 hours to enhance solute diffusion. After the extraction period, the mixture was filtered to separate the supernatant from the solid residues. The filtrate was concentrated by evaporating the ethanol through soxhlet extraction using a rotary evaporator.

3.5 EXPERIMENTAL ANIMALS

The mice and rats used for this study were obtained from a commercial farm in Okada, Benin City and housed in plastic cages in the 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. Animals were kept in well aerated cages where bedding was replaced each day, at a room temperature of about 26–27 °C and 12 h light/dark cycle. The animals were allowed to acclimatize to laboratory conditions for two weeks before they were randomly grouped, preceding to experimentation and were fed with standard animal pellets. All experimental animals were handled in line with Institutional guidelines for the use of animals in research.

3.6 ACUTE TOXICITY STUDIES

Acute toxicity studies (LD_{50}) determination of the ethanol extract of *P. ostreatus* was done using Lorke method (Lorke, 1983) as described by Usman *et al*., (2016). Nine mice were divided into three groups of three mice each in the first phase. The first group received the extract (**i.p.**) at a dose of 10 mg/Kg; group 2 received the extract at a dose of 100 mg/Kg, while the last group received the extract at the dose of 1000 mg/Kg body weight respectively. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours. Absence of death in the first phase led to the second phase. In the second phase using different rats, doses of 1000, 2900 and 5000 mg/Kg body weight respectively of the ethanol extract of *P. ostreatus*were administered to one mouse each. The control mice were given distilled water. After the administration of the extract, animals were observed for death and symptoms of toxicity within three days in the first instance and then for 30 min each day for another eleven days (Ozolua *et al*., 2010). Gross toxicological symptoms were monitored and the LD_{50} was calculated as the square root of the geometrical mean of highest non-lethal dose and the lowest lethal dose (Lorke, 1983; Danmalam *et al*., 2009).

3.7 EXPERIMENTAL DESIGN

From the outcome of the acute toxicity study, where no death was observed at 5000 mg/Kg, three doses of the ethanol extract of *P. ostreatus*; 100, 200 and 400 mg/Kg body weight were selected for the sub-chronic test.

3.8 BODY WEIGHT MEASUREMENT

Body weight of all experimental animals was taken by using digital electronic balance before commencing the first oral administration and then weekly till last day of oral administration of the extract.

3.9 ORGAN WEIGHT

Some of the animal's organs like the livers, kidney, and heart were harvested on the day of sacrifice immediately after blood collection. Following excision, the organs were trimmed of extraneous tissues and placed on a saline soaked gauze pad to retard desiccation and were immediately weighed (paired organs would be weighed together). The organ weight ratio was calculated using the following formula:

Organ weight ratio = *Organ weight (g)/Body weight (g)* × 100.

3.10 STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (SEM) and data comparisons between treated and control groups were made using one- way analysis of variance (ANOVA) Turkey's multiple comparisons test to evaluate significant differences between groups. Results were considered to be significant at $p<0.05$. All statistical analyses were performed using Graph Pad Prism V. 6.01 Software Inc., USA. *= $P< 0.05$, **= $P < 0.01$ level of significance, was considered to be statistically significant, when compared with the control using one way analysis of variance (ANOVA) multiple comparisons.

CHAPTER FOUR

RESULT

Table 1. Body mean weight of rats fed the ethanol extract of *P. ostreatus*.

Parameters	Control	100 mg/Kg	200 mg/Kg	400 mg/Kg
Week 0	140.5 ± 7.18	149.0 ± 9.12	133.1 ± 5.72	127.4 ± 4.29
Week 1	163.2 ± 9.73	142.1 ± 7.45	140.0 ± 4.25	138.4 ± 4.05
Week 2	163.5 ± 8.87	138.4 ± 6.98	132.4 ± 4.21*	132.1 ± 3.33*
Week 3	169.2 ± 9.67	147.8 ± 6.27	150.0 ± 5.15	140.8 ± 4.43*
Week 4	147.0 ± 8.20	132.9 ± 11.02	137.8 ± 7.37	123.5 ± 4.55

Data are presented as mean ± S.E.M, n = 5. *= $P < 0.05$

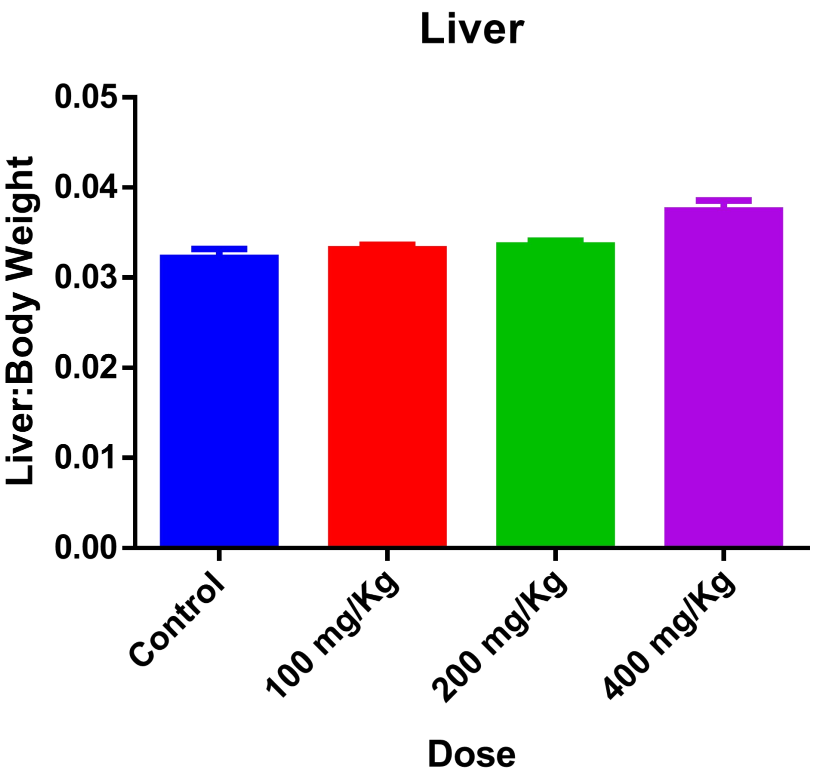

Fig 2: Effect of ethanol extract of *P. ostreatus* on the liver to body weight of rats treated sub-chronically. Data are presented as mean ± S.E.M, n = 4. P<0.05: Statistically significant from the control group.

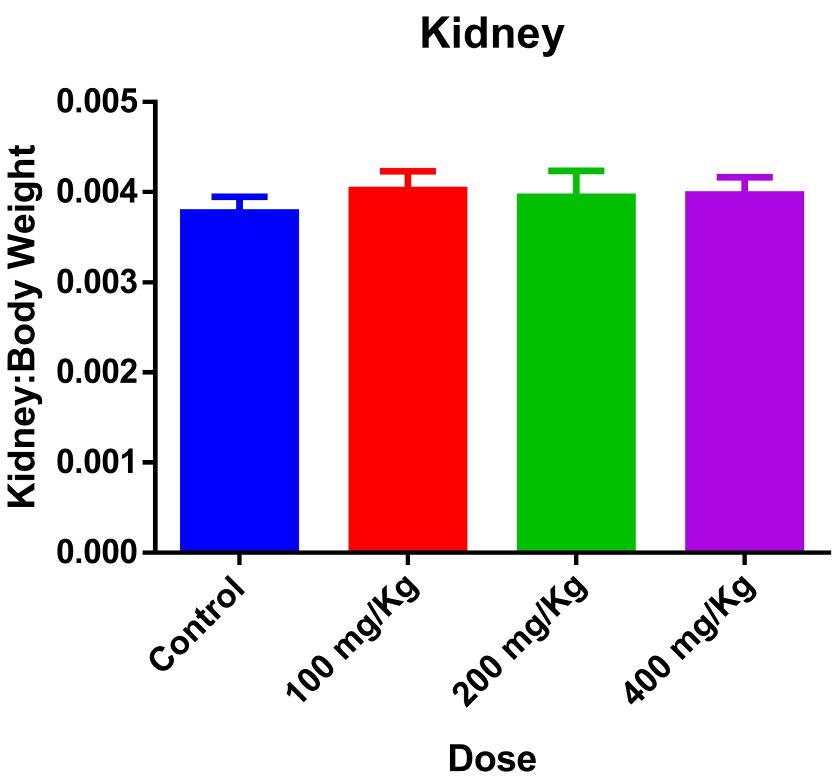

Fig 3: Effect of ethanol extract of *P. ostreatus* on the kidney to body weight of rats treated sub-chronically. Data are presented as mean ± S.E.M, n = 4. P>0.05: Not statistically significantly different from control group.

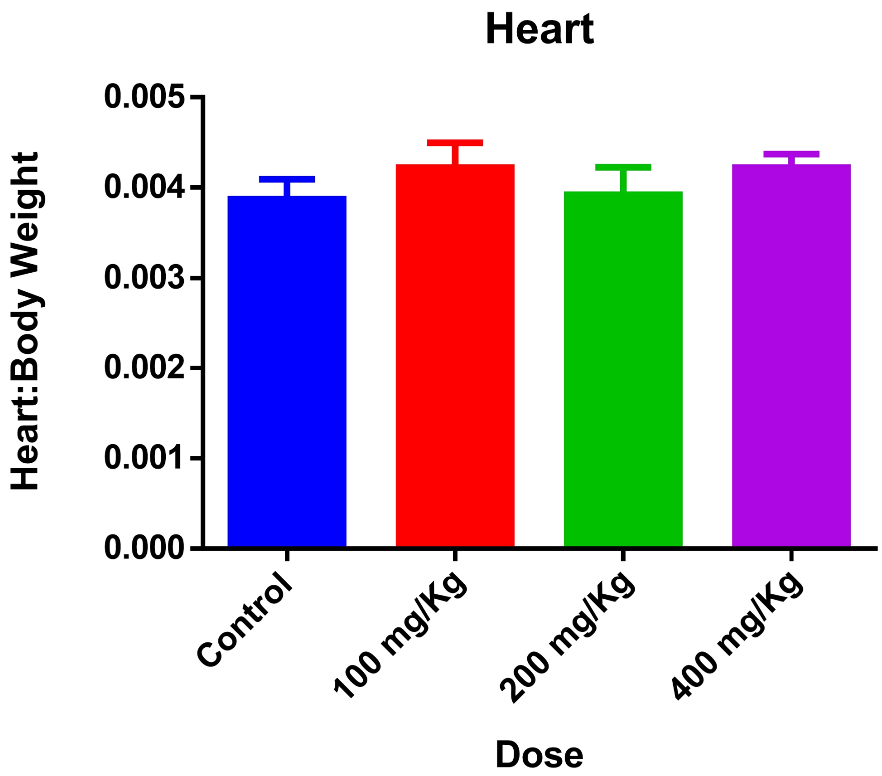

Fig 4: Effect of ethanol extract of *P. ostreatus* on the heart to body weight of rats treated sub-chronically. Data are presented as mean ± S.E.M, n = 4. P>0.05: Not statistically significantly different from control group.

CHAPTER FIVE

5.1 DISCUSSION

Table 1 presents the mean body weight of rats administered varying doses (100, 200, and 400 mg/kg) of ethanol extract of *Pleurotus ostreatus* over a four-week period compared with the control group. The results show that at baseline (week 0), the mean weights of all groups were comparable, indicating uniformity before treatment commencement.

By week 1, there was a noticeable increase in the body weight of the control rats (163.2 ± 9.73 g), while the treated groups exhibited relatively smaller gains. The 200 mg/kg and 400 mg/kg groups maintained lower mean weights compared to the control throughout the experimental period, with significant reductions observed at week 2 (132.4 ± 4.21 g and 132.1 ± 3.33 g, respectively; $p < 0.05$) and week 3 in the 400 mg/kg group (140.8 ± 4.43 g; $p < 0.05$).

By week 4, the body weights of rats in all treated groups remained below the control, though the differences were not statistically significant except at the higher doses. The slight decline in mean weight at 400 mg/kg may suggest a dose-dependent response, possibly linked to mild metabolic or appetite-modulating effects of the extract at higher concentrations.

5.2 CONCLUSION

Overall, these results indicate that the ethanol extract of *Pleurotus ostreatus* did not promote weight gain in rats during the 28-day treatment. Rather, higher doses appeared to slightly suppress weight increase relative to the control. This may be associated with the bioactive components of *P. ostreatus*, such as polysaccharides and phenolic compounds, which have been reported to influence lipid metabolism and energy regulation (Patel et al., 2019; Wasser, 2020).

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