**NUTRITIONAL, ANTIDIABETIC, and ANTILIPIDEMIC STUDIES of ETHANOLIC EXTRACT of P. TUBER-REGIUM in HIGH-FAT-STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR ALBINO RATS.**

.

ABSTRACT

|  |
| --- |
| *Pleurotus tuber-regium*, a nutritionally dense and bioactive-rich edible mushroom prevalent in West Africa, is gaining attention for its potential role in functional foods and complementary strategies for managing metabolic diseases. This study investigated the nutritional composition and therapeutic potential of *P. tuber-regium* in high-fat diet and streptozotocin (STZ)-induced diabetic male Wistar albino rats. Proximate analysis revealed the mushroom to be rich in carbohydrates (68.04%), crude protein (19.92%), and dietary fibre (5.44%), with low fat content (0.97%) and minimal moisture (2.20%), highlighting its suitability for hypocaloric diets. High-performance liquid chromatography (HPLC) analysis showed the presence of essential B-complex vitamins—thiamine (0.006 mg/g), riboflavin (0.003 mg/g), niacin (0.001 mg/g)—and vitamin C (0.008 mg/g), suggesting antioxidant and metabolic support roles. Mineral profiling via inductively coupled plasma-optical emission spectroscopy (ICP-OES) further confirmed the presence of micronutrients essential for enzymatic functions and metabolic homeostasis.Following a 9-week oral administration of *P. tuber-regium* ethanol extract at graded doses and metformin (200, 400, 800, 80 mg/kg respectively), diabetic rats demonstrated significant (p < 0.05) improvements in fasting blood glucose, lipid profile (triglycerides, total cholesterol, High Density Lipoprotein, Low Density Lippprotein), and pancreatic amylase activity. The Histomorphology assessments of pancreatic tissues also revealed restoration of islet architecture in extract-treated groups. Acute oral toxicity testing indicated no mortality or observable toxicological symptoms up to 6000 mg/kg, confirming the safety of *P. tuber-regium* extract.The results suggest that *P. tuber-regium* possesses promising antidiabetic and hypolipidemic properties, potentially mediated by its fibre content, micronutrients, and bioactive constituents. This supports its application as a nutraceutical in the dietary management of Type 2 Diabetes Mellitus. Further mechanistic and clinical studies are warranted to elucidate the pathways involved and to validate translational applicability in human populations.**Keywords:** P. *tuber-regium,* Type 2 Diabetes Mellitus, nutraceutical, Antidiabetic effect, lipid profile. |

1. INTRODUCTION

Edible mushrooms are increasingly being investigated for their nutritional and health benefits. The oyster mushroom (Pleurotus spp.) is one of the most widely cultivated edible mushrooms and is well-known for its therapeutic properties, culinary applications, and uses in biotechnology, environmental management, and pharmaceuticals (Rathod *et al*., 2021; El-Ramady *et al*., 2022; Assemie & Abaya, 2022). These remarkable fungi also grow in the wild, serving as food for both animals and humans (Amara & El-Baky, 2023; Case *et al*., 2022). They are crucial in maintaining ecosystem balance, act as raw materials for various industries, and serve as ingredients in traditional medicines (Bell *et al*., 2022; Panda *et al*., 2024; Ogwu *et al*., 2025). Törős *et al.,* 2022; Carrasco *et al.,* 2021; Adedokun *et al*., 2022 reported that oyster mushrooms are the most commercially cultivated globally.

In Nigeria, among the numerous Pleurotus species, P. tuber-regium commonly known as the tiger milk mushroom—holds a special place in traditional diets, local snacks, as a soup thickener, meat substitute, and bulking agent (Kumar & Netam *et al., 2022*; Afolabi *et al*., 2024; Abdullah *et al.,* 2024). This fungus has gained increasing recognition in recent years, accounting for over 25% of all commercially grown edible mushrooms, due to its ability to grow on agro-waste and its rich nutritional profile (Raman *et al.,2021;* Ghafoor, Niazi *et al*.,2024) P. tuber-regium is regarded as a valuable source of essential amino acids, fat- and water-soluble vitamins, carbohydrates, proteins, dietary fiber, and minerals (Kolawole *et al., 2021;* Raman *et al.,2021)*.

Studies have shown that *P. tuber-regium* is a reservoir of both micro- and macronutrients (Igbokwe *et al.,2015;* Lin *et al., 2020; Ekute* & Nwokocha, 2021). Its amino acid profile, low fat content, vitamin composition, and high dietary fibre make it suitable for managing metabolic disorders such as diabetes, hypertension, and cardiovascular diseases (Adeyi *et al*., 2021). This natural resource is also rich in essential minerals such as calcium, phosphorus, potassium, magnesium, iron, and zinc, which serve as cofactors for enzymes involved in various metabolic processes and immune modulation. It plays a role in blood glucose and lipid regulation as well as the maintenance of cellular function (Ijeh *et al*.,2009; Adeyi *et al*., 2021).

Furthermore, P. tuber-regium contains a wide variety of bioactive compounds, including β-glucans, terpenoids, steroids, and phenolics, which exhibit antimicrobial, immunomodulatory, and antioxidant Properties (Nworu *et al., 2015;* Alaribe *et al.,2018;* Lin *et al., 2020)*. Recently, there has been a growing interest in sustainable, non-animal-based nutrition. With its dense nutritional content, P. tuber-regium presents a promising alternative source of nutrition and a potential functional food ingredient (Lin *et al., 2020;* Adeyi *et al*., 2021; Aswathy *et al., 2024)*.

This study explores the nutritional and bioactive profile of *P. tuber-regium* and its effects on blood glucose levels, lipid profile, pancreatic amylase activity, and pancreatic histomorphology in high-fat-streptozotocin-induced diabetic Wistar albino rats. The findings are expected to contribute to the scientific validation of *P. tuber-regium* as a functional food in the dietary management of diabetes.

1. material and methods
	1. **Collection of mushrooms**

This was done using the modified method as described by Okorie et al. 2021. *P. tuber-regium* is one of the most popular edible mushrooms and can be easily cultivated on various agricultural wastes such as straw, sawdust, coffee grounds, cotton waste, and other lignocellulosic substrates. Composed substrates made from a mix of sawdust and rice bran (9:1) were placed into polyethylene bags, which were sterilized in an automated autoclave at 121°C to eliminate competing microorganisms. These substrates were allowed to cool to ambient temperature before being inoculated with the spawns of *P. tuber-regium* cultivated in the mushroom centre of the Federal Institute of Industrial Research, Oshodi. Inoculation was performed under aseptic conditions. The incubation stage was conducted in darkness to enable mycelium growth through the substrate, forming a network. Fully ramified substrates (17-22 days) were exposed to appropriate environmental conditions (light, humidity, temperature) to induce fruiting and the development of mushroom caps. The mature oyster mushrooms were harvested by cutting clusters at the base of the stem.

**2.2 Nutritional compositions**

The nutritional composition of *P. tuber-regium* was evaluated in this study. The Proximate analysis, vitamins, and mineral elements.

**2.2.1 Proximate analysis**

Proximate analysis plays a vital role in determining the nutritive values of various food substances. It serves as a foundational tool for the development of new food products, assessment of animal feeds, research and development activities, quality assurance, and compliance with regulatory standards. This analytical method is widely applied in the food industry, animal nutrition sector, and research institutions to investigate the properties and behaviour of different materials. It facilitates the optimization of food processing, enhances product safety, and supports accurate nutritional labelling. In this study, proximate composition analysis was performed to quantify the moisture, ash, crude protein, crude fat, and fibre contents, following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005).

* + 1. **Determination of Crude Protein**

The Kjeldahl method (1965) was used to determine the crude protein content of the edible mushroom sample due to its accuracy and aptness for nitrogen analysis. In this procedure, the mushroom sample was digested with concentrated sulfuric acid (H₂SO₄), which decomposed the organic matter and converted nitrogen-containing compounds into ammonium sulfate. The digest was subsequently neutralized with sodium hydroxide (NaOH), releasing ammonia gas (NH₃) during steam distillation. The liberated ammonia was captured in a known volume of boric acid solution. The amount of ammonia absorbed was then quantified by titration with a standardized hydrochloric acid (HCl) solution. The nitrogen content thus obtained was converted to crude protein using a standard conversion factor of 6.25, applicable to most organic materials.

* + 1. **Determination of Crude fibre**

The FOSS Fibertec-2010 apparatus was employed to analyse crude fibre in mushroom samples. The equipment is automated and standardizes the process, ensuring the accuracy and reproducibility of the results obtained*. This process involves a systematic and sequential extraction of fibre components from a sample using chemical treatments, filtration, washing, drying, and weighing to determine the crude fibre content.*

* + 1. **Evaluation of Moisture Content**

The AND-MS70 moisture analyser was utilized to measure the moisture content of the dehydrated mushroom samples. This parameter is essential for maintaining quality control in processed food products and for complying with standards. This method involves evaporating moisture from a sample using halogen heating, resulting in weight loss in the analysed sample. The weight change is expressed as a percentage of the initial weight to determine the moisture content.

* + 1. **Determination of Fat**

The Soxhlet extraction method is a reliable and efficient approach for fat analysis in specified samples. A thimble made of glass or cellulose is used to hold the sample, and a suitable organic solvent such as petroleum ether or hexane is selected based on its ability to effectively dissolve lipids. Additionally, the boiling point of the solvent must be low.

* + 1. **Determination of Ash Content**

The Carbolyte-AAF1100 furnace was used to determine the ash content. This was achieved by heating the dried sample to a temperature between 500-600°C, which incinerates all organic matter and leaves only the inorganic ash. After cooling the inorganic material in a desiccator, it is weighed to calculate the percentage of ash.

* 1. **Determination Of Minerals**

Inductively coupled plasma (ICP) was employed to evaluate the mineral content in the mushroom samples. This approach allows for multi-element analysis, showcasing high sensitivity and accuracy in elemental detection. The Agilent 5800 VDV ICP-OES [Agilent Technologies USA] was equipped with a Sea-Spray nebulizer, a double-pass glass cyclonic spray chamber, and a semi-demountable dual view (DV) injector torch with a 1.8 mm inner diameter. The Agilent SPS 4 autosampler facilitated the rapid and automated sample delivery to the ICP-OES, as outlined in the method of US EPA 200.7.

* 1. **Vitamin Analysis**

High Performance Liquid Chromatography (HPLC) is a common method for analysing a range of compounds, including vitamins. The HPLC system requires an appropriate column, detector, mobile phase, and pumps, which manage the flow rate of the solvent containing the analytes. Before injection, analytes must be concentrated to eliminate possible interfering substances, typically involving processes like extraction, filtration, and dilution. Generally, HPLC techniques for vitamin analysis guarantee accurate, precise, specific, and valid results. Different concentrations of these standards were tested under the same chromatographic conditions as the target compounds, resulting in a calibration curve for each standard.

* 1. **Mushroom Collection and Drying**

Mushroom samples were collected from the biotechnology department of the Federal Institute of Industrial Research Oshodi, Lagos, where oyster mushrooms are cultivated. Mushrooms were dehydrated using a stainless dehydrator (ST-02, 220- 240V, 50Hz, 1500W) at a temperature of 45°C to a constant weight and pulverized with an electric blender (Kenwood blender 3.0L, 8500W, KC-241B, UK-SPEC) and packed with zip-lock bags for further applications.

* 1. **Extraction Of Mushroom**

The extract preparation was performed using a modified method described by Soares et al. (2009). The powdered mushroom sample (500g) was macerated in 2.5 L of absolute ethanol (98.9%) for four days. The resulting mixture was filtered through a clean white muslin cloth, and the filtrate was concentrated at 40°C using a rotary evaporator. The final yield of the extract was 40 g, corresponding to a percentage yield of 12%.

* 1. **Toxicity Study of Mushroom Extract (LD50)**

An acute oral toxicity study (LD₅₀) was conducted following the method described by Obode et al. (2021). Groups of eight Wistar rats were administered extract doses of 1500, 3000, and 6000 mg/kg body weight, respectively. No mortality or observable signs of toxicity were recorded during the study, indicating that the extract was non-toxic at the administered doses.

* 1. **Animals Used in Experiment**

Healthy male albino Wistar rats were housed under standard laboratory conditions at an ambient temperature and maintained on a 12-hour light/dark cycle. During a 72-hour acclimatization period, the rats were fed standardized laboratory chow obtained from Ojuoye Market, Mushin, Lagos, and provided with water ad libitum. The study was carried out with the guidelines approved for the Care and Use of Laboratory Animals by Rhema University, Aba, Abia State.

* 1. **Induction Of Diabetes**

Diabetic conditions were induced by a single intraperitoneal injection (i.p) of 40mg/kg of streptozotocin in about 6 mL of citrate buffer (pH 4.5) and monitored. After 48 hours of streptozotocin injection, some physiological observations were seen, such as frequent urination of experimental animals as reported by Longhurst & Belis.,1986; Akbarzadeh *et al*.,2007. Then an *On Call® Plus II* glucometer with blood glucose test strips (Acon Laboratories, San Diego, USA) were used to check the blood glucose of STZ-induced animals, with serum glucose levels above 200mg/dl considered as diabetic and separated and grouped for treatment.

* 1. **Experimental Design for Study**

Following acclimatization, the animals designated for diabetes induction were fed a high-fat diet and high-fructose syrup for 18 days to promote insulin resistance. The diabetic rats were randomized into five treatment groups (n = 10 per group), and a sixth group served as the normal control. The experimental groups were as follows:

* **Group 1 (Normal Control, NC):** Non-diabetic rats fed standard rat chow and water *ad libitum*
* **Group 2 (Diabetic Control, DC):** Diabetic rats receiving no treatment
* **Group 3 (PTE):** Diabetic rats treated with 200 mg/kg of *Pleurotus tuber-regium* extract
* **Group 4 (PTM):** Diabetic rats treated with 400 mg/kg of *Pleurotus tuber-regium* extract
* **Group 5 (PTH):** Diabetic rats treated with 800mg/kg of *Pleurotus tuber-regium* extract
* **Group 6 (STD):** Diabetic rats treated with 80mg/kg of Metformin

Treatments were administered orally once daily for 9 weeks (63 days). All rats had continuous access to standard chow and water throughout the study. Body weight and fasting blood glucose levels were monitored regularly during the experimental period.

* 1. **Determination of Fasting Blood Glucose**

Fasting blood glucose levels were determined after a 12-hour overnight fast. A small puncture was made at the tip of each rat’s tail, and blood samples were collected using *On Call® Plus II* blood glucose test strips (Acon Laboratories, San Diego, USA). The strips were inserted into the *On Call® Plus II* glucometer to obtain glucose readings, following the method described by Jarald et al. (2013). Blood glucose measurements were taken before the commencement of treatment and subsequently at 7-day intervals throughout the experiment. Following 48 hours of streptozotocin (40 mg/kg) administration, blood samples were collected for serum assay, and the pancreas was harvested for further biochemical and histological analyses.

* 1. **Determination of Blood Lipid Profile**

Blood samples were collected from the rats via the retro-orbital plexus under light anaesthesia. The samples were allowed to clot at room temperature and subsequently centrifuged at 3000 rpm for 15 minutes to separate the serum. Lipid profile analysis was conducted using the serum obtained. Triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were determined using a Biolab assay kit with an automated chemistry analyser (SFRI, BSA, France), following the procedure described by Gebrie et al. (2018), with slight modifications in the analyser model employed. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (Friedewald & Levy, 1972)

LDL Cholesterol = Total Cholesterol – Triglyceride – HDL Cholesterol

 5

* 1. **Determination of Pancreatic Amylase Activity**

Pancreatic amylase is an enzyme secreted by the pancreas that plays a vital role in the digestion of carbohydrates. Blood samples were collected from the animals in EDTA-treated tubes and centrifuged to separate the plasma from the serum. Pancreatic amylase was subsequently extracted using appropriate methods, such as ultracentrifugation, gel filtration, or affinity chromatography, as described by Stiefel and Keller (1973).

* 1. **Statistical Analysis**

Statistical analysis was performed using GraphPad Prism version 5.0d (GraphPad Software, San Diego, CA, USA). Differences between the mean values of the experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc multiple comparison test. Data are presented as mean ± standard error of the mean (SEM). A *p*-value=0.05 was considered statistically significant.

3. results and discussion

**3.0. RESULTS**

**3.1. PROXIMATE ANALYSIS**

**Table1: Proximate composition of *Pleurotus tuber-regium* (% dry weight basis)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Component** | **Moisture** | **Crude protein** | **Crude fibre** | **Crude fat** | **Ash** | **Carbohydrate** |
| ***P. tuberregium* (%)** | 2.20±0.03 | 19.923±0.06 | 5.44±0.003 | 0.97±0.03 | 3.44±0.02 | 68.04±0.012 |
| **Method Used (AOAC 2005)** | AOAC 925.10 | AOAC 920.87 | AOAC 991.43 | AOAC 991.36 | AOAC 923.03 | By difference |

\*Values are mean ± standard deviation of triplicate determinations. *Carbohydrate calculated by difference: 100 - (protein + fat + fibre + ash + moisture).*

**3.2. VITAMIN ANALYSIS**

**Table 2: Vitamin Content of *Pleurotus tuber-regium***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **VITAMIN** | **AMOUNT** | **RDI** | **UNIT** | **METHOD OF ANALYSIS** |
| **Vitamin A(Retinol)** | ND | 0.99 | mg/g | **Agilent 1100 HPLC (Van Wayenbergh *et al 2020*)** |
| **Vitamin B1(Thiamine)** | 0.006 | 1.2 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **VitaminB2 (Riboflavin)** | 0.003 | 1.3 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B3(Niacin)** | 0.001 | 15 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin B5(Pantothenic acid)** | ND | 5 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B6 (Pyridoxine)** | ND | 1.3 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin B9 (Folate)** | ND | 0.5 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B12(Cyanocobalamin)** | ND | 0.002 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |
| **Vitamin C (Ascorbic acid)** | 0.008 | 90 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin D (Cholecalciferol)** | 0.07 | 0.21 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |
| **Vitamin E (Tocopherol)** | 0.013 | 15 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |

**Recommended daily intake (RDI), the unit represents the values per day, Sources- FAO., 2023; CODEX reference standards**

**3.3. MINERAL ANALYSIS**

**Table 3 : Mineral analysis of *P. tuberregium***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Component** | **Calcium** | **Magnesium** | **Potassium** | **Iron** | **Zinc** | **Sodium** | **Manganese** |
| ***P. tuberregium* (%)** | 797.75±5.25 |

|  |
| --- |
| 151.18±5.18 |
|  |

 | 3826.86±9.51 | 2.70±0.10 | 1.88±0.06 | 156.8±3.34 | 0.16±0.07 |
| **Method Used/Instrument**  | **ICP instrumentation/The Agilent 5800 VDV ICP-OES** |

**3.4. HYPOGLYCEMIC EFFECTS OF ETHANOLIC EXTRACT OF *P. tuberrigium***

**3.4.1. Change in Body Weights**

The effect of different doses of the ethanolic fraction of *P. tuberigium* on the body weight of normoglycemic and STZ-diabetic rats was observed for a period of 9 weeks, to ascertain a long-term effect of treatment on the body weight of experimental animals. Figure 1 and 2 shows the body weight changes in experimental animals for 28 days and 63 days.



Fig .1,2. Change in Body Weights in *P. tuberrigium*

**3.4.2. FASTING BLOOD SUGAR**

The fasting blood glucose in the normoglycemic and diabetic rats were evaluated and showed a significant decrease in the glucose levels, after 9 weeks(63days) of the experiments. The PTE-78.2%, PTM-88.4%, PTH-79.0% and STD-79.37%.

 

**Fig.3,4. Bar graph showing fasting blood sugar concentration**

**3.4.3. PANCREATIC AMYLASE**

Pancreatic amylase is an enzyme produced in the pancreas that catalyses the breakdown of carbohydrates into smaller simple sugars like maltose and dextrin. In the study of diabetes, pancreatic amylase is of interest owing to its effect on postprandial glucose levels which may indicate an underlying pancreatic dysfunction. Monitoring the pancreatic amylase levels can provide insight for therapeutic interventions aimed in diabetic management. In this study pancreatic amylase was evaluated in normoglycemic and the STZ-induced experimental animals. From figures below, it was observed that at 28 day, groups PTE (200mg/kg), PTM (400mg/kg) and STD (80mg/kg) had increased pancreatic amylase production while PTH (800mg/kg) was slightly significantly decreased compared to the normoglycemic animals. Furthermore, extended treatment for 63days, regulated the release of pancreatic amylase from the pancreas, therefore regulating the absorption of glucose to the cells, as could be related with the blood glucose values seen on the glucometer.

 

**Fig.5,6. Bar graph showing pancreatic amylase activity**

**3.5. CHOLESTEROL DETERMINATION**

The blood cholesterol (mg/gl) of the treatment groups is presented in Figures 7 and 8. It was observed that at 28 days, there was no significant difference in NC, PTE, and PTH, while there was a decrease in total cholesterol(P<0.01) in all treatment groups of varied doses of ethanolic extract and standard drug (STD)- metformin when compared to NC.

 

Fig.7,8. Bar graph showing cholesterol determination

The HDL levels (mg/dl) of animals in the normal and treatment groups are presented in Figures 9 and 10. It shows that after 28 days of study, there was a significant difference(P<0.05) in PTM, PTH, and STD compared to the normal control (NC). However, a 9-week study revealed a decrease in the HDL levels (P<0.001) in PTH and STD, while PTE and PTM remained comparable to NC.

  

 Fig.9,10. Bar graph showing HDL levels

The triglyceride levels (mg/dl) of animals in the normal and treatment groups are presented in Figures 11 and 12. Groups NC, PTE, and PTH were comparable, while there was a significant decrease (P<0.01) in group PTM and STD. At 63 days of the study, there was an increase in PTH, then PTE, PTM, and STD was comparable to the normal control.

  

Fig.11,12. Bar graph showing triglyceride levels

Fig 13, LDL in group PTH increase significantly (p<0.001) in PTH, while Fig 14 showed a decrease in PTH (P<0.05) and STD (P<0.01) compared to NC.

 

Fig. 13,14. Bar graph showing LDL in group PTH

**HISTOPATHOLOGY**

The pathology of the pancreas was investigated to assess the effects of the treatments on the integrity of the pancreas with a focus on potential tissue damage. This is to further corroborate the findings observed in lipid profiles, blood glucose levels, and pancreatic amylase assays. Figure 13 shows sections of the pancreatic tissues examined using hematoxylin and eosin (H&E) staining at X100 magnification. The pancreatic tissue sections at 28 days showed a mild to moderate distortion of exocrine acini and islet of Langerhans with signs of cellular degeneration, necrosis, or inflammatory infiltration in PTE, PTM, and PTH. At 63 days, the morphology of tissues shows no abnormality or inflammation.



Fig. .15 Histopathology of the pancreas

**DISSCUSSIONS**

The financial burden associated with treating conditions that impair pancreatic β-cell integrity remains a significant global concern (Abdalla, 2024; Saleem et al., 2024). In recent years, numerous studies have highlighted the therapeutic efficacy of natural products, often characterized by minimal adverse effects. This study evaluates the nutritional profile and health-promoting effects of an ethanolic extract of Pleurotus tuber-regium in high-fat diet and streptozotocin (STZ)-induced diabetic male Wistar rats. Emphasis is placed on its potential as a functional food with antidiabetic, cholesterol-lowering, and pancreatic enzyme-modulating properties. Proximate composition analysis indicated that P. tuber-regium is a nutrient-rich mushroom, comprising 68.04% carbohydrates, 19.92% crude protein, and 5.44% dietary fiber. These findings are consistent with previous literature affirming the mushroom’s nutritional value (Chen & Cheung, 2014; Oranus et al., 2014; Igbokwe et al., 2015). These macronutrients play a critical role in energy metabolism, blood glucose regulation, and gut health (Lin et al., 2020; Mo et al., 2024). The composition also supports the mushroom’s traditional use as a meat alternative and bulking agent in local diets, especially within Nigerian communities (Oranus et al., 2014; Igbokwe et al., 2015). Vitamin analysis revealed the presence of both water- and fat-soluble vitamins in low concentrations. Detected vitamins included vitamin D (0.07 mg/g) and vitamin E (0.013 mg/g), with trace levels of thiamine (B1), riboflavin (B2), and ascorbic acid (vitamin C). Despite their low concentrations, these vitamins may act synergistically to support antioxidant defence and immune function (Boa, 2004; Jayachandran et al., 2017; Majesty et al., 2018). Mineral profiling showed high levels of potassium (3826.86 mg/100g), calcium (797.75 mg/100g), magnesium (151.18 mg/100g), and sodium (156.80 mg/100g), all of which are crucial in metabolic regulation, insulin signalling, neuronal function, and cardiovascular health (Tamura, 2021; Basir et al., 2023; Brecht et al., 2023). The hypoglycemic effects of P. tuber-regium were evident in the diabetic rat model, reinforcing its potential role as a dietary adjunct for managing type 2 diabetes and related lipid disorders (Ganesan & Xu, 2019; Shamim et al., 2023). All treatment groups (PTE, PTM, PTH, and STD) demonstrated a significant reduction in fasting blood glucose levels over nine weeks, with the PTM group (400 mg/kg) achieving the most pronounced effect (88.4% reduction). Earlier studies have shown that oyster mushrooms exhibit a non-linear, dose-dependent glycemic effect, likely mediated by bioactive compounds that influence insulin activity and glucose transport mechanisms (Asrafuzzaman et al., 2018; Gochhi et al., 2024; Huang et al., 2012). The ethanolic extract also significantly affected pancreatic amylase (PA) activity, a key enzyme in starch digestion. Elevated PA activity is often associated with increased postprandial glucose spikes (Monago & Okonkwo, 2003; Debnath et al., 2020). After 28 days, enzyme activity increased in the lower-dose and STD-treated groups (200 mg/kg, 400 mg/kg, and 80 mg/kg, respectively), with the highest response observed in the STD group. Conversely, the high-dose group (800 mg/kg) displayed near-normal PA levels, indicating a dose-dependent, biphasic response (Kobayashi et al., 2021). By day 63, all treated groups exhibited a marked reduction in amylase activity—except PTM and STD—suggesting a restorative effect on pancreatic enzyme regulation over time, possibly linked to the extract’s antioxidant or anti-inflammatory actions (Kirk et al., 2006; Massani & Stecca, 2022). The early spike in PA may reflect a compensatory response to STZ-induced pancreatic injury, while the subsequent decline signals functional recovery (Butterworth et al., 2011; Sales et al., 2011; Murtaugh & Keefe, 2015). These enzyme-modulating effects were accompanied by improved glycemic control, particularly in the 400 mg/kg group. Given the relevance of lipid metabolism in diabetes management (Zhang et al., 2022; Bu et al., 2024), serum lipid profiles were also assessed. After 28 days, significant reductions in total cholesterol were recorded in the PTM and STD groups. By day 63, all treatment groups showed lowered total cholesterol compared to the normal control. However, changes in HDL cholesterol levels were more variable. At day 28, HDL levels decreased in PTM, PTH, and STD groups, with PTH and STD showing significant declines by day 63. This suggests that higher doses may not sustain HDL levels long-term and could reflect disrupted lipid transport under diabetic conditions (Femlak et al., 2017). Although the extract lowered overall cholesterol, it may not effectively promote reverse cholesterol transport at elevated doses (Marques et al., 2018; Ouimet et al., 2019). Triglyceride levels dropped significantly in PTM and STD at week 4 but later rose in PTH while continuing to decline in PTE, PTM, and STD by week 9. LDL cholesterol was significantly elevated in the PTH group at 28 days but dropped substantially across all treatment groups by day 63. Histopathological evaluation of the pancreas further validated the biochemical findings. The normoglycemic group showed no structural damage throughout the study, while treatment groups (PTE, PTM, PTH, and STD) exhibited mild to moderate regeneration of β-cell architecture within the islets of Langerhans (Retnakaran et al., 2023; Li et al., 2024). These observations affirm the restorative and protective potential of P. tuber-regium extract on pancreatic tissue, supporting its use as a safe and effective therapeutic agent for long-term diabetes management (Huang et al., 2012; Adeyi et al., 2021).

4. Conclusion

This study has shown persuasive evidence that the ethanolic extract of *P. tuber-regium* is a potent candidate for improving nutritional, antidiabetic, hypocholesterolemic, and pancreatic-protective status in high-fat-streptozotocin-induced Wistar male albino rats. Amongst all treatment groups, the P. tuber-regium extract at 400 mg/kg effectively reduced fasting blood glucose and improved lipid profiles over 9 weeks (63 days). It also modulated pancreatic amylase activity in a dose-dependent biphasic manner and promoted regeneration of β-cells in the islets of Langerhans. These findings highlight *P. tuber-regium* as a nutritionally rich and potentially safe nutraceutical for managing type II diabetes and preserving pancreatic function.

References

Abdalla, M. M. I. (2024). Advancing diabetes management: Exploring pancreatic beta-cell restoration’s potential and challenges. *World Journal of Gastroenterology*, *30*(40), 4339.

Abdullah, E., Amirullah, N. A., Vijayan, H., Abd Rashid, N., Abdullah, N., & Abidin, N. Z. (2024). Chemopreventive role of proteins and polysaccharides from Pleurotus tuber-regium. *Food Bioscience*, 104311.

Adedokun, O. M., Odiketa, J. K., Afieroho, O. E., & Afieroho, M. C. (2022). Importance of mushrooms for food security in Africa. In *Food Security for African Smallholder Farmers* (pp. 343-360). Singapore: Springer Nature Singapore.

Adeyi, A. O., Adams, F. A., & Adenipekun, C. O. (2021). Pleurotus tuber-regium inclusion in diet ameliorates dyslipidaemia in obese-type 2 diabetic rats. *Clinical Phytoscience*, *7*, 1-14.

Akbarzadeh, A., Norouzian, D., Mehrabi, M. R., Jamshidi, S. H., Farhangi, A., Verdi, A. A., ... & Rad, B. L. (2007). Induction of diabetes by streptozotocin in rats. *Indian Journal of Clinical Biochemistry*, *22*, 60-64.

Alaribe, C. S., Lawal, M., Momoh, R., Idaholo, U., Mudabai, P., & Adejare, A. A. (2018). Phytochemical and Toxicological Properties of Sclerotium from the Edible Fungus-Pleurotus tuber-regium: doi. org/10.26538/tjnpr/v2i5. 6. *Tropical Journal of Natural Product Research (TJNPR)*, *2*(5), 235-239.

Amara, A. A., & El-Baky, N. A. (2023). Fungi as a source of edible proteins and animal feed. *Journal of Fungi*, *9*(1), 73.

Asrafuzzaman, M., Rahman, M. M., Mandal, M., Marjuque, M., Bhowmik, A., Rokeya, B., ... & Faruque, M. O. (2018). Oyster mushroom functions as an anti-hyperglycaemic through phosphorylation of AMPK and increased expression of GLUT4 in type 2 diabetic model rats. *Journal of Taibah University medical sciences*, *13*(5), 465-471.

Assemie, A., & Abaya, G. (2022). The effect of edible mushroom on health and their biochemistry. *International journal of microbiology*, *2022*(1), 8744788.

Aswathy, S., Shyamalagowri, S., Hari, S., Kanimozhi, M., Meenambiga, S. S., Thenmozhi, M., ... & Manjunathan, J. (2024). Comparative studies on the cultivation, yield, and nutritive value of an edible mushroom, Pleurotus tuber-regium (Rumph. ex Fr.) Singer, grown under different agro waste substrates. *3 Biotech*, *14*(4), 123.

Bamigboye, C. O., Oloke, J. K., & Dames, J. F. (2019). Development of high yielding strain of Pleurotus tuber-regium: fructification, nutritional and phylogenetic studies. *Journal of Food Science and Technology*, *56*(8), 3597-3608.

Basiri, R., Seidu, B., & Cheskin, L. J. (2023). Key Nutrients for Optimal Blood Glucose Control and Mental Health in Individuals with Diabetes: A Review of the Evidence. *Nutrients*, *15*(18), 3929. Basiri, R., Seidu, B., & Cheskin, L. J. (2023). Key Nutrients for Optimal Blood Glucose Control and Mental Health in Individuals with Diabetes: A Review of the Evidence. *Nutrients*, *15*(18), 3929.

Bell, V., Silva, C. R. P. G., Guina, J., & Fernandes, T. H. (2022). Mushrooms as future generation healthy foods. *Frontiers in Nutrition*, *9*, 1050099.

Boa, E. R. (2004). Wild edible fungi: a global overview of their use and importance to people.

Brecht, P., Dring, J. C., Yanez, F., Styczeń, A., Mertowska, P., Mertowski, S., & Grywalska, E. (2023). How do minerals, vitamins, and intestinal microbiota affect the development and progression of heart disease in adult and pediatric patients?. *Nutrients*, *15*(14), 3264.

Bu, T., Sun, Z., Pan, Y., Deng, X., & Yuan, G. (2024). Glucagon-like peptide-1: new regulator in lipid metabolism. *Diabetes & Metabolism Journal*, *48*(3), 354-372.

Butterworth, P. J., Warren, F. J., & Ellis, P. R. (2011). Human α‐amylase and starch digestion: An interesting marriage. *Starch‐Stärke*, *63*(7), 395-405.

Carrasco, J., Zied, D. C., Navarro, M. J., Gea, F. J., & Pardo-Giménez, A. (2021). Commercial cultivation techniques of mushrooms. In *Advances in Macrofungi* (pp. 11-40). CRC Press.

Case, N. T., Berman, J., Blehert, D. S., Cramer, R. A., Cuomo, C., Currie, C. R., ... & Cowen, L. E. (2022). The future of fungi: threats and opportunities.

Chen, L., & Cheung, P. C. (2014). Mushroom dietary fiber from the fruiting body of Pleurotus tuber-regium: fractionation and structural elucidation of nondigestible cell wall components. *Journal of agricultural and food chemistry*, *62*(13), 2891-2899.

Debnath, G., Das, P., & Saha, A. K. (2020). Characterization, antimicrobial and α-amylase inhibitory activity of silver nanoparticles synthesized by using mushroom extract of lentinus tuber-regium. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *90*, 37-45.

Ekute, B. O., & Nwokocha, L. M. (2021). Nutritive value of the sclerotia of Pleurotus tuberregium: a mushroom. *Science World Journal*, *16*(3), 256-258.

El-Ramady, H., Abdalla, N., Badgar, K., Llanaj, X., Törős, G., Hajdú, P., ... & Prokisch, J. (2022). Edible mushrooms for sustainable and healthy human food: nutritional and medicinal attributes. *Sustainability*, *14*(9), 4941.

EPA METHOD 200.7 DETERMINATION OF METALS AND TRACE ELEMENTS IN WATER AND WASTES BY ICP-OES.

Femlak, M., Gluba-Brzózka, A., Ciałkowska-Rysz, A., & Rysz, J. (2017). The role and function of HDL in patients with diabetes mellitus and the related cardiovascular risk. *Lipids in health and disease*, *16*, 1-9.

Food and Agriculture Organization of the United Nations. (2023). *Codex nutrient reference values* [Internet]. Retrieved from [https://www.fao.org/fao-who-codexalimentarius. Accessed on 14th may,2025](https://www.fao.org/fao-who-codexalimentarius.%20Accessed%20on%2014th%20may%2C2025).

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499-502.

Ganesan, K., & Xu, B. (2019). Anti-diabetic effects and mechanisms of dietary polysaccharides. *Molecules*, *24*(14), 2556

Gebrie, A., Gnanasekaran, N., Menon, M., Sisay, M., & Zegeye, A. (2018). Evaluation of lipid profiles and hematological parameters in hypertensive patients: Laboratory-based cross-sectional study. *SAGE open medicine*, *6*, 2050312118756663.

Ghafoor, A., & Niazi, A. R. (2024). Pleurotus spp: an ultimate solution to the emerging calamities of the world. *New Zealand Journal of Botany*, 1-38.

Gochhi, M., Kar, B., Pradhan, D., Halder, J., Dash, P., Das, C., ... & Rath, G. (2024). A comprehensive review of edible mushrooms for the management of diabetes.

Huang, H. Y., Korivi, M., Chaing, Y. Y., Chien, T. Y., & Tsai, Y. C. (2012). Pleurotus tuber-regium polysaccharides attenuate hyperglycemia and oxidative stress in experimental diabetic rats. *Evidence‐Based Complementary and Alternative Medicine*, *2012*(1), 856381.

Igbokwe, G. E., Nebo, T. L., Ezenwelu, C. O., Nwajiobi, O. J., & Odili, C. L. (2015). Proximate Analysis and Mineral Composition of the Fruiting Body of *Pleurotus tuber-regium* (Mushroom) Cultivars from South East Nigeria. *The Bioscientist Journal*, *3*(1), 88-92.

Ijeh, I. I., Okwujiako, I. A., Nwosu, P. C., & Nnodim, H. I. (2009). Phytochemical composition of Pleurotus tuber regium and effect of its dietary incorporation on body/organ weights and serum triacylglycerols in albino mice. *Journal of Medicinal Plants Research*, *3*(11), 939-943.

Jarald, E. E., Joshi, S. B., Jain, D. C., & Edwin, S. (2013). Biochemical evaluation of the hypoglycemic effects of extract and fraction of Cassia fistula Linn. in alloxan-induced diabetic rats*. Indian journal of pharmaceutical sciences, 75(4), 427*.

Jayachandran, M., Xiao, J., & Xu, B. (2017). A critical review on health promoting benefits of edible mushrooms through gut microbiota. *International journal of molecular sciences*, *18*(9), 1934.

Johnson Afolabi, F., Babafemi Babaniyi, R., Obagunwa, M., Onile, F. O., Ajagun, E. J., Oyedele, O. J., & Adedayo Fasiku, S. (2024). Ethnomycology of Pleurotus tuber-regium and its use in food, medicine and bioremediation. *Botanica Lithuanica*, *30*(4).

Kirk, G. R., White, J. S., McKie, L., Stevenson, M., Young, I., Clements, W. B., & Rowlands, B. J. (2006). Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. *Journal of gastrointestinal surgery*, *10*(4), 499-503.

Kobayashi, M., Akaki, J., Ninomiya, K., Yoshikawa, M., Muraoka, O., Morikawa, T., & Odawara, M. (2021). Dose-dependent suppression of postprandial hyperglycemia and improvement of blood glucose parameters by Salacia chinensis extract: two randomized, double-blind, placebo-controlled studies. *Journal of Medicinal Food*, *24*(1), 10-17.

Kolawole, F. L., Akinwande, B. A., ADE-OMOWAYE, B. O., Shakirah, O. A., & Oyeyinka, S. A. (2021). Amino acid, phytochemicals, and antioxidant activities of gluten-free cookies from orange-fleshed sweet potato and Pleurotus tuber-regium sclerotium.

Kumar, S., & Netam, B. (2022). Study of wild edible mushrooms for improving human health and livelihoods support in Bastar Plateau India. *Plant Archives (09725210)*, *22*(1).

Lara-Arevalo, J. (2023). *Nutrient-Rich Foods in Western African Food Supply: Applying Nutrient Profiling Models to the FAO Food Composition Table for Western Africa (WAFCT 2019)*. University of Washington.

Li, X., & Cheung, P. C. K. (2019). Application of natural β-glucans as biocompatible functional nanomaterials. *Food Science and Human Wellness*, *8*(4), 315-319., P. C. K. (2019). Application of natural β-glucans as biocompatible functional nanomaterials. *Food Science and Human Wellness*, *8*(4), 315-319.

Li, J., Jiang, Q., Wang, X., Hou, L., Wang, L., Lou, K., & Pang, S. (2024). Metformin Preserves Insulin Secretion in Pancreatic β-cells through FGF21/Akt Pathway In vitro and In vivo. *Combinatorial Chemistry & High Throughput Screening*, *27*(18), 2691-2698.

Lin, S., Wang, P., Lam, K. L., Hu, J., & Cheung, P. C. (2020). Research on a specialty mushroom (Pleurotus tuber-regium) as a functional food: chemical composition and biological activities. *Journal of Agricultural and Food Chemistry*, *68*(35), 9277-9286.

Longhurst, P. A., & Belis, J. A. (1986). Abnormalities of rat bladder contractility in streptozotocin-induced diabetes mellitus. *The Journal of Pharmacology and experimental therapeutics*, *238*(3), 773-777.

Majesty, D., Constance, N., Ahamefula, E., Caleb, N., Ijeoma, E., Prince, O., ... & Chieme, C. (2018). Evaluation of nutritional, anti-nutritional and some biochemical studies on Pleurotus squarrosulus (Mont.) singer using rats. *African Journal of Biochemistry Research*, *12*(2), 7-27.

Marques, L. R., Diniz, T. A., Antunes, B. M., Rossi, F. E., Caperuto, E. C., Lira, F. S., & Gonçalves, D. C. (2018). Reverse cholesterol transport: molecular mechanisms and the non-medical approach to enhance HDL cholesterol. *Frontiers in physiology*, *9*, 526.

Massani, M., & Stecca, T. (Eds.). (2023). *Multidisciplinary Management of Acute and Chronic Pancreatitis*. BoD–Books on Demand.

Mo, C., Liu, R., Yang, Z., & Ma, A. (2024). Polysaccharide from Pleurotus tuber-regium mycelium improves DSS-induced colitis in mice by regulating inflammatory cytokines, oxidative stress and gut microbiota. *Food & Function*, *15*(7), 3731-3743.

Monago, C. C., & Okonkwo, J. E. (2003). Amylolytic studies of pleurotus tuber-regium. *Global Journal of Pure and Applied Sciences*, *9*(1), 53-58.

Murtaugh, L. C., & Keefe, M. D. (2015). Regeneration and repair of the exocrine pancreas. *Annual review of physiology*, *77*(1), 229-249.

Nworu, C. S., Ihim, S. A., Okoye, F. B., Esimone, C. O., Adikwu, M. U., & Akah, P. A. (2015). Immunomodulatory and immunorestorative activities of β-d-glucan-rich extract and polysaccharide fraction of mushroom, Pleurutus tuberregium. *Pharmaceutical Biology*, *53*(11), 1555-1566.

Obode, O. C., Adebayo, A. H., & Li, C. (2021). Phytochemical and toxicological evaluations of Prosopis africana (GUILL. and PERR.) extract on albino wistar rats. *Toxicological research*, *37*, 183-195.

Ogwu, M. C., Richard, G., Izah, S. C., Alimba, C. G., & Wangboje, O. M. (2025). Environmental Roles of Edible Mushrooms. In *Bioactive Compounds in Edible Mushrooms: Sustainability and Health Applications* (pp. 1-24). Cham: Springer Nature Switzerland.

Oranusi, U. S., Ndukwe, C. U., & Braide, W. (2014). Production of Pleurotus tuber-regium (Fr.) Sing Agar, chemical composition and microflora associated with sclerotium. *International Journal of Current Microbiology and Applied Sciences*, *3*(8), 115-126.

Ouimet, M., Barrett, T. J., & Fisher, E. A. (2019). HDL and reverse cholesterol transport: Basic mechanisms and their roles in vascular health and disease. *Circulation research*, *124*(10), 1505-1518.

Panda, J., Mishra, A. K., Nath, P. C., Mahanta, S., Sharma, M., Nayak, P. K., ... & Sridhar, K. (2024). Wild edible mushrooms to achieve sustainable development goals: novel sources for food security, health, and well-being. *Food Bioscience*, 104277.

Raman, J., Jang, K. Y., Oh, Y. L., Oh, M., Im, J. H., Lakshmanan, H., & Sabaratnam, V. (2021). Cultivation and nutritional value of prominent Pleurotus spp.: an overview. *Mycobiology*, *49*(1), 1-14.

Rathod, M. G., Gadade, R. B., Thakur, G. M., & Pathak, A. P. (2021). Oyster mushroom: cultivation, bioactive significance and commercial status. *Frontiers in life science*, *2*, 21.

Retnakaran, R., Pu, J., Emery, A., Harris, S. B., Reichert, S. M., Gerstein, H. C., ... & Zinman, B. (2023). Determinants of sustained stabilization of beta-cell function following short-term insulin therapy in type 2 diabetes. *Nature communications*, *14*(1), 4514.

Saleem, B., Hussain, G., Rasul, A., Anwar, H., & Hassan, M. (2024). Antidiabetic Potential of Mushroom‐Based Herbal Formulation in Streptozotocin‐Induced Diabetic Rats*. Scientifica, 2024(1), 7468975*

*Sales, P. M. D., Souza, P. M. D., Simeoni, L. A., Batista, P. D. O. M. D., & Silveira, D. (2012). α-Amylase inhibitors: a review of raw material and isolated compounds from plant source.*

Shamim, M. Z., Mishra, A. K., Kausar, T., Mahanta, S., Sarma, B., Kumar, V., ... & Mohanta, Y. K. (2023). Exploring edible mushrooms for diabetes: unveiling their role in prevention and treatment.*Molecules, 28(6), 2837.*

Soares, A. A., de Souza, C. G. M., Daniel, F. M., Ferrari, G. P., da Costa, S. M. G., & Peralta, R. M. (2009). Antioxidant activity and total phenolic content of Agaricus brasiliensis (Agaricus blazei Murril) in two stages of maturity. *Food chemistry*, *112*(4), 775-781.

Stiefel, D. J., & Keller, P. J. (1973). Preparation and some properties of human pancreatic amylase including a comparison with human parotid amylase. *Biochimica et Biophysica Acta (BBA)-Enzymology*, *302*(2), 345-361.

Tamura, Y. (2021). The role of zinc homeostasis in the prevention of diabetes mellitus and cardiovascular diseases. *Journal of Atherosclerosis and Thrombosis*, *28*(11), 1109-1122.

Törős, G. H., El-Ramady, H., & Prokisch, J. (2022). Edible mushroom of Pleurotus spp.: a case study of oyster mushroom (Pleurotus ostreatus L.). *Environment, Biodiversity and Soil Security*, *6*(2022), 51-59.

Zhang, J., Xiao, Y., Hu, J., Liu, S., Zhou, Z., & Xie, L. (2022). Lipid metabolism in type 1 diabetes mellitus: Pathogenetic and therapeutic implications. *Frontiers in Immunology*, *13*, 999108.