**Comparative Mitigating Effects of Bioactive of *Pleurotus tuber-regium* and *Empagliflozin* on Hepatic Oxidative Damage in Type 2 Diabetic Rats.**

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ABSTRACT

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| **Aims: This study evaluates the hepatoprotective and antioxidant potential of the ethanolic extract of in high-fat-streptozotocin-induced diabetic Wistar rats. *Pleurotus tuber-regium* is an oyster mushroom rich in essential nutrients and possesses anti-inflammatory, hypoglycemic, and hypocholesterolemic properties**.  **Study design:** Type 2 diabetes was induced in rats by a single intraperitoneal injection of 40mg/kg body weight of streptozotocin; rats with >200mg/dl were grouped for immediate intervention to mitigate oxidative stress. Oral doses for 7 days of ethanolic extract of *Pleurotus tuber-regium* (200mg/kg, 400mg/kg, and standard drug Empagliflozin (20mg/kg, 40mg/kg) were administered. Blood samples were collected with sterile EDTA bottles via retro-orbital plexus and serum obtained to analyse for hepatic biomarkers.  **Place and Duration of Study:** The experimental animals were bred in the Physiology department, faculty of medical sciences, University of Lagos, Akoka, Lagos, between January 7 and January 30, 2025.  **Methodology:** Thirty-five male Wistar albino rats (120150g) was used for this experiment. Type 2 diabetes was induced in male Wistar rats via a single intraperitoneal -injection of 40 mg/kg body weight streptozotocin following a high-fat diet. Diabetic rats with fasting blood glucose levels >200 mg/dl were grouped and treated orally for seven days with ethanolic extract of *P. tuber-regium* at doses of 200 mg/kg and 400 mg/kg, while standard drug (Empagliflozin) was administered at 20 mg/kg and 40 mg/kg. Blood was collected via the retro-orbital plexus for hepatic function; analysis alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Liver tissues were harvested, homogenized, and analyzed for antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and lipid peroxidation marker malondialdehyde (MDA).  **Results:** Untreated diabetic rats showed significantly elevated AST and ALT levels, reduced antioxidant enzyme activities (SOD, CAT, GSH), and increased MDA levels, indicating hepatic oxidative stress. Treatment with *P. tuber-regium* extract significantly (p<0.05) improved antioxidant enzyme levels and reduced lipid peroxidation in a dose-dependent manner. ALP showed only mild alterations, suggesting early hepatic stress. These findings indicate biochemical recovery in liver function following extract administration.  **Conclusion:** Ethanolic extract of *Pleurotus tuber-regium* demonstrated hepatoprotective and antioxidant effects in diabetic rats, likely mediated through its bioactive compounds. The extract mitigated oxidative hepatic injury and improved antioxidant status. Further studies with prolonged treatment and higher dosages are recommended to better understand its therapeutic potential in managing diabetes-associated liver dysfunction. |

*Keywords: [Oxidative stress, P. tuber-regium, Empagliflozin, Type 2 diabetes, Lipid peroxidation].*

1. INTRODUCTION

In 2024, the International Diabetes Federation (IDF) atlas reported an alarming rise in the prevalence of approximately 539 million individuals living with diabetes, leading to around 6.7 million global deaths annually. This metabolic disorder has been attributed primarily to insulin resistance and insensitivity, characterized by persistent hyperglycemia, resulting in major complications such as steatosis, hepatic injury, retinopathy, renal dysfunctions, and neuropathy (Bhatti *et al,* 2022; Zhao *et al,* 2023). These conditions are induced by oxidative stress from the production of reactive oxygen species (ROS). Oxidative stress is a leading biomarker for metabolic disorders, contributing to various diseases (Volpe *et al,* 2018; Mukai *et al,* 2022). Hyperglycemia, a precursor state of Type 2 diabetes mellitus, triggers damage to the protective structures of cells (Singh *et al,* 2022; González *et al,* 2023). The defense mechanisms of cells in a diabetic state require enzymatic and non-enzymatic pathways to safeguard against free radical attacks (Ali *et al,* 2023; Bandodkar *et al,* 2025). An imbalance between endogenous antioxidants and the free radicals produced in the body allows oxidative stress to occur (Eddaikra & Eddaikra,2021). In managing Type 2 diabetes, the concerning side effects of insulin-regulating drugs have necessitated the search for safer and more effective therapeutic agents for its management. Edible mushrooms have been known and consumed for centuries for their medicinal and nutritional potential (Assemie & Abaya, 2022). *Pleurotus tuber-regium* is one of nature's most remarkable gifts, abundant in the wild and commercially cultivated, particularly in Asia and sub-Saharan Africa (Febnteh *et al*,2025). They serve as food sources for both animals and humans, providing essential nutrients for a healthy lifestyle (Bell *et al*, 2022). Both the fruity bodies and sclerotia are consumed, providing nutrients that enhance the nutritional and culinary quality of the food. This edible mushroom serves as a bulking agent in local snacks and traditional medicines (Rahman *et al*, 2021; Adetunji *et al,* 2022). In Nigerian soup cuisines, *Pleurotus tuber-regium* is used as a thickener and a meat substitute in vegan diets (Afolabi *et al*, 2024). Niazi *et al*, 2024, reported that fungi are commercially cultivated due to their ability to grow on agro-waste. They contain bioactive compounds such as terpenoids, terpenes, beta-glucans, nitriles, and amides that have demonstrated antidiabetic and hypocholesterolemic effects (Jimenez-Garcia *et al,* 2021*;* Anshika *et al,* 2022). In this study, we compared the fasting blood glucose-lowering effects of three doses of the ethanolic extract of *P. tuber-regium* and Empagliflozin on streptozotocin-induced male Wistar albino rats.

2. material and methods

[**2.1 Mushroom**

Freshly harvested fruity bodies of *P. tuber-regium* were collected from the mushroom house of the Biotechnology Department of the Federal Institute of Industrial Research, Oshodi, Lagos. The mushrooms were dehydrated to a constant weight at 45°C using a stainless-steel dehydrator (Model ST-02, 220–240V, 50 Hz, 1500W).

**2.2 Extraction of Mushroom**

The pulverized dried *P. tuber-regium* (100g) was soaked in 500 mL of absolute ethanol (98.9%) in a 3000 mL beaker and properly covered with aluminium foil, then kept in a film cupboard for 72 hours. The extract was filtered using a No.1 Whatman filter paper and evaporated in a rotary evaporator. The concentrated extract was stored in sample bottles for further use.

**2.3 Animals**

Thirty-five male Wistar albino rats (120-150g), bred in the Physiology department, faculty of medical sciences, University of Lagos, Akoka, Lagos. The animals were fed with pelletized rat chow diet and water *ad libitum* for 72 hours*.* They were housed and maintained under laboratory conditions of light, temperature, and humidity (12-hour light/dark cycle; 25-30°C; 65% relative humidity), respectively. This study was conducted following the Guide for the Care and Use of Laboratory Animals and was approved by the Animal Ethics Committee of Rhema University, Aba, Abia State, Nigeria.

**2.4 Determination of the Fasting Blood Glucose**

The animals were fasted overnight for 12 hours, after which their tails were punctured with a set of OnCall softclix (Acon Laboratories, San Diego, USA) and put on a glucose strip inserted in the On Call® Plus II glucometer. This process was done immediately after acclimatization, pre-induction, and post-induction with streptozotocin, then during treatment and post-treatment.

**2.5 Induction of diabetes**

Diabetes was induced using the protocol according to Furman, 2021, with a single intraperitoneal dose of 40mg/kg body weight of streptozotocin in citrate buffer (pH 4.5). After 48 hours of streptozotocin induction, the animals with serum glucose levels above 200mg/dl were considered diabetic and grouped to be administered various treatments for observation and recovery.



**2.6 Experimental design**

After ascertaining diabetic conditions in the rats, they were further grouped into six groups (including the non-induced), each consisting of eight animals.

Group 1- Normal rats (Pelletized rat chow)-NC

Group 2- Untreated diabetic rats (DC)

Group 3- Diabetic rats treated with 200mg/kg bw ethanolic extract of *P. tuber-regium (PT-1)*

Group 4- Diabetic rats treated with 400mg/kg bw ethanolic extract of *P. tuber-regium (PT-2)*

Group 5- Diabetic rats treated with 20mg/kg bw Empagliflozin (EM-1)

Group 6- Diabetic rats treated with 40mg/kg bw Empagliflozin (EM-2)

The animals were monitored weekly for body weight and blood glucose. The treatment lasted for 21 days and the experiment was terminated. The rats were fasted overnight and sacrificed by cervical dislocation.

**2.7 Preparation of Tissue Homogenates**

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The liver was harvested and rinsed in iced-cold buffer to wash off excess blood, placed in a sieve to drain, and weighed. The liver was homogenized with buffer and centrifuged at 5000 rpm for 20 min, then the supernatant was decanted and stored in the freezer for subsequent analysis. The protein content of the liver tissue fractions was evaluated by Lowry’s method using BSA (bovine Serum Albumin) as standard (Lowry *et al,* 1951)

**2.8 Biochemical parameter (Determination of the Liver Function Parameters)**

The Enzymatic colorimeter method was used to determine hepato-renal parameters (ALT, AST, ALP, Total protein, Albumin, Total cholesterol, HDL, LDL, Triglyceride, and other biochemical parameters) using the Randox kit according to the manufacturer’s protocol.

**2.9 Determination of Oxidative Stress Parameters**

Catalase (CAT) was measured by a method described by Aebi (1984) where the decomposition of hydrogen peroxide (H2O2) was monitored. The superoxide dismutase (SOD) was estimated by a method described by Misra and Fridovich (1972), whose principle is the ability to inhibit autoxidation of epinephrine at an alkaline pH. Glutathione (GSH) was evaluated with a method by Ellman (1959) to measure the level in the reaction involving reduced glutathione with 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow-coloured complex. Lipid peroxidation was determined using a method described by Ohkawa *et al,* measuring malondialdehyde (MDA) formed by the thiobarbituric acid reaction (TBAR).

**2.10 Statistical Analysis**

Statistical analysis was conducted using GraphPad Prism version 5.0d (GraphPad Software, San Diego, CA, USA). Comparisons among group means were performed using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test for multiple comparisons. Results are expressed as mean ± standard deviation of the mean (SD), and differences were considered statistically significant at *p* < 0.05.

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3. results and discussion

**3.0 Results**

Figure 1 shows the fasting blood glucose monitored of six (6) experimental groups through the experiment. All experimental groups reflect baseline blood glucose before induction with streptozotocin from 0-14 days. At 48 hours post-induction, groups (2-6) revealed a spike in blood glucose >200mg/dl, confirming experimental animals to be hyperglycemic, which is a precursor of Type 2 diabetes. Interventions with doses (200mg/kg and 400mg/kg) of ethanolic extract and 20mg/kg and 40mg/kg, showed a gradual decline in the blood glucose levels of treatment groups. The untreated diabetic group (DC) showed high mortality in the experimental animals as a result of underlying physiological complications from hyperglycemic conditions.



**Serum biochemical analysis**

Streptozotocin-induced rats caused an imbalance in the levels of liver function parameters, as shown in Figure 2. There was significant decrease in the levels of ALT and AST in all the treatment groups, the ALP showed a significant decline DC while in treatment groups PT-1, PT-2, EM-1, and EM-2 when compared to the control NC. While ALB and T. Bil showed no significant difference across groups.



**Antioxidant Activities of Experimental Groups**

Figures 3-5 shows antioxidant activities with diabetic induction and various treatments. There was a significant decline in the levels of CAT and SOD activities in the untreated diabetic groups (DC) compared to the normal control. The treatment groups showed increased antioxidant activities (GSH, SOD, and CAT) compared to the normal control (NC) and diabetic control (DC), especially in the treatment groups administered with 200 and 400mg/kg of ethanolic extract of *P. tuber-regium.* Glutathione levels were higher in the diabetic control compared to the normal control, while the there was significant increase in the activity of GSH in the treatment groups, groups PT-1 and PT-2 showed significant more activities than EM-1 and EM-2.





**Lipid Peroxidation**

Malonaldehyde is a biomarker that shows levels of lipid peroxidation. Figure 6 highlights the evaluated levels of MDA in experimental animals. Streptozotocin induction leads to a significant increase in MDA levels. All induced groups showed a significant increase in the MDA levels compared to the normal control group. The treatment groups showed further significant (p<0.05) decrease compared to the untreated diabetic group.

**4.0 Discussion**

The fasting blood glucose of experimental animals was monitored throughout the study (Asiwe *et al,* 2021). All the groups demonstrated baseline glucose levels until 48 hours of post-induction with 40mg/kg streptozotocin (i.p)(Ghasemi & Jeddi, 2023). Rats that showed >200mg/dl were grouped for immediate intervention to salvage possible oxidative stress arising from diabetic conditions (Furman, 2021). Two doses (200mg/kg and 400mg/kg) of ethanolic extract of *P. tuber-regium* and (20mg/kg and 40mg/kg) of Empagliflozin were administered as a regimen. The groups that were treated with 200mg/kg *P. tuber-regium*, 20mg/kg Empagliflozin (PT-1 and EM-1), while 400mg/kg *P. tuber-regium*, 40mg/kg Empagliflozin showed a similar trend, respectively, implying a dose-dependent treatment. Several experimental reports have shown that oxidative stress is a diagnostic marker for diabetes mellitus and various metabolic disorders (De Geest & Mishra, 2022; Krawczyk,2023). Elevated levels of reactive oxygen species (ROS) have also been identified to be the main cause of compromised antioxidant protection of the tissues (Afzal *et al* 2023). The liver tissues are sites for metabolites involved in glucose and lipid metabolism (chandel, 2021; Tappy, 2021). This research demonstrates the variations from dysfunctions of the liver as a result of streptozotocin-induced diabetes and orally administered treatment with doses of ethanolic extract of *P. tuber-regium* (Adeyi *et al,*2021)*.*  There was decline in the levels of CAT and SOD and increased levels of MDA (lipid peroxidation) in the hepatic tissues of the untreated diabetic rats, owing to stress due to oxidation (Igbashio *et al,* 2025). While oxidative stress involves complex pathways, SOD, CAT and GSH have been reported to neutralize the effects of oxidation triggered by the reactive oxygen species (Kıran *et al,* 2023; Chaudhary *et al,* 2023). These oxidative stress enzymes participate in regulating antioxidants in their reduced form (Sadiq, 2023). In a state of cell injury caused by diabetic conditions, lipid peroxidation and elevated levels of MDA are observed (Carmo de Carvalho e Martins *et al*, 2022). The treatment of experimental animals was administered daily for 7 days, where the elevation in CAT, GSH and SOD levels of treatment groups reveals a stabilization in tissue damage recovery in treatment groups compared to the untreated diabetic control groups (Mohammad *et al* 2022). The lipid peroxidation (MDA) in Figure 6 showed a decrease level in the treatment groups, compared to the diabetic control, which could be a result of the response of the therapeutics to the recovery of hepatic cells (Bae *et al,* 2023; Lee *et al*, 2022). The cascade reaction of these enzymes aids the physiological balancing of oxygen donors by expelling organic peroxides and increasing the dismutation of free radicals (Leyane *et al,* 2022). Although the increased activities of GSH, SOD, and CAT in figures 3-5 showed an effective physiological maintenance of tissues especially with the significant increase (p<0.05) observed in the 200mg/kg and 400mg/kg doses of ethanolic extract of *P. tuber-regium* (Adeyi *et al,* 2021)*.* A typical review of a hepatic injury will include assaying the blood serum for AST, ALT, ALP, albumin, total bilirubin, and total protein. The AST and ALT are transaminases that catalyse the transfer of an amino group from an amino acid to α-ketoglutarate, which is then channelled into the tricarboxylic cycle for ATP production (Kalas *et al,* 2021; No, 2024). Figure 2 above illustrates the effects of streptozotocin-induced diabetes and its treatment with 200 and 400 mg/kg of P. tuber-regium, compared with the standard drug Empagliflozin, in the management of diabetes-induced hepatic tissue injury. Studies have shown that after administering an inducer of hepatocyte cytosis, levels of ALT are initially low but increase later in the course of cell injury (Carmo de Carvalho e Martins *et al*, 2022). When damage occurs as a result of injury inflicted, there is release of functional enzymes into the cytoplasm, which are transported into the bloodstream, and when diagnosed, shows increased enzyme activity peculiar to oxidative stress as reported in several research findings (Sadiq, 2023; Igbashio *et al,* 2025). The untreated diabetic group DC showed a significant decrease(p<0.05) in the levels of ALT, AST, and ALP, while total bilirubin, total protein, and albumin showed no significant difference across treatment groups compared to the normal and untreated diabetic control. ALT and AST are specific for indicating hepatic injury, and ALP release is a response to the accumulation of cholestasis (Ghenu *et al*, 2022). Though the was no significant difference seen across the treatment groups compared to the NC and DC, the spikes in the ALP levels may indicate an early hepatic injury, bone disorders, and intestinal dysfunctions (Shu *et al,*2022). Research has shown that for proper functioning of the liver, there is a complex interplay of this assayed biomarker that is dependent on age, nutritional status, sex, and hormonal changes. *P. tuber-regium* has been reported to have beneficial bioactive compounds that possess potential therapeutic effects in oxidative stress management (Li *et al*, 2023). Therefore, a need for further investigation into the effects of higher doses and longer treatment times to ascertain the full benefits of *P. tuber-regium* in the management of metabolic disorders caused by oxidative stress.

4. Conclusion

TThis finding highlights the role of oxidative stress in the pathophysiology of streptozotocin-induced diabetes and enumerates the hepatoprotective potential of Pleurotus tuber-regium ethanolic extract. The decrease in the activities of SOD and CAT, and spikes in lipid peroxidation (MDA) revealed in the diabetic control group confirms oxidative induced hepatic injury. Therapeutic intervention with *P. tuber-regium* at 200mg/kg and 400mg/kg significantly enhanced hepatic antioxidant protection, as shown in elevated levels of GSH, SOD, and CAT, suggesting decrease of oxidative damage and improved hepatic tissues. The lipid peroxidation was persistently high, probably due to short-term treatment, though the trends indicate a progressive improvement of redox imbalance of hepatic tissues. Additionally, biochemical analysis for liver function revealed some promising effects, mostly the reduced levels of transaminases (AST and ALT) in the diabetic control group, which may be a reflection of early hepatic dysfunction. Although, there was no significant difference in the ALP and protein parameters across treatment groups, the two doses of extract administered showed significant (p<0.05) higher potentials in stabilizing hepatic enzyme activity similar to the standard drug, Empagliflozin. Overall, this study supports the *P. tuber-regium* as a promising nutraceutical with potentials in mitigating hepatic oxidative damage in diabetes. However, further studies involving higher extract doses, longer treatment durations and broader biomarker analysis are recommended to fully elucidate its mechanism and optimize its application in managing diabetes-associated hepatic and metabolic dysfunctions.

Ethical approval (where ever applicable)

Tthe Research was Conducted Following the Guidelines of the National Institutes of Health (NIH) for Care and Use of Laboratory Animals was Approved by the Ethical Committee, Rhema University, Aba, Abia State.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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