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Phytochemical evaluation and acute oral toxicity of crude methanol extract of *Pleurotus tuber-regium* (Fr.) Singer in laboratory mice (*Mus musculus*)

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ABSTRACT

This research aimed to evaluate the phytochemical components of the crude methanol extract (CME) of *Pleurotus tuber-regium* and its acute toxicity in mice, *Mus musculus*. Before being filtered and evaporated, the crushed mushroom was macerated in 70% methanol for 72 hours. The phytochemical screening and acute oral toxicity were carried out using standard procedures. The CME consists of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids. When given orally, the LD_{50} was shown to be greater than 5000 mg/kg with no outward symptoms of toxicity. Haematology showed a significant ($p < 0.05$) decrease in packed cell volume, hemoglobin concentration, total red blood cell, and neutrophil counts in the treatment group as compared to the control group, while total white blood cell and lymphocyte counts significantly ($p < 0.05$) increased. On serum biochemistry, a significant ($p < 0.05$) increase in aspartate aminotransferase, alanine transferase, alanine phosphatase, blood urea nitrogen, and creatinine was observed in the treated group. There was however no significant ($p > 0.05$) difference in serum albumin and total protein. In conclusion, 5000 mg/kg of extract had a significant influence on the hematological and biochemical profiles of mice but didn't cause irreparable liver and kidney damage.

1. Introduction

Pleurotus tuber-regium, (Pleurotaceae) can be found on dead trees and fallen logs of *Daniella oliveri* in the wild (Vishwakarma et al., 2018). The mushroom produces a sclerotium, or underground tuber and both the sclerotium and mushroom are edible. When ripe, the cap curls upward to form a cup-like shape, similar to that of an oyster mushroom (*Pleurotus ostreatus*) (Lin et al., 2020). In Nigeria, sclerotium is nutritiously consumed as food, with folkloric claims that they have potent medicinal properties against asthma, high blood pressure, cancer, inflammation, hyperlipidemia, and hyperglycemia (Okolo et al., 2020; Vishwakarma et al., 2018). Plant-botanical interactions can cause poisoning, which can end in harm or death. Toxicological information can be utilized to forecast the effects of plant botanicals used by humans and animals (Shirish, 2011). Nonetheless, complementary and alternative medicine

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(CAM) continues to be marginalized in global discourses about public health (Aliyu et al., 2017). There's also the assumption that because herbal therapies are derived from nature, they don't have the same unpleasant or dangerous side effects as pharmaceuticals (Olaniyan et al., 2016). Despite positive ethnomedical reports from all around the world, the safety profile of several medicinal plants used in complementary and alternative therapies is unknown (Muia et al., 2020). Toxicity should be evaluated for proper and documented herbal medical products, as it is for conventional orthodox pharmaceuticals that have been properly researched and manufactured, according to Olaniyan et al. (2016); nevertheless, the toxicity of traditional herbal therapies is rarely assessed. As a result, consumers frequently focus on herbal drugs' medical benefits while ignoring their negative effects on other organs. *P. tuber-regium* was investigated for its qualitative phytochemical components and acute oral toxicity.

2. Materials and methods

2.1. P. tuber-regium collection, identification, and processing

P. tuber-regium was obtained from Yaba College of Technology's Mushroom Research and Training Laboratory. A mycologist (Dr. Ofodile Lauretta) from the Department of Botany taxonomically identified the mushroom with the voucher number of YCT 001 2021. The mushroom was cleansed in a salt solution to remove any microbial contamination before being transported and preserved in the laboratory for future use.

2.2. Preparation of crude methanol extract

The whole mushroom was air-dried at room temperature for 2 weeks in the laboratory. Thereafter, normal milling equipment (Model E Scale, Model Number ES0, China) was used to pulverize 500 g of the *P. tuber-regium*. The pulverized material was macerated in 5000 ml of 70% methanol for 72 hours (Itodo et al., 2022). The filtrate was then concentrated with a rotary evaporator (Model BUCHI, Model Number R110, England) and evaporated to dryness on a water bath (Model BATH, Model Number B11, China) at 50 °C, and the percentage yield was calculated (weight of dry extract/weight of dry plant × 100). Before further experimentation and analysis, the dried extracts were properly stored in an airtight container at 4 ± 2 °C in a refrigerator.

2.3. Phytochemical screening of crude methanol extract

The crude methanol extract was screened for qualitative phytochemicals using standard laboratory protocols (Khandelwal, 2005).

2.4. Source of experimental animals

Adult male mice (*Mus musculus*), weighing 15-18 g, were obtained from the Veterinary Pharmacology and Toxicology animal house at Ahmadu Bello University in Zaria. The experimental animals were housed at 25 – 27 °C temperature and 50-60% humidity in 15 × 30 cm cages with metal tops that were thoroughly cleaned, the bedding materials consisted of wood shavings (sawdust) which were changed twice a week. The experimental animals were allowed to acclimatize for two weeks before the commencement of the study. They were fed ordinary mice pellets and allowed access to unrestricted clean water.

2.5. Acute toxicity study

The up-and-down acute toxicity test procedure was utilized according to the guidelines of the Organization for Economic Cooperation and Development (OECD) 425 (OECD, 2008). Ten mice were randomly distributed into two groups of five each. The mice were fasted for 12 hours before the commencement of the oral administration of extracts. Using an orogastric tube, five mice (*M. musculus*) were sequentially administered 5.000 mg/kg body weight of *P. tuber-regium* once while the remaining five served as control. The mice were observed for any change in behavior and physiological signs of toxicity for short-term effects of 24 hours and, delayed effects of 14 days. Following light ether anesthesia, the mice were euthanized by jugular venesection, and blood was collected into labeled EDTA and plain sample bottles for hematology and serum biochemistry procedures.

2.6. Haematological assessment

The hematological parameters that were assessed were packed cells volume (PCV), hemoglobin concentration (HC), and red and total white blood cell counts (RBC and TWBC) as described by standard procedures (Dacie & Lewis, 1991; Schalm et al., 1975). Blood samples were collected from mice in both the treated and control groups.

2.7. Serum biochemistry

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), albumin (ALB), total protein levels (TP), blood urea nitrogen (BUN) ranges were determined using the Randox assay kit (Randox Laboratories Ltd, Ardmore, Antrim, UK) according to the manufacturer's instructions.

2.8. Histopathological examination

The kidney and liver were harvested from sacrificed mice following the termination of the experiment to check for lesions associated with toxicity such as hemorrhage, shrunken glomerulus, and central vein. The harvested organs were immediately fixed in 10% formalin (Sigma-Aldrich, Inc., St. Louis, MO, USA) solution and then prepared for histopathological examination, viewed under a light microscope, and the microscopic field images were captured (Luna, 1968).

2.9. Data analysis

Data were represented in tables. The quantitative data were presented in the form of a mean ± standard deviation (SD). GraphPad InStat version 3.1 was utilized to analyze data from the acute oral toxicity trial using an independent sample *t*-test. Statistical significance was defined as a $p \leq 0.05$.

3. Results and discussion

3.1. Phytochemical screening

In the methanol extract of *P. tuber-regium*, nine (9) secondary metabolites were found (Table 1). Alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids were among the metabolites discovered. Anthraquinone, on the other hand, was not found in the extract. The result of the phytochemical screening in this study agrees with previous research (Adeyi et al., 2021; Ijeh et al., 2009) on saponins, alkaloids, flavonoids, and steroids.

3.2. Acute oral toxicity study

The acute oral toxicity of the methanol extract of *P. tuber-regium* indicated that at 5000 mg/kg, the extracts caused no mortality in the first 24 hours and then for the next 14 days after being given orally to the mice. No change in clinical or behavioral signs was observed (Table 2). Acute toxicity tests, among other toxicity tests, were used to investigate the safety of herbal products in laboratory animals (Ebbo et al., 2020). Plants produce secondary metabolites for a variety of reasons, including growth regulation, inter- and intra-specific interactions, and defense against predators and pathogens. Many of these natural compounds have been demonstrated to have fascinating biological and pharmacological activity, and they are used as chemotherapeutic agents or as the foundation for the development of modern pharmaceuticals (Ukwuani et al., 2012).

Table 1. Qualitative phytochemical screening of methanol extract of *P. tuber-regium*

No	Phytoconstituents	Test	Inference
1	Alkaloids	Dragendorff test	+
2	Cardiac Glycosides	Keller-Kiliani test	+
3	Saponins	Frothing test	+
4	Phenolic compounds	Lead acetate test	+
5	Tannins	Ferric chloride test	+
6	Steroids	Salkowiski test	+
7	Carbohydrates	Molisch test	+
8	Flavonoids	Shinoda test	+
9	Terpenoids	Liebermann Burchard test	+
10	Anthraquinones	Borntrager's test	-

Keys: (+) = Present, (-) = Absent

Table 2. Acute oral toxicity of methanol extract of *P. tuber-regium* in mice

Experimental mice	Mice label	Dose (mg/kg)	Short-term effect (24 hrs)	Delayed effect (14 days)
A	I	5000	No death	No death
B	II	5000	No death	No death
C	III	5000	No death	No death
D	IV	5000	No death	No death
E	V	5000	No death	No death

At 14 days of observation, a 5000 mg/kg methanol extract of *P. tuber-regium* demonstrated no deleterious influence on the behavioral reactions of the examined rats. There was no death at the tested level which suggests that the plant is relatively safe at the dose used for the study. This is in concert with the work of Adeyi et al. (2021) who reported no death or toxicity sign in rats administered *P. tuber-regium* extract.

3.3. Haematology

There was an observed significant ($p < 0.05$) decrease in the packed cell volume (PCV), hemoglobin concentration (HGB), and total red blood cells (TRBC) in the treated group when compared to the control group. However, there was a significant ($p < 0.05$) increase in the total white blood cell (TWBC), lymphocytes, and monocytes of the treated group (Table 3). The hematopoietic system is one of the most vulnerable systems to hazardous substances, notably in the bone marrow, where red blood cell formation takes place (Kifayatullah et al., 2015). According to Choudhari and Deshmukh (2007), the lower RBC count and Hgb concentration could be due to deteriorated hematopoiesis, restricted erythropoiesis, or an increase in red blood cell breakdown. Haematology revealed a significant change in PCV, Hgb concentration, and RBC in the treated group, which could be due to the type of solvent used for extraction. Methanol extract may have lowered hemoglobin content and PCV count by inhibiting RBC synthesis thereby causing the induction of anemia. An increase in WBCs signals the activation of the immune response, which serves as a protective mechanism against foreign chemicals (Kifayatullah et al., 2015). The ability of the extract to evoke immune status in the mice could explain the substantial increase in total white blood cells and lymphocytes seen in the treated group. The phytochemical constituents present in the extract may be responsible for the protection of the mice against toxicities. According to Oluba et al. (2020), mushroom polysaccharides have been demonstrated to stimulate cellular immunity, increase antibody formation, and boost cytokine release, resulting in a significant shift in neutrophil count. The extract did not affect the other parameters in the hematological examination.

Table 3. Effect of the methanol extract of *P. tuber-regium* on haematological parameters at the limit dose

Parameters	Control	Test
PCV (%)	40.0 ± 1.12	30.7 ± 0.9[a]
HGB (g/dl)	13.2 ± 0.5	10.2 ± 0.3[a]
TWBC (*10^9/l)	7.5 ± 0.5	16.8 ± 1.4[a]
TRBC (*10^9/l)	4.6 ± 0.2	3.6 ± 0.2[a]
NEUT (%)	36.0 ± 0.6	15.0 ± 1.6[a]
LYMPHO (%)	63.0 ± 1.2	81.3 ± 0.9[a]
MONO (%)	1.0 ± 0.6	3.7 ± 2.0[a]
EOSINO (%)	N.D.	N.D.
BASO (%)	N.D.	N.D.
BAND (%)	1.0 ± 0.6	1.0 ± 1.0

Data are expressed as mean ± SD, $n = 5$. [a]:With different superscript across each row are statistically significant ($p < 0.05$) when compared to control. Key: PCV (packed cell volume), HGB (Haemoglobin), TWBC (Total white blood cells), TRBC (Total red blood cells), NEUT (Neutrophils), LYMPHO (Lymphocytes), MONO (Monocytes), EOSINO (Eosinophils), BASO (Basophils), N.D. (Not detected).

Table 4. Effect of the methanol extract of *P. tuber-regium* on blood serum biochemistry at the limit dose

Parameters	Control	Test
AST (u/l)	232.0 ± 15.6	288.0 ± 9.6[a]
ALT (u/l)	78.0 ± 4.04	91.7 ± 2.0[a]
ALP (u/l)	73.0 ± 2.3	111.0 ± 7.5[a]
TP (g/l)	61.0 ± 2.8	69.7 ± 0.3
BUN (mg/dl)	56.7 ± 0.9	81.0 ± 1.2[a]
ALB (g/l)	2.5 ± 0.29	2.9 ± 0.1[a]
CREAT (mg/dl)	0.7 ± 0.1	1.0 ± 0.1[a]

Data are expressed as mean ± SD, $n = 5$. [a]:With different superscript across each row are statistically significant ($p < 0.05$) when compared to control. Key: AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), TP (Total protein), BUN (Blood urea nitrogen), ALB (Albumin), CREAT (Creatinine).

3.4. Serum biochemical analysis

There was an observed significant ($p < 0.05$) increase in the serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen, and creatinine in the treated group when compared to the control. Serum albumin and total protein showed no significant changes when the treated group was compared to the control group ($p > 0.05$) (Table 4). The liver and kidney are the two principal organs involved in the detoxification process, with the kidney being more vulnerable to drug-induced toxicity. When tissue is destroyed, liver function marker enzymes (AST, ALT, and ALP) are released into the bloodstream, and their amount is proportional to the extent of hepatic damage (Jisha et al., 2019). The considerable increase in AST, ALT, and ALP levels in the treatment group (Table 4) after receiving a high dose of 5000 mg/kg extract could be due to some of the extract's ingredients. Ojiako and Igwe (2008) produced a similar report. They stated that when saponin, flavonoids, and tannin are consumed by animals, they may cause unfavorable metabolic

reactions. Albumin is the most important protein in plasma generated by the liver in terms of quantity, and it serves as a useful biomarker of hepatic function (Kifayatullah et al., 2015). Albumin synthesis is influenced by a variety of factors, including dietary conditions, hormonal balance, and osmotic pressure. Creatinine, the most extensively used biomarker for assessing renal damage, is a leftover product of creatine produced at a nearly constant rate in the body, which is filtered freely by the glomeruli and not reabsorbed in renal tubules (Sá et al., 2015). When compared to the control group, the results demonstrated a considerable change in creatinine levels in the treated group. Although creatinine is not expected to be reabsorbed, all of the creatinine filtered in the glomerular filtrate travels through the tubular system and is eliminated in urine. Creatinine is reabsorbed rather than expelled in this condition. According to previous reports by Nogaim et al. (2011), mushrooms at higher concentrations tend to evoke an increase in creatinine levels and that was observed in this study.

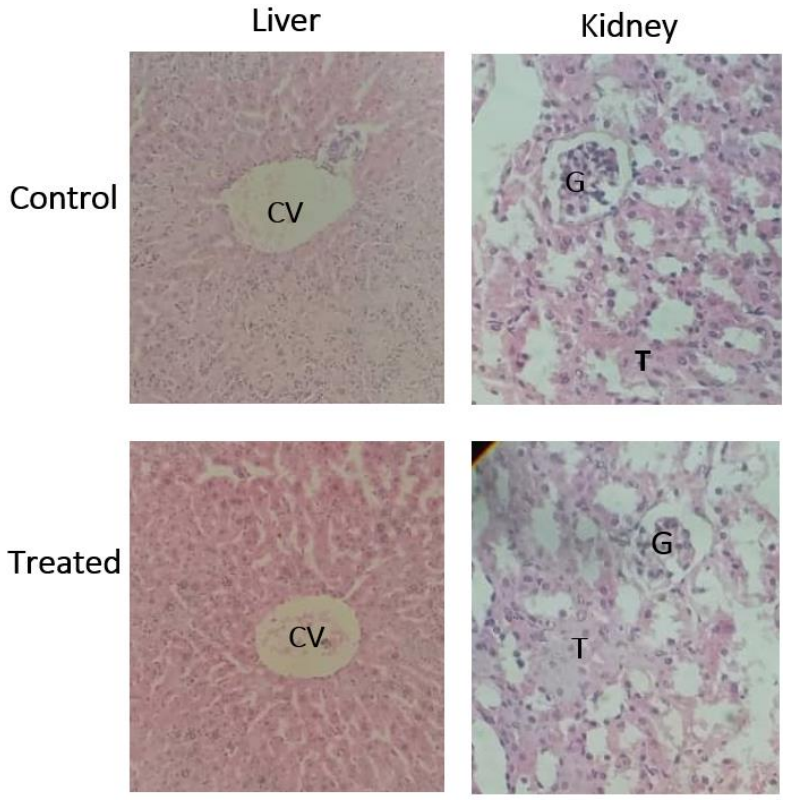

Figure 1. Photomicrograph of liver and kidney from mice showing normal central vein (CV), intact glomerulus (G) and tubules (T) (H & E x 200)

3.5. Histopathological evaluation

On histopathology, the liver section showed a normal central vein without any visible pathology while the examination of the kidney revealed intact glomerulus and tubules (Figure 1). This could be attributed to the mixture of flavonoids and phenols in the plant which has membrane-stabilizing and antioxidant activities, it promotes hepatocyte regeneration, reduces inflammatory reactions, and inhibits fibrogenesis (Soares et al., 2013).

4. Conclusions

In conclusion, qualitative phytochemical screening revealed the presence of nine secondary metabolites. Acute oral toxicity using the up and down procedure showed no deleterious effect at the administered dose of 5000 mg/kg. Significant influence on various hematological and biochemical profiles of *M. musculus* was observed, but not potent enough to cause irreparable liver and kidney damage.

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None.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were following the ethical standards of the Ahmadu Bello University, Zaria Committee on Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2022/011.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Supplementary File

None.

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