
**HAEMATOLOGICAL AND BIOCHEMICAL PROPERTIES OF
Pleurotus ostreatus MUSHROOM SPECIES**

***Oyekanmi, B. A.,¹Onifade, A. K.,¹Osho, I. B.² and Adetuyi, F. C.¹**

¹Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

²Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria.

*Corresponding author: bolapeoyekanmi@yahoo.com

Phone no: +2348034345654

ABSTRACT

Pleurotus ostreatus species is known for its broad spectrum antimicrobial and antioxidant potentials. Investigations were conducted to examine the effect of methanol extract of *P. ostreatus* on the haematological and serum biochemical parameters in albino rats after seven days of oral administration. *P. ostreatus* methanol extract LD₅₀ was determined and graded doses of the extract (625, 1250 and 2500 mg/kg body weight) was administered on apparently healthy Wistar albino rats. The LD₅₀ obtained was greater than 5000 mg/kg body weight. The weight gain of test rats was lower (1.15- 4.76 %) as compared with the control (13.36 %). Total leukocyte count was slightly high in 2500 mg/kg dose (15.27±3.64×10⁹/L) but with neither neutrophilia nor lymphocytosis. Haematocrit level was best in 2500 mg/kg (43.67±1.20 %) treatment and least in normal control (38.00±4.04 %), although all treatment and control were within the same range at P<0.01. Alanine amino transaminase, alkaline phosphatase and aspartate amino transaminase of test were within normal and of lower values as compared with normal control while urea and creatinine level in tests and control were within the same range statistically. The mushroom species possesses some bioactive components of benefits to blood circulatory system and did not have deleterious effect on liver and kidney enzyme biomarkers. Further studies of its bioactive principles and cytotoxicity profile could be a lead to future development of new tonic for blood and liver.

Key words: mushroom, *Pleurotus ostreatus*, haematocrit, alanine amino transaminase, liver, kidney

INTRODUCTION

Infectious disease remains a leading cause of death worldwide. This is because antimicrobial resistance has become a continuous public health problem globally and more importantly in developing Countries. Some of the contributing factors are: rapid technological development that leads to increased environmental pollution, consumption of chemical drink and unhealthy meals; inability to afford full course of treatment due to low income; and ageing process which leads to deterioration of vital body organs. Consequently, there is a call for renewed strategies on treatment and prevention; the need to fortify vital body organs and the immune system against future health challenges. Mushrooms are macro fungi, they possess fruiting body known as cap that can be seen by the naked eyes and plucked with hands. Pharmacological and medicinal studies of fungi have shown that the Basidiomycetes and Ascomycetes divisions are an immense source of biologically active components, although the antimicrobial and therapeutic importance of mushrooms has not been fully explored. *Pleurotus ostreatus* species is commonly known as oyster mushrooms and grow in the wild, tropical, subtropical and temperate regions (Chirinang and Intarapichet, 2009). *P. ostreatus* is one of the edible

mushrooms mostly consumed globally. Nowadays, mushroom extracts are sold commercially as dietary supplements for their nutritional properties, mainly for the enhancement of immune function and antitumor activity (Guillamón *et al.*, 2010; Finimundy *et al.*, 2013; Kalač, 2013; Valverde *et al.*, 2015). This study therefore focuses on the effect of *P. ostreatus* mushroom species on haematological and serum biochemical parameters of albino rats.

MATERIALS AND METHODS

Collection and identification of mushrooms:

Fresh fruiting bodies of *P. ostreatus* were harvested from AgrikkMatas farm at Aisu Ede (latitude 07° 46' 15.74 North and longitude 04° 33' 25.13" East) in Nigeria from April to August 2018. It was cultivated on rice straw and wood dust under controlled humidity. The mushroom samples were then transported in a cellophane bag to Microbiology laboratory in the Department of Microbiology, Federal University of Technology, Akure, Nigeria. The mushroom was identified by a Botanist at Obafemi Awolowo University, Ile-Ife, Nigeria and further identified based on its sequence data as described by Huang *et al.* (2009).

Preparation of extracts:

The mushroom samples were dried in a well-ventilated place at ambient temperature for 2 weeks, and then

ground into fine powder using clean and dry electric blender (MarlexElectroline, domestic electric food mixers, Dabhel Domain). Mushroom powder was processed using cold extraction method; 500 g of ground powder was soaked in 5 L methanol (98 %) for 72 h. Methanol (98 %) was prepared, the mouth of the beaker was covered with foil paper and the mixture was allowed to stand at room temperature (28 to 30 °C) for 48 h with frequent agitation. The supernatant was filtered using muslin cloth and filtrates concentrated in a rotary evaporator (Rotary evaporator, Laborata 4000 efficient, Heidolph WB eco) at 40°C, then evaporated to dryness in small vials at 40°C in the laboratory oven (Lab-Tech). A 500 mg/ml concentration was prepared using sterile physiological saline (0.85 %) as diluent, filtered through membrane filter (0.45 mm diameter) and labeled as stock. From the stock different dosages for treatment were prepared as required and preserved at 4°C

Experimental animals: White albino rats of weight range 60 to 100 g were obtained from the animal house in the Department of Animal Production and Health (APH), Federal University of Technology, Akure, Nigeria. The animals were fed with commercially prepared feed pellets once in a day and clean drinking water. The room

temperature was maintained under 12 hr light and 12 hr darkness. Twelve healthy albino rats were randomly selected and allowed to acclimatize in 7 days.

Determination of lethal dose:

Studies were performed on laboratory animal according to guidelines approved. Lorke's method as described by Deora *et al* (2010) was used. It involves 2 phases.

Phase 1: Nine albino rats were divided into three groups of 3 animals each. Each group was administered with different doses (10, 100 and 1000 mg/kg) of *P. ostreatus* extract. The extract was filtered through 0.45 Millipore membrane filter and required dosages prepared using injection water. The rat was carefully handled and turns so that abdomen is exposed. Then with the aid of sterile tuberculin 1cc syringe, required volume was administered intraperitoneally (IP). The IP injection was given at the lower right of abdomen trying to avoid hitting bladder, liver, or other internal organs. The animals were then observed for 24 hours to monitor their behavior as well as if mortality would occur. The result was recorded.

Phase 2: Three animals were divided into 3 groups of one animal each. Animals was administered with higher doses (1650, 2900 and 5000 mg/kg) of

extract intraperitoneally and observed for 24 hours. The result was recorded and used to determine the graded doses administered in the toxicological study.

Experimental design for seven days chronic toxicity study:

Twelve albino rats were distributed into 4 groups of 3 and were allowed to acclimatize in 7 days. The animals were fed with commercial feed pellets once in a day and clean drinking water. The room temperature was maintained and light was 12 h light and 12 h darkness. Graded doses of the extract (625 mg/kg, 1250 mg/kg and 2500 mg/kg, body weight) were administered orally to apparently healthy albino rats for 7 days. Normal control group was given 0.85 % sterile physiological saline (NaCl). Animals were starved for 12 h and sacrificed using cervical dislocation method. Blood sample was collected from the heart for haematological parameters and serum biochemical analysis.

Evaluation of haematological parameters of *P. ostreatus*:

Blood sample was released inside Ethylene diamine tetra acetic acid (EDTA) bottle and mixed gently. The samples were analyzed to determine the haematocrit level, red cell count, total leukocyte count and differential leukocyte count using standard

operating procedures (Cheesbrough, 2000).

Haematocrit level was determined using Micro-haematocrit Hawsley, Model: haematospin 1400, and non-heparinized micro haematocrit tubes. The blood capillary tubes were spun at 3000 rpm for 5 minutes and reading taken with the aid of micro-haematocrit reader (Hawsley).

Red blood cells (RBC's) count was carried out by making 1:200 dilution of blood sample in red cells diluent (formol citrate); 10 µl blood was added to 2 ml RBC fluid in a glass test tubes. The suspension was mixed gently, the Haemocytometer (Improved Neubauer chamber) was charged, cells were allowed to settle in 2 minutes and RBC's present in 80 smallest squares were counted with the aid of a binocular microscope (Olympus) using ×40 Objective.

Red cell count (per l) =

$$\frac{\text{No of cells counted}}{\text{Volume counted } (\mu\text{l})} \times \text{dilution factor} \times 10^6 / \text{L}$$

Cells in 80 smallest squares were counted, thus total volume equals 0.02 µl and blood is diluted 1:200.

Therefore Red cell count =

$$\frac{\text{No of cells counted}}{0.02} \times 200 \times 10^6 / \text{L.}$$

$$= N \times 10,000 \times 10^6 / \text{L.}$$

$$= 10,000N \times 10^9 / \text{L of blood (Ochei and Kolhatkar, 2007)}$$

Leukocyte total count was carried out by making 1:20 dilution of properly mixed blood {20 μ l of blood to 0.38 ml diluting fluid (2 % Glacial acetic acid)} in a 75 \times 10 mm glass tube and mixed by gentle tapping. The Haemocytometer (Improved Neubauer chamber) was charged, cells were allowed to settle in 2 minutes and WBC's in the larger corner squares were counted with the aid of a binocular light microscope (Olympus) using $\times 10$ (low power) objective. The number of WBC counted per litre of blood was calculated using this formula:

$$\text{Count/l} = \frac{\text{No. of cells counted}}{\text{Volume counted } (\mu\text{l})} \times \text{Dilution factor} \times 10^6$$

Where N= cells are counted in 0.1 μ l, then the leukocyte count per litre equals $N \times 10 \times 200 \times 10^6 / \text{L}$ (Ochei and Kolhatkar, 2007)

Blood film for leukocyte differential count was prepared by placing a drop of properly mixed blood on one end of a clean grease free glass slides, spread to make a thin blood film, then fixed for 2 minutes and stained for 10 minutes using Leishman stain (Ochei and Kolhatkar, 2007). The stained film was washed, dried and observed using binocular light microscope with $\times 100$ objective. One hundred (100) WBCs were counted and individual cells expressed in percentage.

Evaluation of biochemical parameters:

The serum obtained from the blood sample of the rat was subjected to biochemical analysis using enzyme maker kits from Randox. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total protein, Urea and Creatinine, were analyzed.) Blood was collected inside a plain bottle, allowed to clot and spun using a bench centrifuge, at 2,500 rpm for 3 minutes. The serum was separated and refrigerated at 4 $^{\circ}$ C. The serum was analyzed according to the guidelines on standard operating procedure described by Cheesebrough (2000)

Statistical analysis:

The results of three replicates were pooled and expressed as Mean \pm Standard error of mean. Data obtained were subjected to two-way analysis of variance and treatment means were compared using Tukey's Multiple Comparison test with the aid of Graph Pad Prism 5 at $P \leq 0.01$ level of significance.

RESULTS

P. ostreatus mushroom species used in this study is edible and commonly known as oyster mushroom. It was of accession number MG819739.1 and 97 % molecular identity with *Pleurotus ostreatus* isolate DMRP-20 as described in Table 1.1 The acute administration of methanol extracted *P. ostreatus* at dosages ranging from 10 mg/kg – 5000 mg/kg body weight neither caused abnormal behavioral pattern nor mortality in experimental animals (Table 1.2).

In table 1.3, after seven (7) days administration a varied temperature change was observed in the experimental animals with the highest increase in the normal control rats (from 34.53°C to 37.30°C) and least in 2500 mg/kg dose (from 34.07°C to 35.10°C) although not statistically significant $P < 0.01$.

In table 1.4 the percentage weight change was higher in normal control

(13.36 %) than the treatment (1.15 % to 4.76 %) but not significant at $P < 0.01$. In table 1.5, leukocyte total count was highest in 2500 mg/kg dose ($15.27 \pm 3.64 \times 10^9$ /L), and least in 1250 mg/kg ($6.67 \pm 0.47 \times 10^9$ /L); neutrophil count was highest in normal control (77.00 ± 3.61 %) and within normal range in 625 mg/kg dose (54.00 ± 2.00 %) to 2500 mg/kg dose (69.67 ± 4.70 %); lymphocyte count was lowest in normal control (22.67 ± 3.71 %) and highest in 625 mg/kg dose (45.00 ± 2.00 %); eosinophils, basophils and monocytes were within normal range in test and control (0.00 - 2.00 %). The haematocrit level was numerically highest in 2500 mg/kg (43.67 ± 1.20 %) and least in the normal control (38.00 ± 4.04 %); red cell count was significantly highest in 625 and 2500 mg/kg treatments (8.33 ± 1.38^b ; $8.08 \pm 0.24^b \times 10^{12}$ /L respectively) followed by normal control and 1250 mg/kg doses (4.95 ± 0.36^a ; $3.63 \pm 1.28^a \times 10^{12}$ /L respectively) at $P < 0.01$.

After 7 days consecutive oral administration of the mushroom extract, Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) levels were significantly higher in normal control (58.67 ± 8.33^b IU/L; 113.0 ± 8.74^b IU/L respectively) and least in 2500 mg/kg treatment (15.50 ± 0.50^a IU/L; 40.50 ± 0.50^a IU/L respectively); Alkaline phosphatase (ALP), total protein (TP), serum urea

and creatinine were statistically within the same range in both normal control and tests at $P < 0.01$ (Table 1.6)

DISCUSSION

Mushrooms have a long history of consumption as protein supplement in Nigeria and in traditional medicine. Due

Table 1.1: Molecular identity of *Pleurotus ostreatus*

CODE	<i>Pleurotus ostreatus</i>
Habitat/ Season found	Substrate: rice straw with wood dust/ January- December
Common names	Oyster mushroom
Genus	<i>Pleurotus</i>
Organism matched	<i>Pleurotus ostreatus</i> isolate DMRP-20
Percentage Identity	97.00
Accession Number	MG819739.1
Identity	<i>Pleurotus ostreatus</i> MG819739.1

Table 1.2: LD₅₀ of *P. ostreatus* methanol extract

Group	Dosages administered (mg/kg body weight)	Number of rat treated	Morbidity
Phase 1			
1	10	3	0
2	100	3	0
3	1000	3	0
Phase 2			
1	1650	1	0
2	2900	1	0
3	5000	1	0

0 = No mortality; LD₅₀ is the lethal dose causing 50 % death of all test animals.

Table 1.3: Effect of 7 days administration of *P. Ostreatus* on the temperature of albino rat

Treatment	D1 (°C)	D3 (°C)	D10 (°C)
NCT	37.30 ± 0.35	37.00 ± 0.00	34.53 ± 0.79
AT	36.07 ± 0.26	34.20 ± 0.70	36.50 ± 0.40
BT	35.10 ± 0.10	34.63 ± 0.52	34.77 ± 0.77
CT	34.47 ± 1.03	35.10 ± 0.35	34.07 ± 0.62

AT = 625 mg/kg dose administered for 7 days; BT = 1250 mg/kg administered for 7 days; CT = 2500 mg/kg administered for 7 days; D = day; different superscripts along the same vertical axis is a significant difference at P<0.01

Table 1.4: Effect of *P. ostreatus* on the weight of albino rats after 7 days administration

T	D1(g)	D3 (g)	D10 (g)	Wc = $\frac{(W10-W1)}{W1} \times 100$ (%)
NCW	87.33±18.94	98.67±18.66	99.00 ±19.29	13.36
AW	130.0±3.51	132.7± 6.36	132.5 ±1.50	1.92
BW	139.7±9.60	143.7±12.47	141.3 ±11.98	1.15
CW	109.3±14.38	114.3± 14.90	114.5± 6.06	4.76

T= treatment; AW = average weight when 625 mg/kg dose was administered; BW = average weight when 1250 mg/kg dose was administered; CW = average weight when 2500 mg/kg dose was administered; D = day; Wc = average weight change in percentage; different superscripts along the same vertical axis is significant at P<0.01

Table 1.5: Effect of *P. ostreatus extract* on haematological parameters of albino rats after 7 days administration

T (P<0.01)	WBCT ($\times 10^9$ /L)	N (%)	L (%)	E (%)	M (%)	B (%)	HCT (%)	RBC ($\times 10^{12}$ /L)
NC	10.87±1.3	77.00±3.6	22.67±3.7	0	0.33±0.3	0	38.00± 4.0	4.95±0.4 ^a
A	8.20±0.6	54.00±2.0	45.00±2.0	0	1.50±0.5	0	42.00±2.0	8.33±1.9 ^b
B	6.67±0.5	62.33±4.5	37.00±4.0	0	0.67±0.7±	0	41.67±2.9	3.63±1.3 ^a
C	15.27±3.6	69.67±4.7	29.67±4.5	0	0.67±0.7±	0	43.67±1.2	8.08±0.2 ^b

WBCT = total white blood cell count; RBC = red cell count; N = neutrophil; NC = normal control that received 0.85 % NaCl; T= treatment; L = lymphocyte; E = eosinophil; M = monocyte; B = basophil; A = 625 mg/kg *P. ostreatus*; B = 1250 mg/kg *P. ostreatus*; C = 2500 mg/kg *P. ostreatus*; n = 3; different superscripts along the same vertical axis is significant at P<0.01

Table 1.6: Effect of *P. ostreatus* on serum enzyme biomarkers of Wistar albino rat after 7 days administration

T	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	UREA (Mmol/L)	CREAT (μmol/L)
NC	58.67±8.33 ^b	113.0±8.74 ^b	52.67±6.49	83.00±8.50	8.67±0.61	106.7±2.40
A	17.00±0.00 ^a	59.50±0.50 ^a	43.50±0.50	72.50±0.50	6.85±0.05	105.5±0.50
B	35.00±1.00 ^b	59.33±5.67 ^a	41.67±6.01	82.33±3.93	7.47±0.80	108.0±16.70
C	15.50±0.50 ^a	40.50±0.50 ^a	49.50±0.50	73.50±0.50	6.55±0.05	115.5±0.50

ALT = Alanine amino transaminase; AST = Aspartate amino transaminase; ALP = Alkaline phosphatase; TP = Total protein; CREAT = Creatinine; T= treatment; NC= Normal control; A = treatment with 625 mg/kg; B = treatment with 1250 mg/kg; C = treatment with 2500 mg/kg; different superscript along the same vertical line is significant at $P < 0.01$

to the great biodiversity of mushrooms, classification based on morphological characteristics might not be sufficient; consequently there is the need for identity based on their nucleotide sequences. The *Pleurotus* species used in this study has close identity with *Pleurotus ostreatus* isolate DMRP-20 in the Gen bank. The current study is similar to the work of Debnath *et al.* (2017) who reported the identity of different isolates of ten *Pleurotus* species based on their morphology and sequence data.

Methanol extracted *P. ostreatus* had a high LD₅₀ which is indicative of high tolerance of its bioactive components in the body system of the experimental animal. The dose that would cause death in 50 % of the animals could be above 5 kg/kg body weight. This study agrees with the report of Deepalakshmi and

Mirialini (2014) that LD₅₀ of *P. ostreatus* ethanol extract is greater than 5000 mg/kg in 72 hours oral administration.

Normal rectal temperature range for rats is 36°C to 40°C (Ochei and Kolhatkar, 2007); seven days oral intake of *P. ostreatus* extract did not elicit an elevated temperature, which means it might not have ill effect on the nerves. *P. ostreatus* is known to be a low calorie food but highly proteinous and rich in vitamin B. The insignificant weight gain at $P < 0.01$ and feed conversion efficiency of test which was 8.6 % to 35.6 % of the normal control indicate that *P. ostreatus* did not encourage weight gain and might be of value in limiting obesity and maintaining normal body weight in health. The insignificant weight gain can be due to the effect of the polysaccharide component of the

mushrooms in lowering cholesterol level (Mao *et al.*, 2015) This study is similar to the work of some researchers (Chang *et al.*, 2015; Delzenne and Bindels, 2015; Murphy *et al.* 2015) who reported the benefits of some mushrooms including *Pleuroteus* sp. in regulating the microbiomes in the gut which consequently leads to reduction in inflammation and weight gain. The present study also corroborates the study of Toghyani *et al.* (2012) who reported lower body weight in chickens fed with 2 % mushroom supplement.

Leukocytes are the body soldiers that are involved in eliciting immune response against foreign substance in the body system (Brooks *et al.*, 2010) and leukocytoneutrophilia is seen in bacterial infection, leukaemia and when the immune system is stimulated by drugs. This study was investigated on healthy albino rats; it could be inferred that high dose (2500 mg/kg) of *P. ostreatus* was the cause of the slight leukocytosis. Therefore the leukocytosis in the test animal might be due to the ability of the extract to stimulate immune response which led to a release of leukocytes from the bone marrow into the peripheral circulation. The current study is in agreement with Fardet *al.* (2014) who reported improvement in some immune parameters in chickens fed with oyster mushroom supplement. Red blood cells count (RBC) and

haematocrit are important parameters for blood health status and are low in anaemia. In this study normal haematocrit with significantly raised RBC could indicate a positive effect of *P. ostreatus* on erythropoiesis. The mushroom extract had no ill effect on the haemopoietic cells; it agrees with the study of some researchers who reported that mushrooms improve blood circulation (Hu *et al.*, 2006). The favorable contribution of *P. ostreatus* to the blood cells could also be associated with the nutritious values and health benefits derived from mushrooms as reported by Ergönül *et al.* (2013)

Alanine amino transaminase (ALT), Alkaline phosphatase (ALP) and Aspartate amino transaminase (AST) are liver biomarkers and are raised in hepatocellular damage (Adeneye, 2009). Hepatocellular damage due to drug hypersensitivity may be shown by continuous increase in plasma transaminase. In the current study ALT and AST were reduced significantly in *P. ostreatus* treatment $P < 0.01$ and were indicative of no hepatocellular damage but healthy contributions of the mushroom species to the liver cells. This is similar to the study of Jayakumar *et al.* (2006) who reported that *P. ostreatus* has biological agents that protect major organs, including liver and heart against oxidative stress. The liver detoxifies many metabolic products,

hormones, drugs and toxins often prior to their excretion in the kidney. Urea and creatinine are kidney biomarkers and are raised in renal damage, when there is impairment to filtration (Ochei and Kolhatkar, 2007). In this study urea and creatinine level in both test and normal control were within the same range which implies that *P. Ostreatus* might not be deleterious against kidney enzymes. Total protein includes albumin and globulin, when present in appropriate proportion has positive effect on the blood circulatory system. Albumin is globular protein found in blood plasma; regulates blood osmotic pressure thereby maintains blood volume; also act as carriers for molecules of low water solubility thereby isolating their hydrophobic nature. A low total protein level is suggestive of liver or kidney disorder, or a disorder in which protein is not digested or absorbed properly, for example severe malnutrition, celiac disease or inflammatory bowel disease (Ochei and Kolhatkar, 2007). Total protein level in the present study was within normal control range, it is indicative of healthy contribution of *P. ostreatus* to the hepatocytes, nephron and the cellular components of the blood. The current study corroborates the report of Sirag (2009) that *P. ostreatus* has no adverse effects on the

biochemical and haematological parameters of albino rats.

CONCLUSION AND RECOMMENDATIONS

Mushrooms have longed contributions of health benefits to human and *P. ostreatus* is one of the mostly consumed edible mushrooms globally. Methanol extract of *P. ostreatus* has bioactive agents of high tolerance to body cells and of no deleterious effect on peripheral blood, liver and kidney enzymes in 7 days acute administration. Further study of *P. ostreatus* for 28 days chronic toxicity profile could form the basis for development of new tonic for blood, healthy liver and kidney.

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