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Haematological, Serum Biochemical and Histopathological Changes in Acute and Sub-Chronic Aqueous Extract of Oyster Mushroom in Male Wistar Rats

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Abstract

This study aimed at evaluating toxicological implications of aqueous *P. ostreatus* extract (POE) in male Wistar rats. POE was prepared in 1:10 (pulverized *P. ostreatus* : distilled water). In acute toxicity test, single oral dose of 2 mL/kg of POE was administered and observed for 28 days. The sub-chronic toxicity study was conducted by daily oral administration of graded doses (0.25, 0.50 and 0.75 mL/kg b.w) of the extract for 28 days. Clinical signs of toxicity, hematological, serum biochemical parameters and histopathological studies were subsequently evaluated. No treatment-related signs of toxicity or mortality in the animals were recorded in both toxicity tests. Rats administered with lowest dose of POE (25 mL/kg) had highest percentage weight gain. POE had no significant difference ($P>0.05$) on Red Blood Cell, White Blood Cell (WBC) and differential WBC, and serum biochemistry across all the treated groups when compared to the controls. The result of photomicrographs of stomach, spleen, heart, lung, kidney and liver showed a well outlined arrays of normal tissues in both acute and sub-chronic doses connoting that POE had no toxic effect on them. In view of these, POE may be concluded to be non-toxic within the tested doses and period of investigation.

Keywords: Haematology; Histopathology; Mushroom; *Pleurotus ostreatus*; Toxicity.

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1. Introduction

Mushrooms are edible group of fungi which can be cultivated or grown wild. The cultivation involves biotechnological process for lignocellulosic organic waste recycling

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[1]. They can be grown on a wide range of agro-waste that are rich in lignin and cellulose. Edible mushrooms can be grown on varieties of substrates such as sawdust, paddy straw, banana leaves, cassava peels, wheat straw and rice bran. The substrate can be utilized individually or in combination to provide enough and required nutrients that support mycelial running and emergence of fruiting bodies [2].

Pleurotus ostreatus is an important edible mushroom and second most cultivated worldwide after *Agaricus bisporus* [3]. It gained the feat due to its numerous nutritional and medicinal benefits. Generally, *Pleurotus* mushrooms are a good source of dietary fibres and carbohydrate which are present as glycoproteins and polysaccharides. The most dominant polysaccharides are α- and β-glucans, hemicellulose and chitin [4]. *P. ostreatus* contains high quality protein that can be incorporated into foods, improving their nutritional and functional values [5]. Also, it is a good source of protein for strict vegetarians who rely solely on plant protein. In addition, Mushrooms are used to cure epilepsy, cardiovascular diseases, rheumatoid arthritis, skin diseases, wound, gall bladder disorder, dysentery and as vermicides [6]. *P. ostreatus* is known for its anticancer, antiviral, antibiotic and anti-inflammatory, antidiabetic, antihypertensive, antihypercholesterolemic, immunodulatory, hepatoprotective and antioxidant activities [7].

Edible mushrooms may contain toxic substances that are lethal to biological system. Flammutoxin from Flammulina was identified as a cardiotoxic protein which can also cause respiratory failure and lung bleeding [8]. The aqueous extract of oyster mushroom also instigated an abnormal contraction of nonvascular tracheal smooth muscle. Ostreolysin, a protein from *P. ostreatus* caused abnormal cardiorespiratory effects in rat when injected intravenously into the rodents [9]. Therefore, the evaluation of toxic effect of *P. ostreatus* on haematological and serum biochemical parameters as an integral component of toxicity becomes necessary. In view of foregoing scientific investigations, the present study was designed to evaluate acute and sub-chronic toxicological implications of aqueous extract of *P. ostreatus* on haematological, serum metabolic markers and histopathological changes in male Wistar rats.

2. Materials and Methods

2.1. *Collection of Pleurotus ostreatus*

Fresh fruiting bodies of *P. ostreatus* (2 kg) were procured from Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria (FIIRO). They were collected into sterile polythene bags and taken to Plant Pathology Laboratory, Department of Plant Biology, University of Ilorin, Ilorin. The authentication of the taxonomy was confirmed in the Herbarium unit, Department of Plant Biology, University of Ilorin, Ilorin.

2.2. *Preparation of Pleurotus ostreatus extract*

The fruiting bodies of *P. ostreatus* chopped into small pieces, air-dried at room temperature for 8 days to a constant weight and subsequently pulverized using pestle and mortar (Thomas Scientific) was used for the study. The powdered sample (100 g) was suspended in 1 litre of distilled water for 48 hrs with continuous shaking at 25 °C using orbital shaker (Ginotech^{R}) at 3000 rpm. The solution obtained was filtered with Whatman No. 1 filter paper and the resulting filtrate was concentrated in a water bath (Ginotech^{R}) at 60 ºC. The slurry obtained was then stored in a desiccator for further use.

2.3. *Experimental animals*

Eighteen (18) healthy adult male Wistar rats weighing between 154-257 g were obtained from Animal Holding Unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were allowed to acclimatize to laboratory conditions for 7 days. They were kept in animal house of Department of Plant Biology, University of Ilorin with 3 rats in 100 cm x 50 cm rectangular cage and room temperature maintained at 25 ± 2 °C and 12 h day/night cycles. The rats were fed with growers mash (Vita feed^{R}) and provided drinking water *ad libitum* during the acclimatization period.

2.4. *Experimental protocol*

The present study was conducted according to research ethics of University of Ilorin, Ilorin, Nigeria. The oral acute toxicity study was conducted using six rats according to Organization for Economic Co-operation and Development (OECD) guideline 423 [10]. All the experimental rats were fasted overnight but had free access to water before the experiment. The six (6) rats used were randomized into two groups of 3 rats per cage. Animals in group I were given 2 mL/kg body weight single oral dose of POE while group II comprised rats administered with 2 mL/kg distilled water and served as control. Subsequent to this treatment, all the rats were closely observed (for behavioural changes, symptoms of toxicity and mortality) for the first 4 h (critical hours), then over a period of 24 h. and thereafter once daily for 28 days. While the feed and water intakes of the rats were monitored and recorded on daily basis [11]. Changes in the body weights of the rats were recorded on weekly basis throughout the experimental period.

For sub-chronic toxicity study, twelve (12) rats were used. They were divided into 4 groups (n=3 per cage); one control group and three treatment groups. While groups 1-3 were rats administered with 0.25, 0.50 and 0.75 mL/kg body weight doses of POE for 28 days, group 4 rats were given distilled water and served as control. All administrations were done once daily via oral gavage. The rats were observed individually and special attention was given to the treatment groups. The cage-side observations (changes in skin, and eyes; respiratory effects; autonomic effects, including salivation, diarrhea, and urination; central nervous system effects, including tremors and convulsions; changes in

the level of activity, gait and posture; reactivity to handling or sensory stimuli; and altered strength) and mortality were monitored daily throughout the study period. The weekly body weight changes of all the animals were also taken and recorded.

2.5. *Blood collection, haematological and serum biochemical analyses*

Twenty-four (24) h after the last doses for both acute and sub-chronic treatments, the animals were humanely euthanized under diethyl ether (for its quick and efficient action) and subsequently bled via cardiac venipuncture. Blood samples were separately collected in either sample bottles containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA) or plain bottles and used for hematological or biochemical analyses respectively. Hematological analysis was performed using an Automated Hematological Analyzer (Sysmex KX21) and parameters including red blood cell (RBC) or erythrocyte count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) or leukocyte count and platelet count (PLT) were determined. For the biochemical analysis, the blood samples were allowed to clot and thereafter centrifuged (3000 rpm, 15 min) prior to serum aspiration into new sample bottles and stored at –20 °C. The serum samples were analyzed to determine the levels of albumin (Alb) as well as the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and total protein (TP) using an Automated Biochemistry Analyzer (ADVIA 2400, Japan).

2.6. *Histological procedure*

Following animal sacrifice, the stomach, spleen, heart, lung, liver and kidney were respectively and carefully extracted from the rats and treated with 10 % formaldehyde (fixation) in order to preserve both the structure and molecular composition [12]. The organs were dehydrated by bathing it successively in rated mixture of ethanol and water (70–100 %). The ethanol was replaced with a solvent mixable with the embedding medium. The tissues were penetrated with xylene and it became transparent (clearing). The instilled tissue with xylene was placed in melted paraffin in an oven and maintained at 58–60 °C (embedding). The heat allowed the solvent to evaporate and the spaces within the tissues became filled with paraffin. The tissue together with its impregnating paraffin hardened following removal from the oven. The sections (5 μm) were then floated on water and transferred to a glass slide and stained with haematoxylin and eosin stains. The slides were viewed under light microscope with magnification X10.

2.7. *Data analyses*

Data were expressed as mean ± SE. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Duncans' Multiple Range Test

(DMRT). Statistical estimates were done at confidence interval of 95 %. Probability Values less or equal to 0.05 ($P \leq 0.05$) were considered significant.

3. Results and Discussion

3.1. *Toxicity evaluation of POE on weight gain, haematological and serum biochemistry*

The general result of this study showed that *Pleurotus ostreatus* is a safe mushroom due to absence of mortality in all the treated groups (acutely and sub-chronically). Our findings conform to the study that determined the toxicological assessment of *P. ostreatus* in Sprague Dawley rats to ascertain its safety and toxicity [13].

The outcome of percentage weight gains obtained in this study showed that POE improved the percentage weight gain on acute dosing (28 days) as observed in 5.3 % weight gain as compared with 3.1 % seen in the control group (Table 1). The same trend of observation was seen in sub-chronic dosing (28 days) which also showed increase in percentage weight gain (38.65 %) in 0.25 mL/kg of *P. ostreatus* extract and (27.8 %) 0.50 mL/kg of *P. ostreatus* extract as against 10.17 % observed in the control group (Table 2). Weight gain observed in administered rats is an indication that there is an increase in nutritional state of the rats. *P. ostreatus* is very rich in protein with well distributed essential and non-essential amino acids and this might be the rationale behind improved body weight of the rats [14]. Thus, group dosed with 0.75 mL/kg of *P. ostreatus* extract showed decrease in percentage weight gain (8.22 %) when compared with the control and other groups. This may however be attributed to the presence of tannins in *P. ostreatus* which has anti-nutritive effects and as such reducing feed consumption especially at higher dosage. This observation was in line with the report that *P. ostreatus* showed variability in weight gain when used as feed supplement in pigs [15].

Table 1. Percentage weight gain of rats following 28 days Acute treatment with POE.

Acute	Initial	WK 1	WK 2	WK 3	WK 4	% wt. gain
GROUP 1 (2 mL POE)	226.33±2.84	223.00±8.00	222.67±4.41	233.33±5.70	235.33±7.67	5.30
Control	257.33±6.84	220.67±12.84	245.33±13.97	241.67±14.97	265.33±13.30	3.11

Table 2. Percentage weight gain of rats following 28 days sub-chronic treatment with *Pleurotus ostreatus* extract.

	Initial	WK 1	WK 2	WK 3	WK 4	% wt. gain
0.25 mL	163.00±28.43	215.67±14.62	202.67±19.89	220.00±20.55	226.00±20.13	38.65
0.50 mL	154.67±10.67	164.00±8.66	178.33±15.19	190.00±10.97	197.67±11.87	27.80
0.75 mL	194.67±19.23	164.00±8.66	183.33±20.22	178.67±14.31	210.67±24.66	8.22
1 mL dist. Water (control)	200.00±12.12	198.33±7.31	213.67±10.53	215.33±10.73	220.33±10.33	10.17

Assessing haematological parameters is a marker to determine the extent of deleterious effects of xenobiotics such as plant extract on blood constituents of an animal and can further be used to explain haemo-relating function of the extract [16]. This study indicated that the haematological parameters; Packed Cell Volume (PVC), RBC, haemoglobin (HB) and platelets (PL) had no significant change ($P>0.05$) in all the treated groups (acutely and sub-chronically). Likewise, RBC, WBC and differential WBC (neutrophils, lymphocytes and monocytes) had no significant ($P>0.05$) effect across all the treated groups when compared with the controls (Tables 3 and 4). Blood is a patho-physiological reflector of the body and haematological analysis reveals the health status of the animals [17]. Since no significant changes on the haematological parameters was observed within the tested doses, it can be inferred from this study that POE does not compromise health status.

Table 3. Effects of acute oral administration of *P.ostreatus* on haematological parameters of Wistar rats (n=3, Mean ± SEM).

Parameters	Group 1 (2 mL POE)	Group 2 (control)
WBC×10^9 L	5.90±0.06	7.30±1.21
NEUT %	55.33±2.19	56.00±1.15
LYMPH %	43.33±2.40	42.67±0.88
MONO %	1.33±0.33	1.33±0.03
RBC×101^2 L	4.97±0.65	3.62±0.02
HGB g/dL	9.87±1.51	6.87±0.88
PCV %	34.33±5.46	24.67±0.33
MCV FI	68.00±4.16	67.33±0.33
MCH pg	19.83±1.13	18.53±0.29
MCHC g/dL	28.77±0.19	27.97±0.33
PLT×10^9 L	300.67±17.00	346.67±19.92

WBC: White blood cells, NEUT: Neutrophiles, LYMPH: Lymphocytes, MONO: Monocytes, RBC: Red blood cell, HGB: Haemoglobin, PCV: Packed cell volume, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, PLT: Platelets

Table 4. Effect of sub-chronic oral administration of *P.ostreatus* on haematogical parameters of Wistar rats (n=3, Mean ± SEM).

PARAMETERS	GROUP 1 (0.25 mL POE)	GROUP 2 (0.50 mL POE)	GROUP 3 (0.75 mL POE)	GROUP 4 (control)
WBC×10^9 L	7.33±0.81	5.73±0.12	8.83±2.00	6.27±1.36
NEUT %	52.00±2.89	44.00±4.51	62.33±6.69	54.33±6.33
LYMPH %	45.33±3.17	53.33±4.70	36.00±6.11	43.67±6.33
MONO %	2.33±0.33	2.33±0.33	2.00±0.57	2.00±0.00
RBC×10^{12} L	4.50±0.68	5.45±0.38	5.27±0.24	4.90±0.32
HGB g/dL	8.27±1.50	10.27±1.39	9.30±0.57	8.87±0.48
PCV %	27.67±3.33	36.33±4.48	32.00±1.54	33.33±2.96
MCV FI	60.67±2.90	65.67±4.26	60.67±1.45	62.33±2.33
MCH pg	18.20±0.86	18.63±1.36	17.60±0.32	18.13±0.87
MCHC g/dL	29.73±0.56	28.16±0.78	29.00±0.85	29.17±0.98
PLT×10^9 L	288.67±0.05	333.67±0.15	276.00±0.50	293.33±0.37

WBC: White blood cells, NEUT: Neutrophiles, LYMPH: Lymphocytes, MONO: Monocytes, RBC: Red blood cell, HGB: Haemoglobin, PCV: Packed cell volume, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, PLT: Platelets

Hepatocellular damage was always monitored by serum activities of ALT and AST which had leaked from hepatic tissues [18]. The result of serum chemistry in this study showed that hepatic enzymes had no significant (P>0.05) effect when compared with the control (Tables 5 and 6). The outcome further showed that the albumin and total protein had no significant (P>0.05) effects in all the treated groups (acutely and Sub-chronically) when compared with the control. The two transaminases (AST and ALT) are biologically important as they involve in interconversion of highly important metabolites and are index of liver cell injury. The increase of these enzymes indicates damage in liver parenchyma cells [19,20]. There is no significant difference in the ALP level across all groups when compared with the control indicating that it is well hydrolyzed and eliminated in the bile. Elevation in ALP is caused by inability to excrete it via bile as a result of obstruction or blockage of the biliary tract [13]. From this study, since there is no significant difference in the serum biochemistry, it can be inferred that POE does not have any adverse effect on the hepatic functions.

Table 5. Effect of acute oral administration of *P. ostreatus* extract on blood serum of Wistar rats (n=3, Mean ± SEM).

Parameters	Group 1 (2 mL POE)	Group 2 (Control)
AST nmol/L	26.97±1.62	31.43±4.33
ALT mmol/L	28.50±0.95	30.10±0.32
ALP mmol/L	43.07±2.21	37.47±3.91
Albumin g/L	35.67±0.88	35.00±4.36
T. Protein g/L	82.33±1.45	81.67±1.45

AST: Aspartate Aminotransminase, ALT: Alanine Aminotransaminase, ALP: Alkaline Phosphate, and Total Protein

Table 6. Effect of sub-chronic oral administration of *P. ostreatus* extract on blood serum of wistar rats (n=5, Mean ± SEM).

PARAMETERS	Group 1 (0.25 mL POE)	Group 2 (0.50 mL POE)	Group 3 (0.75 mL POE)	Group 4 (Control)
AST mmol/L	30.90±1.35	29.83±0.64	26.47±0.62	27.53±1.86
ALT mmol/L	29.23±0.55	29.77±0.55	25.67±1.98	30.83±0.83
ALP mmol/L	35.00±2.58	36.37±2.14	35.57±3.50	33.37±4.42
Albumin g/L	34.33±1.53	31.33±3.18	32.33±4.26	37.33±2.67
T. Protein g/L	80.00±2.30	77.67±1.45	80.00±2.08	74.67±4.70

AST: Aspartate Aminotransminase, ALT: Alanine Aminotransaminase, ALP: Alkaline Phosphate, Albumin and Total Protein.

3.2. *Histopathological toxicity evaluation of POE*

The histopathological outcome showed that both acute and sub-chronic doses of POE had no toxic effects on the various organs (stomach, spleen, kidneys, lung, liver and heart) of the experimental animals within the tested doses (Plate 1-12). The results of the photomicrographs of these organs showed a well outlined arrays of normal tissues in both acute and sub-chronic doses. This observation agreed with the study concluding that the

liver of Dawley rat treated with various doses of *P. ostreatus* showed normal lobular architecture with central vein and radiating hepatic cords without marked histological changes in all the treated groups [13].

Most of the toxicological studies report that toxic effects due to the use of medicinal plant and mushroom are associated with hepatotoxicity. Others includes toxic effects of the kidney, nervous system, blood and cardiovascular system; Thus, numerous advance biological experimental techniques have been used as standard safety test prior to the efficacy study. It has been noted that *P. ostreatus* exhibited significant effects for its anticancer, antiviral, antibiotic, anti-inflammatory, antidiabetic, antihypertensive, hepatoprotective and antioxidant activities indicating that it is a safe substance to be used as both food and medicinal agents [7].

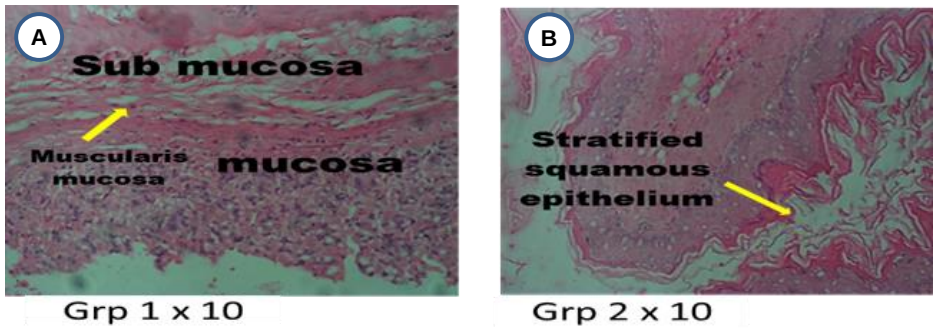

Plate 1: Photomicrograph of stomach of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E). The mucosa (arrow) appeared normal and showed no visible lesion (NVL).
B: Administered with sterile distilled water acutely (H and E) epithelium (arrow) appeared normal and showed no visible lesion (NVL).

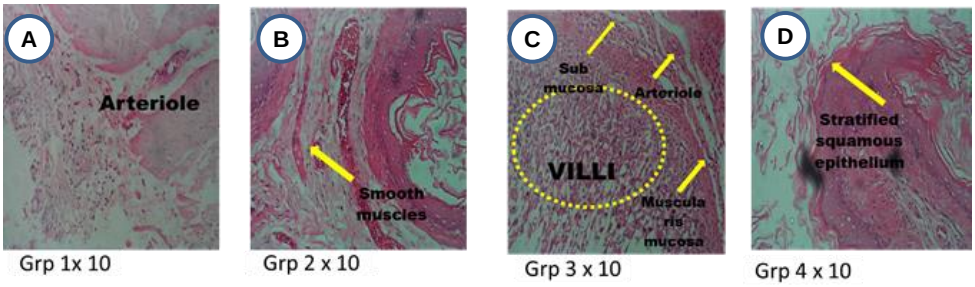

Plate 2: Photomicrograph of stomach of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg POE (H and E) the arteriole appeared normal without any visible lesion (NVL)
B: Administered with 0.50 mL/kg POE (H and E) the smooth muscle (arrow) appeared normal without any visible lesion (NVL).
C: Administered with 0.75 mL/kg POE (H and E) villi (doted circle), arteriole and muscle mucosa (arrows) all appeared normal and they showed no visible lesion (NVL)
D: Administered with sterile distilled water (H and E) stratified muscle mucosa (arrows) appeared normal and showed no visible lesion (NVL)

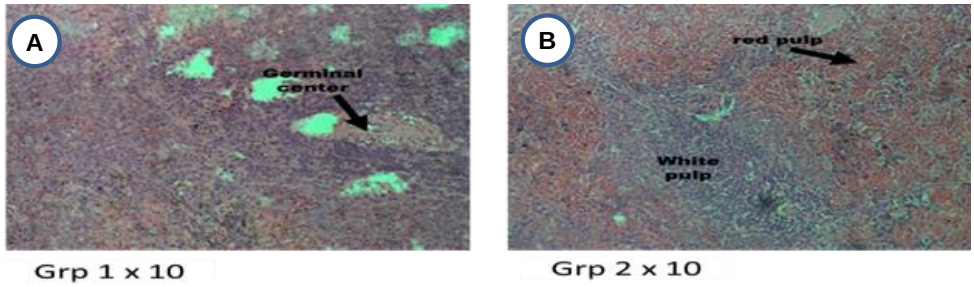

Plate 3: Photomicrograph of spleen of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E) the germinal center (arrow) appeared normal and showed no visible lesion (NVL)
B: Administered with sterile distilled water (H and E) showing both the red and white pulp (arrow) appearing normal without any visible lesion (NVL)

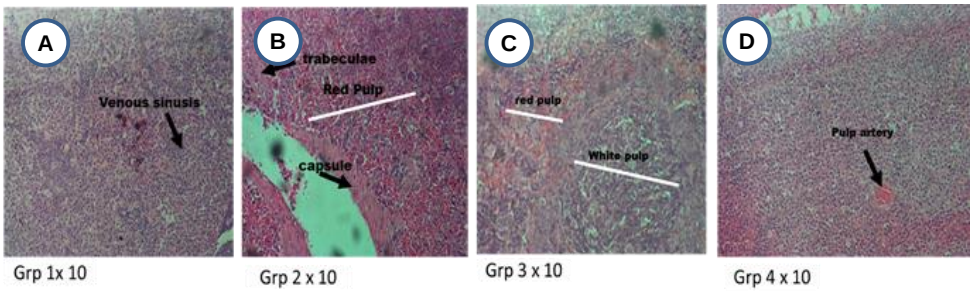

Plate 4: Photomicrograph of spleen of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg of POE (H and E) the venous sinusis (arrow) appeared normal without any visible lesion (NVL)
B: Administered with 0.50 mL/kg of POE (H and E) the trabeculae and capsule (black arrows), the red pulp (white line) all appeared normal without any visible lesion (NVL).
C: Administered with 0.75 mL/kg of POE (H and E) the red pulp and white pulp (white lines) both appeared normal and they showed no visible lesion (NVL)
D: Administered with sterile distilled water (H and E) pulp artery (arrows) appeared normal and showed no visible lesion (NVL)

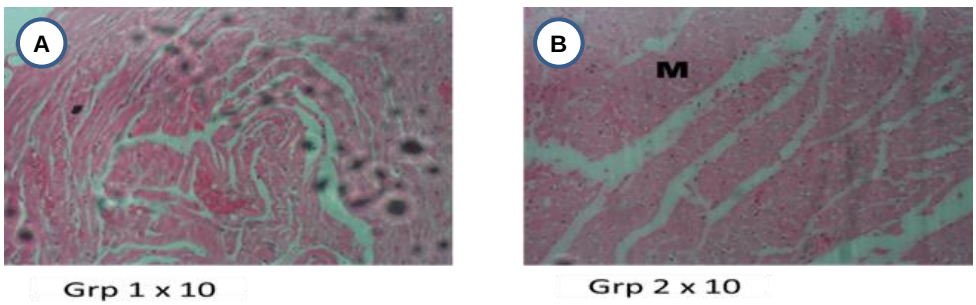

Plate 5: Photomicrograph of heart of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E). The myocardium (black dot) appeared normal and showed no visible lesion (NVL)
B: Administered with sterile distilled water (H and E) showing both the red and myocardium (black M) appearing normal without any visible lesion (NVL)

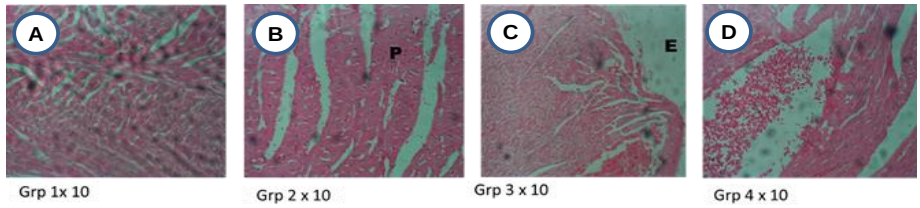

Plate 6: Photomicrograph of heart of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg of POE (H and E) myocardium (black dot) appeared normal without any visible lesion (NVL)
B: Administered with 0.50 mL/kg of POE (H and E) the pericardium (black P) appeared normal without any visible lesion (NVL).
C: Administered with 0.75 mL/kg of POE (H and E) the endocardium (black E) appeared normal without any visible lesion (NVL)
D: Administered with sterile distilled water (H and E) cardiac artery (blue arrows) appeared normal and showed no visible lesion (NVL)

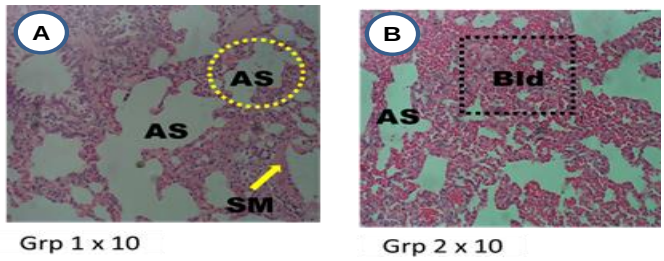

Plate 7: Photomicrograph of lung of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E) the walls of the alveolar ducts were lined by alveoli that directly open into Clusters of alveoli that surround and open into Alveolar sac (AS) and appeared normal and showed no visible lesion (NVL)
B: Administered with sterile distilled water (H and E) The. Pulmonary artery (Bldv) was well demonstrated and branched as they accompany the bronchi and the bronchioles into the lungs.

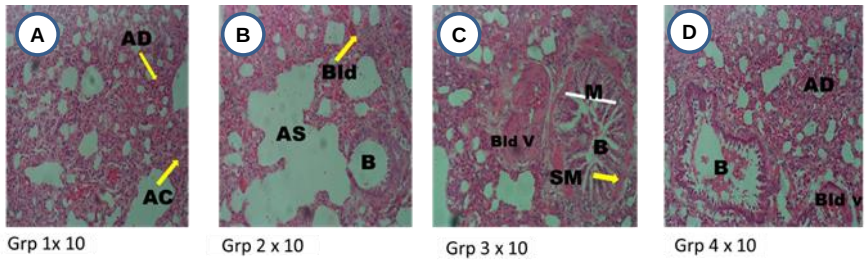

Plate 8: Photomicrograph of lung of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg of POE (H and E) the alveoli duct (AD) (yellow arrow) and alveoli capillary (AC) (yellow arrow) appeared normal without any visible lesion (NVL)
B: Administered with 0.50 mL/kg of POE (H and E.) the pulmonary artery (yellow arrow), alveoli sac and the bronchus (B) appeared normal without any visible lesion (NVL).
C: Administered with 0.75 mL/kg of POE (H and E) Mucosal folds (M), the bronchus (B), the pulmonary artery (Bidv) and the smooth muscle (SM) (yellow arrow). All appearing normal without any visible lesion (NVL)
D: Administered with distil water (Hand E) the pulmonary artery (Bidv), the bronchus (B) and the alveoli duct (AD). All appearing normal without visible lesion (NVL)

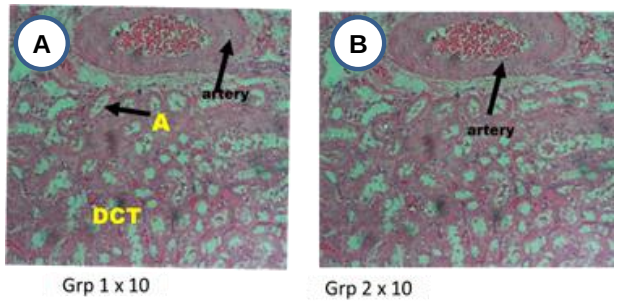

Plate 9: Photomicrograph of kidney of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E), the distal convoluted tubules (black arrows) appeared normal and showed no visible lesion (NVL)
B: Administered with sterile distilled water (H and E) the distal convoluted tubules (black arrows) appeared normal and showed no visible lesion (NVL)

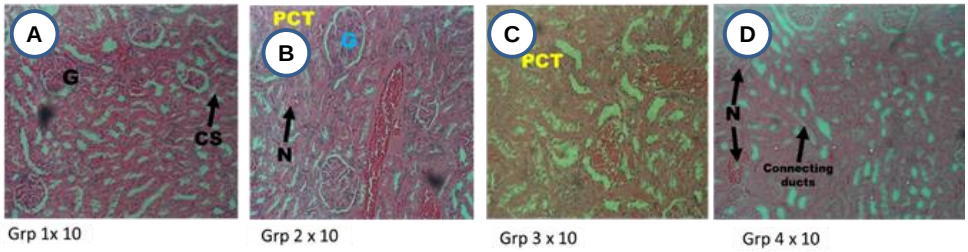

Plate 10: Photomicrograph of heart of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg of POE (H and E) the glomeruli (G) and capsular space (CS) (black arrow) appeared normal without any visible lesion (NVL)
B: Administered with 0.50 mL/kg of POE (H and E) the glomeruli (G) showing area of necrosis (N) with minimal lesion.
C: Administered with 0.75 mL/kg of POE (H and E) Proximal convoluted tubule (yellow text) appearing normal without any visible lesion (NVL)
D: Administered with sterile distilled water acutely (H and E) Proximal convoluted tubule (black arrows) appearing normal without any visible lesion (NVL)

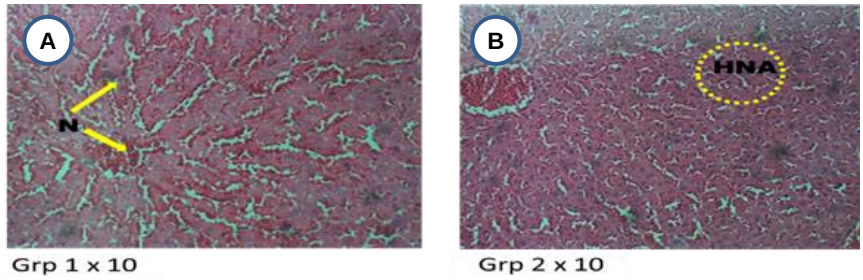

Plate 11: Photomicrograph of liver of Wistar rats treated with acute dose of POE
A: Administered with 2 mL/kg of POE (H and E) showed progressive active hepatic nuclear activities HNA and N (Yellow arrows) with nuclear degeneration, condensation, fragmentations and exhibited significant cytotoxicity.
B: Administered with sterile distilled water acutely (H and E) showing hepatic nuclear activities (doted HNA) appearing normal without any visible lesion (NVL)

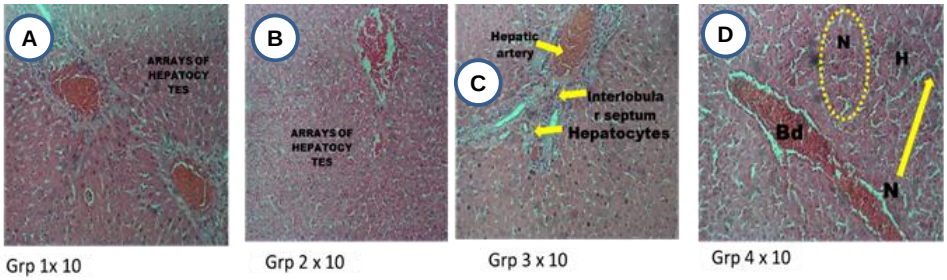

Plate 12: Photomicrograph of liver of Wistar rats treated with sub-chronic doses of POE
A: Administered with 0.25 mL/kg of POE (H and E) the hepatic nuclear activities (black dots) appearing to show necrosis with minimal visible lesion.
B: Administered with 0.50 mL/kg of POE (H and E) the hepatic nuclear activities (black dots) appearing to show necrosis with minimal visible lesion.
C: Administered with 0.75 mL/kg of POE (H and E) the hepatic nuclear activities (black dots) appearing to show necrosis with marked visible lesion.
D: Administered with sterile distilled water acutely (H and E) the hepatic nuclear activities (HN), the hepatic artery appearing. All appearing normal without visible lesion (NVL)

4. Conclusion

Pleurotus ostreatus is relatively safe as no mortality was recorded throughout the experimental period. POE showed no detectable detriment on the haematological parameters which are patho-physiological reflector of the health status. No hepatocellular damage was observed through serum analysis. Histopathological evaluation revealed that POE has no harmful effects on stomach, spleen, kidneys, lung, liver and heart within the tested doses. Therefore, it can be inferred from the study that *P. ostreatus* is suitable and safe to be used directly as food and indirectly as feed supplement and for therapeutic purposes.

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