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EFFECT OF ETHANOL EXTRACT OF THE FRUITING BODIES OF *PLEOROTUS OSTREATUS* ON THE SERUM HEPATO-SPECIFIC ENZYME MARKERS AND LIVER HISTOLOGY OF HIGH SUCROSE-HIGH FAT DIET-STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The effect of ethanol extract of the fruiting bodies of Pleurotusostreatus on the serum hepato-specific enzyme markers and liver histology were determined in high sucrose-high fat diet-streptozotocin induced diabetic rats. The pharmacological model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (intraperitoneal) Streptozotocin (STZ) induced diabetic rat model, with the fruiting body ethanol extracts administered orally at 50, 150 and 300mg/kg b.w. After 6 weeks of treatment, alkaline phosphatase activity (ALP) of the treated groups and the reference treatment groups were significantly ($p<0.05$) higher than the D group but after the 9th week of treatment, the ALP activity of the diabetic groupstreated with fruiting body ethanol extract at 50mg/kg b.w., 150mg/kg b.w. and the reference treatment group were significantly lower ($p<0.05$) than the D group. All the treated groups had aspartate amino transferase activity that was significantly higher ($p<0.05$) than the D group at the end of 3 weeks but at the end of the 6th week of treatment, the AST activity of all the treated groups was significantly lower than the D group ($p<0.05$). At the end of the first 3 weeks of treatment, the alanine amino transference (ALT) activity of the diabetic group treated with fruiting body ethanol extract at 150mg/kg b.w. was significantly ($p<0.05$) lower than the D group. After 6 weeks of treatment, ALT activity of all the treatment groups as well as the reference treatment groups were significantly lower ($p<0.05$) than the D group but the values of the diabetic group treated with fruiting body ethanol extract at 150mg/mg b.w. and diabetic group treated with metformin hydrochloride at 150mg/kg b.w. were not significantly different ($p>0.05$) from the normal control. At the end of 9 weeks of treatment, the ALT activity of the diabetic group treated with fruiting body ethanol extract at 300mg/kg b.w. and diabetic group treated with fruiting body ethanol extract at 50mg/kg b.w. were significantly lower ($p<0.05$) than the D group although not significantly different than the normal control.The extract at 300mg/kg b.w. reversed the fatty liver and periportalinflammation caused by HS-HFD-streptozotocin induced diabetes in the rats to normal, indicating dose dependent protective effects of the extract against HS-HFD-streptozotocin induced hepatotoxicity alterations. The reduction in the serum levels of these liver enzymes by the extract suggests that they may be used to reverse the incidence of liver function test irregularities common in diabetic patients. The results suggest that ethanol extract of fruiting bodies of Pleurotusostreatus may be employed in the management of liver diseases associated with diabetes mellitus.

Keywords: Serum hepatospecific-enzyme markers, liver histology, Streptozotocin, diabetes, rats, high fat diet

INTRODUCTION

Diabetes mellitus has been reported as a burden of disorder in the structure and function of biological systems (Jamaludinet al., 2016). According to Okorohet al., (2021), the disease is a major factor in the endocrine region of the biological system responsible for the crisis in the metabolism of biomolecules such as fats, carbohydrates and proteins. Diabetes mellitus has been reported to be linked with abnormalities in the liver such as abnormal glycogen deposition, non-alcoholic fatty liver disease, fibrosis, cirrhosis, abnormal increase in liver enzymes levels, acute liver disease etc. (Guvenet al., 2006) When too much fat accumulates in the liver, insulin resistance may be critical causing serious irregularities in metabolism (Levinthal and Tavill, 1999). The liver is one of the major organs in the body that suffers the negative effects of hyperglycemia-induced oxidative stress (Bugianesiet al., 2005; Palsamyet al., 2010). Diabetes mellitus has been implicated in histopathological changes in the liver such as micro vesicularsteatosis and macro vesicularsteatosis (Mukhlif et al.,2020). Diabetes mellitus is a contributory factor to impaired vision, stroke, kidney failure, cardiovascular diseases (WHO, 2016), its prevalence has been on the increase particularly among middle- and low- income nations like Nigeria. The World Health Organization has stated that diabetes mellitus will be the 7^{th} leading cause of deaths by 2030. The implication is that diabetes mellitus presents a major challenge to researchers and health care systems around the globe. Diabetes mellitus is defined as a group of metabolic diseases of endocrine origin caused by high glucose level in the blood over a prolonged period because of complete or relative lack of insulin resulting from the impairment of insulin secretion, insulin action or both (WHO,2014). Its symptoms include osmotic diuresis, increased thirst, hunger, and high concentration of lipids in the blood (WHO, 2013).High blood sugar level as a result of insulin resistance causes alteration in the metabolism of fats, proteins and carbohydrates resulting to non-alcoholic steatohepatitis, cirrhosis and hepatocellular carcinomas (Jamaludinet al.,2016).Diabetes has been indicated to cause pathological changes in the liver (Lucchesiet al., 2015). Alanine aminotransferase, aspartate amino transferase and alkaline phosphatase are biomarkers of hepatocyte damage and are involved in various reactions in the liver. Hepatocyte injury has been revealed by the levels of AST and ALT in the plasma or serum andhigh levels of ALP indicate biliary tree obstructions (Lee et al., 2012). Synthetic drugs such as sulfonylurea, biguanides and thiazolidinedionesused to treat diabetes are expensive and have side effects (Lee et al., 2012).

Pleurotusostreatus belongs to the family of mushrooms known as *Pleurotaceae* (Kuo, 2005).*P.ostraetus* is also called tree oyster mushroom (Stamets, 2000). The people from Japan call it Hiratake which means flat mushroom (Hall,2010).The Igbo-speaking people of South-East Nigeria, call it Eroatakata because it has very tough texture on mastication (Akpajaet al., 2003). The mushroom has quality nutritional value, numerous medicinal properties and many other beneficial effects. It has been used as food and as means of treating ailments by numerous people all over the globe for many years (Finimundyet al.,

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2013). *P. ostreatus* is rich in dietary fiber, sterol, proteins, macro-minerals and trace-elements. It has been reported that the macro fungi, due to the presence of mychochemicals in them coupled with their antioxidative properties may be used to cure ailments associated with viruses, bacteria, high cholesterol level in blood, it has hematological characteristics as well as the capacity to enhance immune functions (Finimundyet al., 2013; Markopoulosetal., 2012). This is because it is a source of important mineral nutrients such as selenium, potassium, magnesium, copper, calcium, vitamins like riboflavin, niacin, vitamin D,tocopherol, vitamin C, folic acid, vitamin K and dietary fiber to humans (Maria et al., 2014). The present study was conducted to determine the effect of ethanol extract of the fruiting bodies of organically cultivated *P.ostreatus* on the serum hepato-specific enzyme markers and liver histology of HS-HFD-streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Preparation of High Calorie Density Diet

High calorie density diet was prepared according to formulation by Okorohet al., (2021) as shown below;

Table1: High Sucrose-High Fat Diet (HS-HFD %)

Composition	Proportion (%)
Normal diet	60.0
Sucrose	20.0
Lard	20.0
Total	**100.0**

* Diet was prepared daily to avoid microbial contamination and fed to the animals *ad libitum*, throughout the period of the experiment.

Collection of Mushroom Materials and Preparation of Mushroom Ethanol Extracts

Pleurotusostreatus fruiting bodies were obtained from the samples cultivated using organic supplements at the Research Unit Demonstration Farm of the University of Port Harcourt, Rivers State, Nigeria. Ethanol extract of *P.ostreatus* was prepared according to the method reported by Okorohet al. (2021)

Collection of Experimental Animals, Induction of Diabetes and Determination of Blood Glucose and Body Weight

A total of 54 normoglycemic female Wistar albino rats were used for this study. The animals were purchased from the Animal House, Department of Biochemistry, Faculty of Science, University of Port Harcourt, kept and maintained in a house that is well-ventilated, having a 12hour light / 12hour dark cycle in propylene cages, at room temperature. Food and water were adequately given to the animals. The animals were acclimatized to laboratory conditions, 7days prior to starting of experiment. After acclimatization of the animals for a period of 7 days, the nine animals in Normal control group were placed on normal diet of guinea growers mash diet while the other rats in the remaining five groups (n=9) were fed with High Sucrose-High Fat Diet (HS-HFD) throughout the experimental period. The forty-five rats (n=9 rats/group) in the other five groups were placed on HS – HFD for 21 days, fasted overnight and induced diabetes using a single intraperitoneal injection of streptozotocin (35mg/kg bw). Stroptozotocin (Sigma, USA) at a dose of

35mg/kgbw was prepared in fresh and cold normal saline solution and administered immediately to the animals. The animals were first weighed using an electronic scale (TH 500) and their base line fasting blood glucose level taken using Fine Test Auto-coding™ Premium Blood Glucose Monitoring System and Blood Glucose Strips via tail vein cut before they were injected with streptozotocin (Okorohet al.,2021).

Experimental Design

The experimental model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (intraperitoneal) streptozotocin (STZ) induced diabetic rat model .The Metformin HCl and ethanol extract were given once daily(1ml per animal) by intragastric gavage to the reference treatment and experimental groups respectively at doses 150mg/kg b.w., 50mg/Kg b.w, 150mg/kg b.w. and 300mg/kg b.w. respectively while the normal control received saline solution for 88days. The rats (3 from each group) were sacrificed after 3, 6 and 9 weeks of treatment. Blood samples and liver were collected for analysis. The extracts and metformin HCl (reference drug) were kept in plastic bottles with cap tightly sealed before and after each use, stored in the refrigerator, protected from direct sunlight to prevent spoilage throughout the time of animal treatment. (Okorohet al.,2021). Ethical guidelines for the use of animals (Mary and Paul, 2016) was followed in this research

Determination of Serum Hepato Specific Enzyme Markers and Liver Histopatology

Alkaline phosphatase activity , alanine transaminase activity and aspartate transaminase in the serum were estimated using Randox test kits (Randox Laboratories Test Crumlin, England, UK) (Reitman & Frankel, 1957).Histopathology was carried out at the Department of Anatomical Pathology, University of Port Harcourt Teaching Hospital. Small pieces of liver tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in wax of paraffin. Sections of about 5µm in thickness were cut, mounted on slide and stained with hematoxylin and eosin. The sections were then observed under light (Opticphot -2, Nikon, Tokyo, Japan) at x200 and x400 magnifications respectively.

Statistical Analysis

Data was statistically analyzed by a one way analysis of varience (ANOVA) using SPSS/PC + package. Multiple comparisms of differences between means were conducted using Fisher's Least Significance Difference (LSD). Significance was accepted at a p-value of less than 0.05.

RESULTS AND DISCUSSIONS

The initial and most important biomarkers in assessing liver injury include the levels of serum ALT, AST and ALP (Longo et al., 2011).The results of this scientific study (Table 1, Table 2 and Table 3, respectively) revealed that the extracts of *P.ostreatus* at all doses significantly ($p< 0.05$) lowered ALP level after 3 weeks of treatment, while *Pleurotusostreatus*extract

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(POE) at 50 and 150mg/kg significantly ($p<0.05$) reduced ALP levels of the treated rats after 9 weeks. The ALT activity was significantly ($p<0.05$) lowered after 3 weeks of treatment with extract dose of 150mg/kg. It was also significantly ($p<0.05$) lowered after 6 weeks of treatment at all doses of POE, and reduced ALT activity was observed after 9 weeks of treatment with extract doses of 300 and 50mg/kg respectively. The AST levels were significantly ($p<0.05$) reduced after 6 weeks of treatment by extracts at 50 and 300mg/kg doses respectively. Diabetes has been indicated to cause pathological changes in the liver (Lucchesiet al., 2015). Alanine aminotransferase, aspartate amino transferase and alkaline phosphatase are biomarkers of hepatocyte injury and these enzymes are involved in various reactions in the liver. Hepatocyte injury has been revealed by the levels of AST and ALT in the plasma or serum. A high level of ALP indicates biliary tree obstructions (Lee et al., 2012). Haris (2005), in a clinical study reported that people suffering from type 2 diabetes mellitus show more liver function test irregularities when compared to the individuals that are normal. The reduction in the activities of these liver disease marker enzymes in the serum by *P.ostreatus*ethanol extracts suggests that they may be used to reverse the incidence of liver function test irregularities common in diabetic patients. The AST/ALT ratio is vital in medical diagnosis for elevated transaminases to differentiate between the causes of liver damage. An AST/ALT ratio equal to 1 may be sign of acute viral hepatitis or drug related liver toxicity while an AST/ALT ratio higher than 1 means that there is liver cirrhosis (Nyblom et al., 2006).

The mushroom extract at a dose of 150mg/kg b.w. was more effective in lowering ALP, AST and ALT levels compared to metformin hydrochloride (reference standard drug) after 3 weeks of treatment. The extract at a dose of 300mg/kg b.w. was less effective than the reference standard drug in lowering ALP and AST but the effect of the extract and the reference standard drug in lowering ALT level was comparable.

After 6weeks of treatment, the extract at a dose of 150mg/kg b.w. was more effective than the reference standard drug in lowering AST and ALT levels but the extract at 300mg/kg was less effective than the reference standard drug in lowering ALT level.

However, after 9 weeks of treatment, the extract at 300mg/kg b.w. was only more effective in lowering ALT level compared to the reference standard drug.

The reduction in the serum levels of these liver enzymes by the mushroom extract observed in this study suggests that they may be used in place of metformin hydrochloride to reverse the incidence of liver function test irregularities common in diabetic patients

Table 2: Effect of ethanol extract of the fruiting bodies of *Pleurotusostreatus* on the serum hepato-specific enzyme markers of HS-HFD-Streptozotocin induced diabetic rats after three weeks of treatment.

Treatment group	Magnitude			
	Alkaline phosphatase activity (U/L	**Aspartate transaminase activity (U/L)**	**Alanine transaminase(U/L)**	**AST:ALT ratio**
NC	90.47 ± 21.32^{a}	49.67 ± 2.52^{af}	11.67 ± 1.53^{a}	4.26±1.34
DC	209.67 ± 18.50^{b}	31.67 ± 3.79^{a}	10.00 ± 2.00^{ad}	3.17±2.11
D+POE_{50}	84.26 ± 8.65^{a}	67.33 ± 9.02^{bf}	16.33 ± 208^{b}	4.12±1.94
D+POE_{150}	49.43 ± 18.36^{ce}	$53/00\pm13.53_{cf}$	7.0 ± 1.00^{cd}	7.57±1.00
D+POE_{300}	108.16 ± 17.24^{a}	56.00 ± 20.00^{df}	10.00 ± 2.00^{ad}	5.6±2.35
D+MET_{150}	52.80 ± 2.69^{de}	54.00 ± 4.58^{ef}	10.00 ± 2.00^{ad}	5.4±2.35

Values are means ± SD, n=9per group. Values in the same column with different superscripts are significantly different at $p<0.05$. NC=normal control, DC=diabetic control, D+POE_{50}, D+POE_{150} , D+POE_{300} , D+MET_{150} are diabetic groups treated with *Pleurotusostreatus* extracts and metformin at different doses.

Table 3: Effect of ethanol extract of the fruiting bodies of Pleurotusostreatus on the serum hepato-specific enzyme markers of HS-HFD-Streptozotocin induced diabetic rats after six weeks of treatment.

Treatment group	Magnitude			
	Alkaline phosphatase activity (U/L	**Aspartate transaminase activity (U/L)**	**Alanine transaminase(U/L)**	**AST:ALT ratio**
N	92.23 ± 6.99^{a}	38.33±2.52	8.67 ± 1.15^{a}	4.4
D	94.10 ± 8.05^{a}	54.67±10.79	14.0 ± 3.61^{bd}	3.9
D+POE_{50}	99.60 ± 11.90^{af}	51.67±16.26	13.67 ± 1.53^{cd}	3.7
D+POE_{150}	101.53 ± 13.37^{ag}	43.67±8.02	8.67 ± 1.54^{a}	5.0
D+POE_{300}	99.50 ± 11.45^{ah}	51.67±4.73	11.00 ± 1.73^{ad}	4.6
D+MET_{150}	113.60 ± 11.43^{bfgh}	55.33±11.93	9.00 ± 2.65^{a}	6.1

Values are means ± SD, n=9, per group. Values in the same column with different superscripts are significantly different at $p<0.05$. NC=normal control, DC=diabetic control, D+POE_{50}, D+POE_{150} , D+POE_{300} , D+MET_{150} are diabetic groups treated with *Pleurotusostreatus* extracts and metformin at different doses.

Table3: Effect of ethanol extract of the fruiting bodies of *Pleurotusostreatus* on the serum hepato-specific enzyme markers of HS-HFD-streptozotocin induced diabetic rats after nine weeks of treatment.

Treatment group	Magnitude			
	Alkaline phosphatase activity (U/L	**Aspartate transaminase activity (U/L)**	**Alanine transaminase (U/L)**	**AST:ALT ratio**
N	100.37 ± 0.12^{a}	39.67 ± 1.53^{a}	10.67 ± 0.58^{a}	3.7
D	106.40 ± 11.86^{ac}	46.67 ± 5.88^{af}	12.67 ± 2.52^{ad}	3.6
D+POE_{50}	103.63 ± 16.13^{ad}	66.33 ± 10.97^{bg}	11.00 ± 1.00^{a}	6.0
D+POE_{150}	105.17 ± 4.56^{ae}	66.00 ± 2.65^{cg}	14.00 ± 1.73^{bde}	4.7
D+POE_{300}	120.43 ± 0.12^{bcdef}	59.20 ± 17.49^{dfg}	9.00 ± 1.00^{a}	6.6
D+MET_{150}	103.20 ± 12.73^{af}	72.67 ± 6.35^{eg}	16.33 ± 1.53^{ce}	4.4

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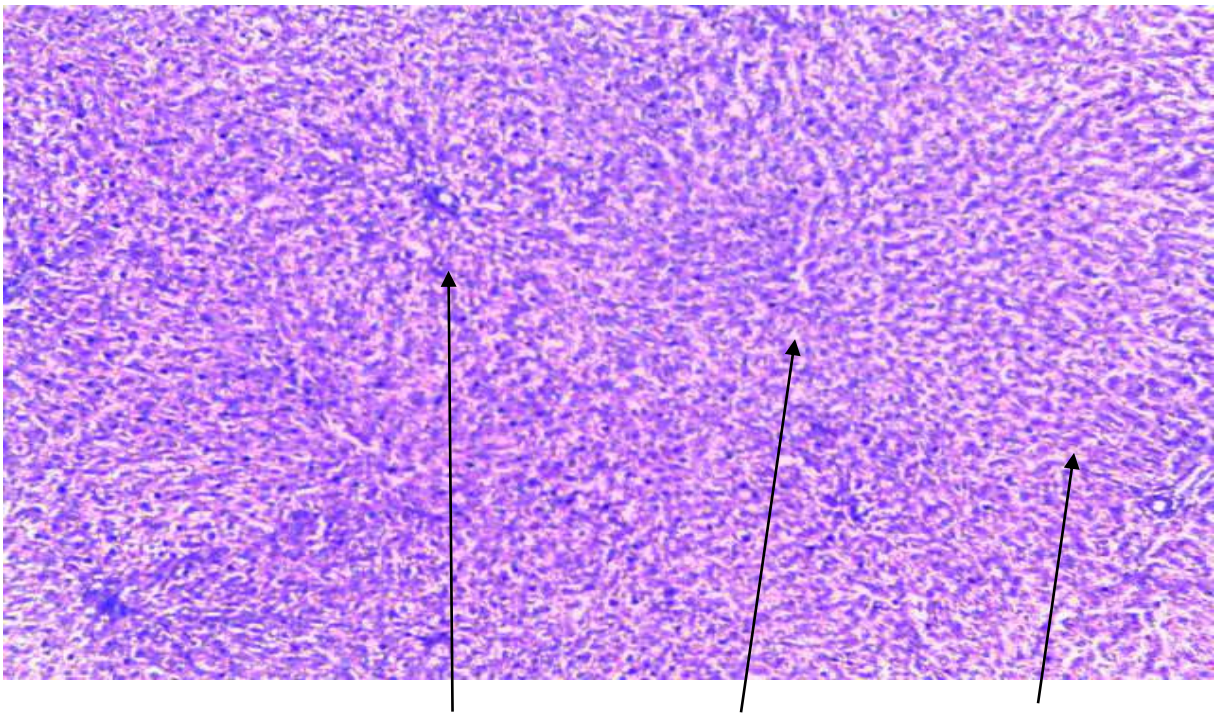
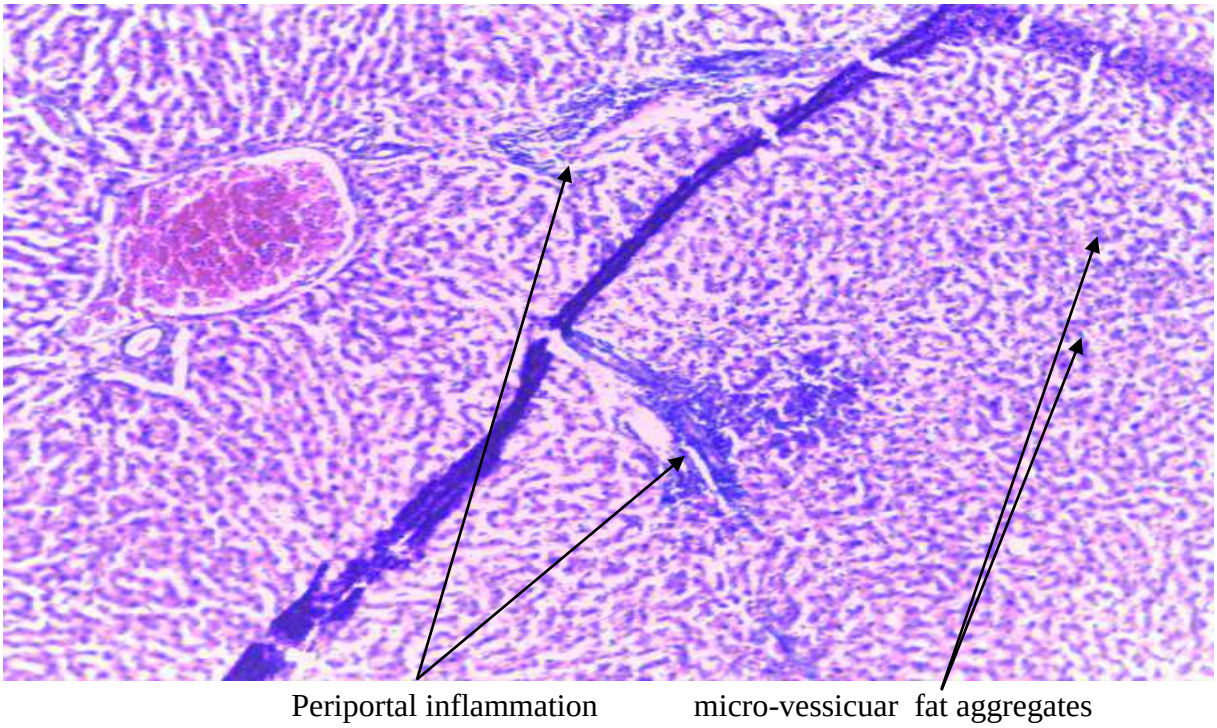
Values are means ± SD, n=9, per group. Values in the same column with different superscripts are significantly different at $p<0.05$. NC=normal control, DC=diabetic control, D+POE_{50}, D+POE_{150} , D+POE_{300} , D+MET_{150} are diabetic groups treated with *Pleurotusostreatus* extracts and metformin at different doses.

Histopathological changes of *P.ostreatus*ethanol extract in HS-HFD-streptozotocin induced diabetic rats were investigated as shown in the histologic section of the liver in plates 1-6. In diabetes mellitus, the liver has been a major organ of focus because of its critical role in carbohydrate and lipid metabolism as well as blood sugar regulation and detoxification functions. In some diabetic incidences, manifestations in the changes in the liver hepatocytes have been reported (Singh et al., 2017). The histologic section of the diabetic rat's revealed fatty liver and periportal inflammation compared to the normal control which showed normal histological features of the liver. Treated diabetic rats (POE_{50} group), (POE_{150} group) and the reference treatment group (MET_{150}) also manifested fatty change in hepatocytes and periportal inflammation. This may imply that the treatment at these dosages could not reverse these complications. Kumeet al., (1994) also reported the accumulation of fat into hepatocytes in streptozoticin induced diabetic mice. Fatty changes in hepatocytes were also reported by Noman (2009) in the work on histopathological liver changes in streptozoticin induced diabetic mice. However, an interesting finding in this study is that there was no obvious histologic change in the liver of diabetic rats treated with extracts at dosage level of 300mg/kgb.w. This clearly showed that the extract at this dose reversed to the control the fatty liver and periportal inflammation the liver caused by HS-HFD-streptozotocin induced diabetes. The reason for this hepatoprotective effect of the extract may be due to the pharmacological activities of this mushroom against insulin resistance and hyperlipidemia as a result of the presence of bioactive substances of medicinal value in the macro fungi such as the steroids. This study therefore has shown that *Pleurotusostreatus* extract may dose dependently act as a bio protective agent against HS-HFD-Streptozotocin induced hepatotoxicity alterations indicated in this study normal.



Group N Liver H&E X 200

Plate 1: Histologic section of the liver of normal rats showing normal histologic features.



Group D Liver H &E X 400

Plate 2: Histologic section of the liver of diabetic rats showing inflammation and fatty change

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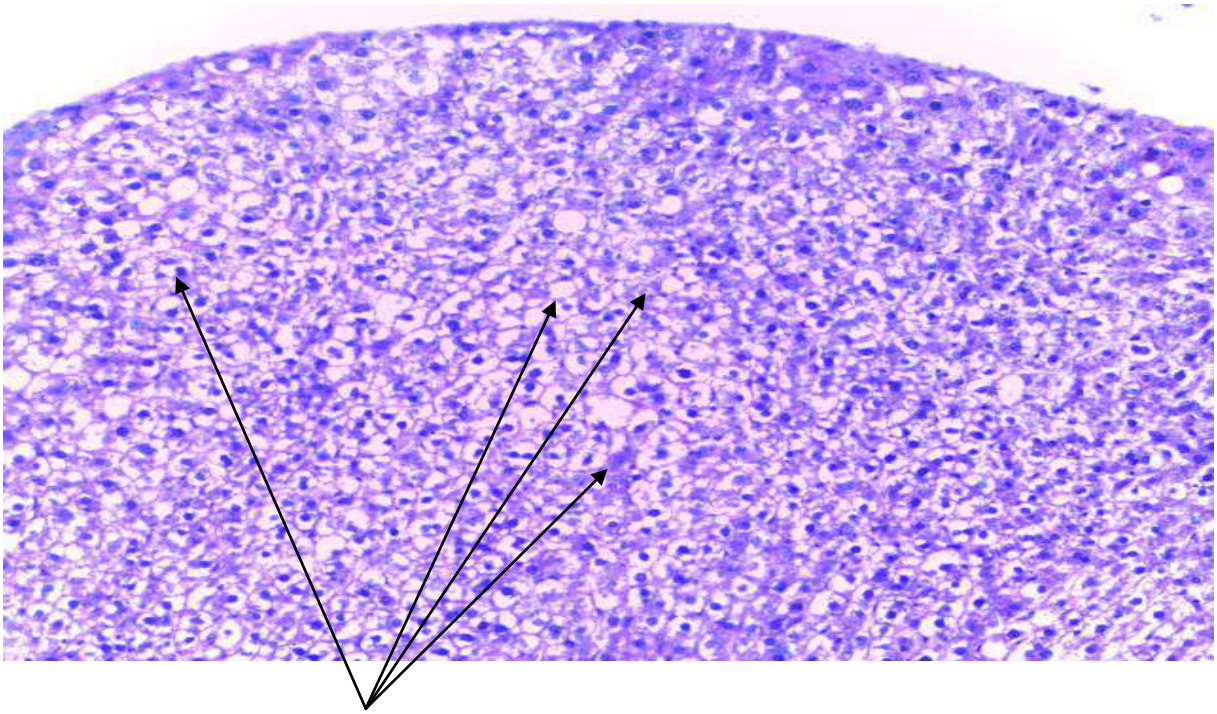
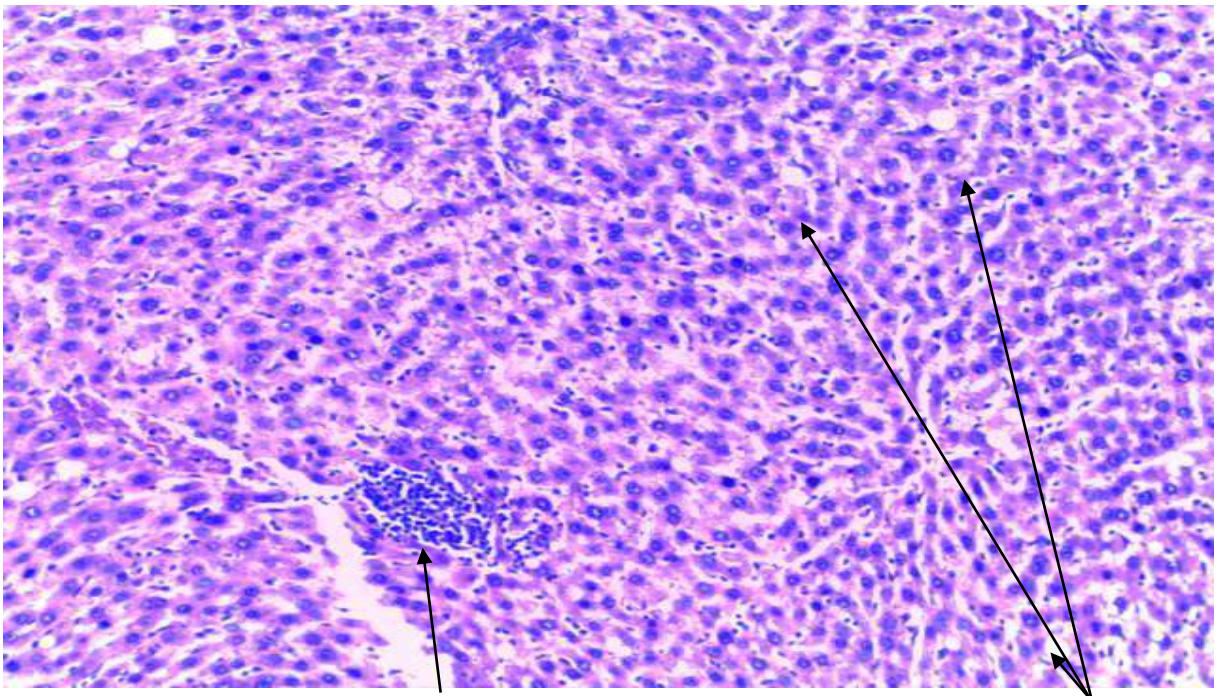


Fat vessicles

Group D+POE$_{50}$Liver H &E X 200

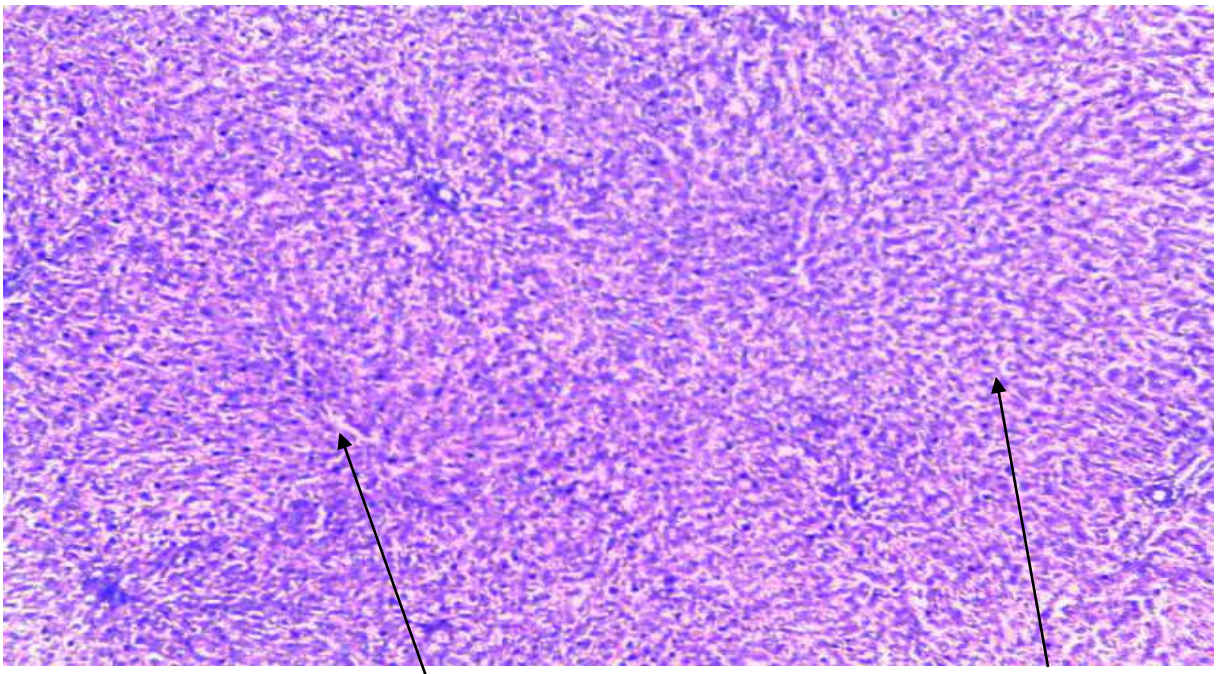
Plate3: Histologic section of the liver of diabetic rats treated with extracts at dosage of 50mg/kg indicating marked fatty change in hepatocytes



Inflammatory cells fat cells

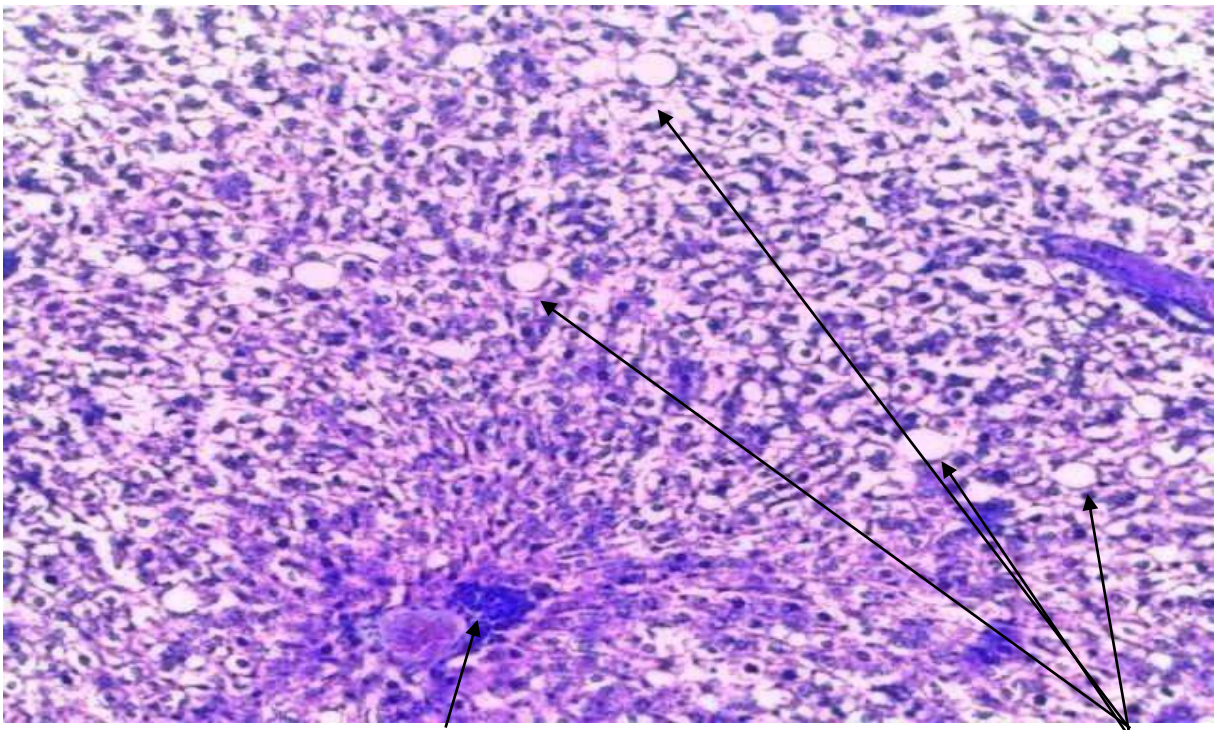
Group D+POE$_{150}$ Liver H&E X 400

Plate4: Histologic section of the liver of diabetic rats treated with extracts at dosage of 150mg/kg showing fats cells and inflammatory cells within the liver parenchyma.



Group D+POE$_{300}$ Liver H &E X 200

Plate5: Histologic section of the liver of diabetic rats treated with extracts at dosage of 300mg/kg highlighting no obvious histologic changes.



Group D+MET$_{150}$Liver H &E X 400

Plate6: Histologic section of the liver of diabetic rats treated with metformin HCl (150mg/kg) showing marked fatty change and periportal inflammation.

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CONCLUSION

The results in this study showed that ethanol extracts of P.ostreatus time and dose dependently caused a reduction in the serum levels of liver enzymes (ALP, ALT and AST) suggesting that they may be used to reverse the incidence of liver function test irregularities common in diabetic patients. The extracts reversed the fatty liver and periportal inflammation pathological insults on the liver caused by HS-HFD-Streptozotocin induced diabetes in the rats to normal, indicating dose dependent bio protective features of the extract against HS-HFD-Streptozotocin induced hepatotoxicity alterations. The results suggest that ethanol extract of organically cultivated *Pleurotusostreatus* may be employed in the management of liver diseases associated with diabetes mellitus.

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Competing Interests

The authors hereby declare that there was no conflict of interest or financial inducement which may have negatively influenced them in writing this scholarly article

Authors' Contributions

P.N.O. and S.C.O. designed the study, drafted the manuscript and carried out the experiments; K.O. collected resource materials and conducted statistical analysis; A.A.U and C.C.M supervised laboratory work, revised and edited the manuscript. The authors read and approved the manuscript.

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