



Effect of Ethanol Extract of the Fruiting Bodies of *Pleurotus Ostreatus* on Serum Lipid Profile and Atherogenic Indices of HS-HFD-STZ Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PNO and AAU designed the study and performed the statistical analysis, they also prepared the first draft. Author SO managed the literature searches. Author CYU managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

The effect of ethanol extract of the fruiting bodies of *Pleurotus ostreatus* on serum lipid profile and atherogenic indices of high sucrose high fat diet streptozotocin (HS-HFD-STZ) induced diabetic rats was determined by standard methods. All the groups were fed high sucrose-high fat diet except the normal group. The Metformin HCl and ethanol extract was given once daily by gavage to the reference and experimental groups respectively at doses of 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. The HDL-cholesterol level was higher than the value of the disease group after 6 weeks of administration of extract at 300mg/kg and after 9 weeks administration of extract at 150mg/kg. HDL cholesterol concentration increased by 22.2% after 6 weeks of extract administration at 300mg/kg and by 16.7% and 28.3% respectively after 9 weeks of extract administration at 150mg/kg and

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50mg/kg respectively, indicating that the POE extract has the capacity to reduce cardiovascular diseases. At all the doses of extract administered for treatment at 3 weeks, 6 weeks and 9 weeks intervals, there was dose and time dependent lowering of LDL: HDL ratio even below the recommended risk limit of ≤ 2.5 compared to the test control with value above 2.5. After 3 weeks of treatment with extract at administration concentration level of 150mg/kg, atherogenic indices were lowered and extract levels of 150mg/kg and 300mg/kg reduced atherogenic index value after 6 weeks of administration while after 9 weeks of treatment, extract at 150mg/kg concentration reduced atherogenic indices. The atherogenic indices of the diabetic animals under treatment were dose-and time dependently reduced by POE treatment as observed in this study. These results suggest a possible use of the extracts in the management of hyperlipidemic conditions, hypertension and associated complications.

Keywords: Atherogenic; hyperlipidemia; HDL-cholesterol; lipid.

1. INTRODUCTION

Our world is fast becoming obesogenic because of human consumption of high density calorie foods and sedentary life style. Obesity has now become a spring board to diseases such as diabetes, cardiovascular diseases, hypertension, arteriosclerosis, hence a major contributor of deaths, complications in the bio system, disability and economic problem of nations of the world. According to [1], diabetes has been tagged a disease of global burden. [2], reported that diseases such as obesity, diabetes, hypertension, hypercholesterolemia and hyperlipidemia are risk indicators of the development and progression of cardiovascular diseases. [3], highlighted that cardiovascular disease is the route course of death in the world today. High level of cholesterol in the blood has been revealed as an indicator of increased risk of heart attack, stroke and atherosclerosis. The deposition of cholesterol in the walls of the artery, diabetes, and cardiovascular disease are linked to hypercholesterolemia as risk indicator. Epidemiological studies revealed that hyperlipidemia and hypercholesterolemia are the risk factors of cardiovascular diseases [4].

There is a consistent body of evidence from numerous clinical trials which has established that the lowering of lipid can bring about a reduction in the incidence of cardiovascular diseases and stroke in a broad spectrum of human subjects [5]. Therefore, it may be possible to use any nutritional and nutraceutical intervention to improve abnormal lipid metabolism and reduce the risk of cardiovascular diseases [6]. There are a lot of synthetic drugs used in the management of hyperlipidemia and hypercholesterolemia and these include statins, niacin, resins, and fibrates. Statins are seen to be the most effective lipid-lowering drugs but

they have side effects [7]. It is therefore necessary to conduct a scientific research on other agents having lipid-lowering and atherogenic-reducing characteristics but with utmost pharmacovigilance.

The mechanism of action of Streptozotocin is such that it enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD⁺ and ATP. The rats that are diabetic are determined by measuring glucose level using a glucometer [8,9]

The resource mushroom, *Pleurotus ostreatus* belongs to the family of mushrooms known as pleurotaceae [10]. *P.ostreatus* is also called tree oyster mushroom [11] or grey oyster mushroom and this name marks it out from the other species in the genus. Some people call it straw mushroom. The people from Japan call *Pleurotus ostreatus* Hiratake which implies flat mushroom [12]. The Igbo-speaking people of South-East, Nigeria, call it Ero atakata because it has very tough texture on mastication [13].

Pleurotus ostreatus is one of the most common mushrooms that local people hunt from the wild. The macro fungi can also be cultivated by people using saw dust as substrate. The mushroom has bitter sweet aroma of benzoic aldehyde [14]

Mushroom can be used as food supplement for patients that suffer hypercholesterolemia because it contains high level of Lovastatin [15] as well as bioactive phenolic compounds such as gallic acid, hesperetin, protocatechaic acid, naringenin, biochanin-A and chlorogenic acid,

[16]. It has also been reported that the macrofungi, due to the presence of mycochemicals in them coupled with their antioxidative properties may be used to cure ailments caused by high cholesterol level in blood. It provides important mineral nutrients such as selenium, potassium, magnesium, copper, and calcium, vitamins like riboflavin, niacin, vitamin D, tocopherol, vitamin C, folic acid, vitamin K and dietary fiber to humans [17]

Metformin was chosen over the other diabetic drugs because metformin improves blood sugar levels by working on enzymes in the liver to lower glucose absorption from the diet and the production of new glucose. Metformin does not usually cause hypoglycemia [18].

The purpose of this study was to investigate the effect of ethanol extract of the fruiting bodies of *Pleurotus ostreatus* on serum lipid profile and atherogenic indices of HS-HFD-STZ induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Preparation of High Calorie Density Diet

High calorie density diet was prepared according to formulation by [19]

The high calorie density diet was prepared using normal animal diet, sucrose and lard in the combination ratio of 3:1:1. The basic composition of the High Sucrose-High Fat Diet (HS-HFD) is shown in Table 3,4.

Table 1. 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

2.2 Experimental Design

2.2.1 Preparation of *P.ostreatus* Ethanol Extract

The dried macrofungi materials was pulverized with a manual grinder and weighed with an

electronic balance to obtain a mass of 944g (ground dry weight sample) which was well packaged and labeled. Ethanol extraction was carried out at the Organic and Inorganic Pharmaceutical Chemistry laboratory, University of Port Harcourt. To every mass of 100g of the pulverized macrofungi material, 300ml of ethanol were used for soaking and the bottles were shaken intermittently. First filtration process was done using clean white cotton material already immersed into the ethanol. Second filtration was done using What man No.1 filter paper. The filtrate was concentrated using a rotary evaporator at a temperature of 55°C and the concentrate was subjected to evaporation using a water bath regulated at a temperature of 55°C until a dark brown paste which weighed 54.02g was obtained as extract. The paste was stored in a refrigerator until further experimental use. The percentage yield was 6.0% (w/v). This was calculated as follows:

$$\text{Extract percentage yield (\%)} = \frac{\text{weight of extract}}{\text{weight of dry ground power}} \times \frac{100}{1}$$

2.2.2 Experimental Animals

A total of 54 normoglycemic female wistar albino rats were used for this research. The animals were purchased from the Animal House, Department of Biochemistry, Faculty of Science, University of PortHarcourt, and 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 till the experimental research commenced. The animals were acclimatized to laboratory conditions, 7days prior to starting of experiment.

2.2.3 Induction of Diabetes and Determination of Blood Glucose and Body Weights of Rats

All experiments have been examined and approved by the appropriate ethics committee. After acclimatization of the animals for a period of 7 days, the nine animals in Normal 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 (Sigima, 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 streptozocin. The animals in normal control group were injected normal saline alone. After 72hr of streptozotocin administration, the rats were again fasted and blood collected via tail cutting and their fasting blood glucose level were tested which confirmed hyperglycemia. Treatment of the animals with the ethanol extracts of *Pleurotus ostreatus* cultivated by substrate organic supplementation and Metformin HCl reference drug was done immediately after the last streptozotocin injection. Blood samples were drawn after 3rd week, 6th week and 9th week of commencement of treatment during the study. 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.

2.3 Experimental Design

The experimental model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (via intraperitoneal) streptozotocin (STZ) induced diabetic rat model. The Metformin HCl and ethanol extract was given once daily (1ml per animal) by intragastric gavage to the reference 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. The rats (3 from each group) were sacrificed after 3, 6 and 9 weeks of treatment. Blood samples and major organs were collected for analysis.

2.4 Determination of Plasma Lipid Profile

Plasma total cholesterol (TC), HDL-Cholesterol (HDL-C) and triglyceride (TG) were determined enzymatically with Randox test Kits (Randox Laboratores, Crumlin, England). Plasma LDL- and VLDL-Cholesterol (LDL-C and VLDL-C) were calculated using the Friedewald equation [20] as follows:

- i. $LDL-C \text{ (mmol/L)} = TC - [HDL-C] - \frac{[TG]}{2.2}$
- ii. $[VLDL-cholesterol] \text{ mm/L} = \frac{[Triglyceride]}{2.2}$

While the plasma non-HDL-Cholesterol concentration was determined as reported by [21]:

$$[\text{Non-HDL-cholesterol}] = [\text{Total cholesterol}] - [\text{HDL cholesterol}]$$

The atherogenic indices were calculated as reported by [22] as follows;

- i. Cardiac Risk ratio = $\frac{[\text{Total cholesterol}]}{[\text{HDL cholesterol}]}$
- ii. Atherogenic coefficient = $\frac{[\text{Total cholesterol}] - [\text{HDL - cholesterol}]}{[\text{HDL - cholesterol}]}$
- iii. Atherogenic Index of Plasma = $\frac{[\text{Triglyceride}]}{[\text{HDL - cholesterol}]}$

2.5 Statistical Analysis

Experimental data were statistically analyzed by a one way analysis of variance (ANOVA) using SPSS/PC + package. Multiple comparison of differences between means were conducted by using Fisher's Least Significance Difference (LSD). Significance was accepted at a p-value of less than 0.05 ($P < .05$).

3. RESULTS

3.1 The Effect of Ethanol Extract of the Fruiting Bodies of *Pleurotus Ostreatus* on Serum Lipid Profile of Diabetic Rats

The effect of ethanol extract of fruiting bodies of *pleurotu sostreatus* on the serum lipid profile of high sucrose-high fat diet-streptozotocin-induced diabetic rats during the period of treatment are shown in tables below. After 3 weeks of treatment, the cholesterol levels of D + POE₅₀, D + POE₁₅₀ and D + POE₃₀₀ (treated groups) were significantly lower ($P < .05$) than the value of the D group (diabetic control).

After 9 weeks of treatment at the highest dose of 300mg/kg POE and the reference treatment (150mg/kg metformin), cholesterol levels of the animals reduced by 26.1% and 27.7% respectively. The HDL- cholesterol levels of the treated groups were not significantly different ($P > .05$) from that of the D group after 3 weeks of treatment. Between 3 weeks and 6 weeks of

treatment, the HDL-cholesterol level increased by 22.2% in D + POE₃₀₀ group and between the 3 weeks and 9 weeks of treatment, there was a rise in HDL-cholesterol levels by 16.7% and 28.3% respectively in D + POE₁₅₀ and D + POE₅₀ groups.

The non-HDL-cholesterol levels of the treated groups were significantly lower ($P < .05$) than the test control after the first 3 weeks of treatment but the values of the D + POE₁₅₀ group and the normal were not significantly different ($P > .05$).

The triglyceride levels of the D + POE₁₅₀, D + POE₃₀₀ and the reference treatment groups were significantly lower ($P < .05$) than the D group after 3 weeks of treatment but significantly higher ($P < .05$) than the normal control.

After 3 weeks of treatment, the LDL: HDL ratios of the D + POE₅₀ and D + POE₁₅₀ were significantly lower ($P < .05$) than that of the D-group. The LDL: HDL ratio of the D + POE₅₀ and D + POE₁₅₀ group was below the recommended 2.5 limit while that of the test control was above 2.5. At the end of the 6 weeks of treatment, the LDL: HDL ratio of the two treatment groups D + POE₁₅₀ and D + POE₃₀₀ were lower than the recommended 2.5 value and also significantly lower ($P < .05$) than the D group. However, long term treatment of 9 weeks caused the LDL: HDL ratio to be significantly lower than ($P < .05$) the diabetic groups in all the treatment group and even the reference treatment group. The values of LDL: HDL ratio of all treatment groups and the normal did not exceed the acceptable level of ≤ 2.5 while that of the diabetic group exceed. A value of 2.5 and above portends a risk of heart disease.

3.2 The Effect of the Ethanol Extract of the Fruiting Bodies of *Pleurotostreatus* on the Atherogenic Indices of Diabetic Rats

The effect of the ethanol extract of the fruiting bodies of *Pleurotostreatus* on the atherogenic indices of high sucrose-high fat diet-streptozotocin induced diabetic rats during the period of treatment are shown in Tables 5,6 and 7 respectively.

After the first 3 weeks of treatment, only the atherogenic index of the D+POE₁₅₀ group was found to be significantly lower ($P < .05$) than the D group and the value of atherogenic index (AI) of

the D+POE₁₅₀ and the normal control were not significantly different ($P > .05$). At the end of the 6 weeks of treatment, the AI of the D+POE₁₅₀, D+POE₃₀₀ and the reference treatment group were significantly lower than the value of the diabetic group ($P < .05$) although the AI of D+POE₁₅₀ and the reference treatment group were not significantly different ($P > .05$). The AI values of these groups were lower than the risk cardiovascular disease range of 0.11-0.21. The AI of the D+POE₁₅₀, after 9 weeks of treatment was significantly lower than the value of the D group ($P < .05$) although the values of the reference treatment group, D+POE₅₀ and D group were not significantly different ($P > .05$).

The cardiac risk ratio values of all the other groups were not significantly different ($P > 0.05$) but were significantly lower than the cardiac risk ratio (CRR) value of D + MET₁₅₀ ($P < .05$) after the first 3 weeks. At the end of 6 weeks of treatment, the CRR values of D+POE₃₀₀, D+POE₁₅₀ and D+POE₅₀ were significantly lower than that of the D group ($P < .05$) and the CRR value of the D group was higher than the acceptable moderate value of 5.5. The CRR values of D+POE₅₀ and normal were not significantly ($P > .05$) different. After 9 weeks of treatment, the CRR values of the treatment groups, reference treatment group were significantly lower than ($P < .05$) the D group and the CRR values of D+POE₁₅₀, D+MET₁₅₀, D+POE₅₀ and the normal control were not significantly different ($P > .05$). After 3 weeks of treatment, there was no significant difference ($P > .05$) between the atherogenic coefficient of all other groups except the D + MET₁₅₀ which was significantly higher ($P < .05$). At the end of 6 weeks of treatment, the AC of D+POE₃₀₀ was significantly lower ($P < .05$) than the D group.

The AC of D+MET₃₀₀ was significantly higher ($P < .05$) than all other values but the values of the D+POE₅₀, D group and N group were not significantly different ($P > .05$). However, after 9 weeks of treatment, there was significant lowering of the AC values of all the treated groups and the reference treatment compared to the D groups ($P < .05$). Also, at the end of the study, there was no significant difference between the AC of D+POE₅₀ and D+POE₁₅₀ ($P > .05$) compared to the normal control. Comparing the 3rd 6th and 9th weeks of monitoring the atherogenic indices of the experimental animals, there was dose and time dependent, reduction of the values of the atherogenic indices affected by both the POE treatment and metformin Hydrochloride treatment.

Table 2. Effect of ethanol extracts of the fruiting bodies of *Pleurotus ostreatus* on the serum lipid profile of HS-HFD-Streptozotocin-Induced diabetic rats after 3 weeks of treatment

Treatment group	Concentration(mmol/L)						
	Triglyceride	Total Cholesterol	HDL Cholesterol	VLDL Cholesterol	LDL Cholesterol	Non-HDL Cholesterol	LDL:HDL ratio
N	0.70±0.10 ^a	2.17±0.12 ^{ar}	0.47±0.17	0.32±0.05 ^a	1.43±0.12 ^a	1.70±0.10 ^a	3.10±0.56 ^{cei}
D	1.57±0.21 ^{be}	3.40±0.36 ^{bei}	0.70±0.54	0.71±0.09 ^{be}	2.03±0.32 ^{bd}	2.70±0.30 ^{bd}	2.93±0.50 ^{cei}
D + POE ₅₀	1.73±0.59 ^{ce}	2.60±0.20 ^{afgi}	0.60±0.23	0.79±0.29 ^{ce}	1.30±0.35 ^a	2.00±0.26 ^{ae}	2.20±0.60 ^{cej}
D + POE ₁₅₀	0.90±0.10 ^{ag}	2.23±0.25 ^{afg}	0.60±0.28	0.41±0.05 ^{af}	1.27±0.12 ^a	1.63±0.15 ^a	2.13±0.23 ^{cek}
D + POE ₃₀₀	1.33±0.15 ^{def}	2.80±0.62 ^{ctgi}	0.63±0.53	0.61±0.14 ^{def}	1.60±0.56 ^{ad}	2.17±0.59 ^{ad}	2.90±2.08 ^{cei}
D + MET ₁₅₀	1.20±0.45 ^{aefg}	3.00±0.20 ^{defi}	0.50±0.19	0.53±0.07 ^{aef}	2.03±0.15 ^{cd}	2.50±0.17 ^{de}	4.20±0.92 ^{dfi}

Values are means ± SD, of triplicate determinations

Values in the same column with different superscripts are significantly different at $P < .05$

Table 3. Effect of ethanol extract of the fruiting bodies of *Pleurotus ostreatus* on the serum lipid profile of HS-HFD-Streptozotocin-Induced diabetic rats after 6 weeks of treatment

Treatment group	Concentration(mmol/L)						
	Triglyceride	Total Cholesterol	HDL Cholesterol	VLDL Cholesterol	LDL Cholesterol	Non-HDL Cholesterol	LDL:HDL ratio
N	0.83±0.06	2.17±0.06 ^a	0.43±0.06 ^{ac}	0.38±0.03	1.27±0.06 ^a	1.73±0.06 ^a	2.93±0.31
D	0.93±0.06	3.07±0.06 ^{bt}	0.53±0.06 ^{ac}	0.42±0.02	2.07±0.15 ^{be}	2.53±0.12 ^{be}	3.93±0.64
D + POE ₅₀	1.03±0.15	2.90±0.10 ^{cf}	0.57±0.15 ^{ac}	0.47±0.07	1.87±0.06 ^{cef}	2.33±0.12 ^{cef}	3.50±1.06
D + POE ₁₅₀	0.93±0.21	2.33±0.21 ^{ag}	0.60±0.10 ^{ac}	0.42±0.09	1.30±0.00 ^a	1.73±0.12 ^a	2.20±0.40
D + POE ₃₀₀	0.83±0.15	2.73±0.47 ^{dfg}	0.77±0.06 ^{bc}	0.38±0.07	1.57±0.35 ^{afg}	1.97±0.42 ^{afg}	2.00±0.30
D + MET ₁₅₀	0.93±0.15	2.83±0.21 ^{et}	0.60±0.10 ^{ac}	0.42±0.07	1.77±0.23 ^{def}	2.23±0.29 ^{def}	3.00±0.85

Values are means ± SD, of triplicate determinations

Values in the same column with different superscripts are significantly different at $P < .05$

Table 4. Effect of ethanol extract of the fruiting bodies of *Pleurotus ostreatus* on the serum lipid profile of HS-HFD-Streptozotocin-Induced diabetic rats after 9 weeks of treatment

Treatment group	Concentration(mmol/L)						
	Triglyceride	Total Cholesterol	HDL Cholesterol	VLDL Cholesterol	LDL Cholesterol	Non-HDL Cholesterol	LDL:HDL ratio
N	1.03±0.06 ^a	2.03±0.06 ^a	0.57±0.58 ^a	0.47±0.03 ^a	0.90±0.00 ^a	1.47±0.12 ^a	1.60±0.17 ^a
D	0.83±0.06 ^{be}	2.83±06 ^{be}	0.53±0.58 ^a	0.38±0.03 ^{be}	1.87±0.06 ^{eb}	2.30±0.10 ^{bd}	3.53±0.46 ^b
D + POE ₅₀	1.13±0.06 ^a	3.07±0.12 ^{ce}	0.77±0.58 ^{bd}	0.52±0.03 ^a	1.73±0.15 ^{ec}	2.30±0.10 ^{bd}	2.23±0.12 ^{ad}
D + POE ₁₅₀	0.87±0.12 ^{ce}	2.40±0.10 ^{df}	0.70±0.00 ^{acd}	0.39±0.05 ^{ce}	1.27±0.6 ^{fd}	1.70±0.10 ^a	1.83±0.12 ^{ae}
D + POE ₃₀₀	1.10±0.10 ^a	2.07±0.12 ^a	0.47±0.12 ^a	0.50±0.05 ^a	1.07±0.21 ^{la}	1.60±0.20 ^a	2.40±0.85 ^{cdet}
D + MET ₁₅₀	0.80±0.10 ^{de}	2.17±0.29 ^{af}	0.57±0.12 ^a	0.36±0.05 ^{de}	0.97±0.15 ^a	1.60±0.17 ^a	1.73±0.41 ^{af}

Values are means ± SD , of triplicate determinations

Values in the same column with different superscripts are significantly different at $P < .05$

Table 5. Effect of ethanol extract of the fruiting bodies of *Pleurotostreatus* on the atherogenic indices of HS-HFD-Streptozotocin-Induced rats after 3 weeks of treatment

Treatment	Magnitude		
	Cardiac risk Ratio (CRR)	Atherogenic coefficient (AC)	Atherogenic index (AI)
N	4.70±0.56 ^a	3.70 ±0.56 ^a	0.18±0.03 ^d
D	4.90±0.46 ^a	3.90±0.46 ^a	0.35±0.05 ^a
D+POE ₅₀	4.43±0.93 ^a	3.43±0.93 ^a	0.45±0.19 ^b
D+POE ₁₅₀	3.77±0.21 ^a	2.77±0.21 ^a	0.18±0.06 ^d
D+POE ₃₀₀	4.90±2.25 ^a	3.90±2.25 ^a	0.34±0.12 ^a
D+MET ₁₅₀	6.13±1.03 ^b	5.13±1.03 ^b	0.37±0.06 ^c

Values are means ± SD, of triplicate determinations. Values in the same column with different superscripts are significantly different at P<.05

Table 6. Effect of ethanol extract of the fruiting bodies of *Pleurotostreatus* on the atherogenic indices of HS-HFD-Streptozotocin-Induced diabetic rats after 6 weeks of treatment

Treatment	Magnitude		
	Cardiac risk Ratio (CRR)	Atherogenic coefficient (AC)	Atherogenic index (AI)
N	5.07±0.59 ^a	4.07±0.59 ^a	0.29±0.02 ^a
D	5.80±0.69 ^{ac}	4.80±0.69 ^a	0.28±0.06 ^a
D+POE ₅₀	5.37±1.48 ^a	4.37±1.48 ^a	0.27±0.19 ^a
D+POE ₁₅₀	3.93±0.31 ^{ade}	2.93±0.31 ^c	0.19±0.04 ^{ac}
D+POE ₃₀₀	3.53±0.40 ^{bef}	2.53±0.40 ^c	0.03±0.05 ^{bc}
D+MET ₁₅₀	4.83±1.06 ^{at}	8.40±3.15 ^b	0.19±0.14 ^{ac}

Values are means ± SD, of triplicate determinations
Values in the same column with different superscripts are significantly different at P<.05

Table 7. Effect of ethanol extract of the fruiting bodies of *Pleurotostreatus* on the atherogenic indices of HS-HFD-Streptozotocin-Induced diabetic rats after nine weeks of treatment

Treatment	Magnitude		
	Cardiac risk Ratio (CRR)	Atherogenic coefficient (AC)	Atherogenic index (AI)
N	3.60±0.52 ^a	2.60±0.52 ^a	0.26±0.07 ^a
D	5.37±0.59 ^{bc}	4.37±0.59 ^{bc}	0.19±0.07 ^{ad}
D+POE ₅₀	4.03±0.25 ^a	3.03±0.25 ^a	0.17±0.05 ^{ad}
D+POE ₁₅₀	3.43±0.15 ^a	2.43±.15 ^a	0.09±0.05 ^{bd}
D+POE ₃₀₀	4.60±1.15 ^{ac}	3.60±1.15 ^{ac}	0.38±0.11 ^{ac}
D+MET ₁₅₀	3.87±0.23 ^a	2.87±0.23 ^c	0.16±0.10 ^{ad}

Values are means ± SD, of triplicate determinations
Values in the same column with different superscripts are significantly different at P<.05

4. DISCUSSION AND CONCLUSION

High levels of lipids in the blood brings about high levels of glucose in the blood of diabetic patients and the features of this condition include increase in TG, LDL, TG, VLDL and fall in HDL. High concentration of TG in the plasma can be an independent factor for cardiovascular diseases. It can as well combine action with other risk factors [23] and this factor is often linked with high blood pressure [6], obesity,

insulin insensitivity, diabetes mellitus and abnormal lipid metabolism. POE extract that is administered at 100mg/kg and 300mg/kg respectively, significantly lowered the TG level of the diabetic rats after 6 and 9 weeks of administration. [24] also reported that *Pleurotus ostreatus* extract lowered serum TG and TC in hyperlipidemic rats and increased HDL-C levels. According to [25], a post- treatment of diabetic mice with *Pleurotus ostreatus* extract reduced serum TG, LDL-C, total cholesterol and

significantly increased serum HDL-cholesterol. [26] indicated that high plasma levels of VLDL-cholesterol is a major risk factor for cardiovascular disease which often go with obesity [27] as well as diabetes mellitus. POE extracts at 300mg/kg and 150mg/kg doses respectively reduced the VLDL-cholesterol of treated group compared to the control after 3 weeks of treatment in this study. At the end of the 9 weeks of treatment, 300mg/kg POE reduced the cholesterol level of diabetic rats by 26.1% . The hypolipidemic effect of *Pleurotus ostreatus* may be attributed to the presence of steroids detected during the preliminary mycochemical analysis. Mushrooms are known to be source of steroids called ergosterol found in their cell membrane and are a precursor of vitamin D₂. Phytosterol have been revealed clinically to block cholesterol absorption sites in the intestine of humans. By this way, they help to lower cholesterol concentration. The American Heart Association has recommended that people diagnosed with high concentration of cholesterol in their blood should take plant sterols as a supplement.

[28] also highlighted that dried fruiting bodies of oyster mushroom at 5% level, added to diets rich in cholesterol effectively lowered the accumulation of cholesterol in the liver and plasma of experimental rats. According to [28],cholesterol was redistributed to favor HDL-cholesterol while LDL-cholesterol, VLDL-cholesterol and TC production were lowered, then the rate of absorption of cholesterol as well as the activity of HMG-CoA-reductase in the liver were drastically reduced by the macrofungi. The extracts of these mushrooms may therefore be recommended as natural cholesterol reducing agents in the human diet.

Clinical data has revealed that when plasma HDL-cholesterol concentration increases, the risks associated with the heart and its blood vessels reduces [29]. HDL-cholesterol mediates its protective effect by reducing the rate at which cholesterol enters the cell through LDL-cholesterol and increasing the rate at which cholesterol is released from the cell. It may also block the oxidation of LDL as well as the atherogenic influence of oxidized LDL .This may be because it has anti-oxidative [20,26] and anti-inflammatory features [26]. In this study, it was observed that HDL-cholesterol level was higher than the value of the diabetic group after 6 weeks of administration of extract at 300mg/kg and after 9 weeks administration of extract at 150mg/kg. HDL cholesterol concentration increased by

22.2% after 6 weeks of extract administration at 300mg/kg and by 16.7% and 28.3% respectively after 9 weeks of extract administration at 150mg/kg and 50mg/kg respectively, indicating that the POE extract has the capacity to reduce cardiovascular diseases.

At all the doses of extract administered for treatment at 3 weeks, 6 weeks and 9 weeks intervals, there was dose and time dependent lowering of LDL: HDL ratio even below the recommended risk limit of ≤ 2.5 compared to the test control with value above 2.5.A value of LDL: HDL ratio ≥ 2.5 Portend a risk of heart disease. According to [30] low LDL: HDL ratio and low atherogenic indices are protective against heart disease within the blood vessels of the heart. After 3 weeks of treatment with extract at administration concentration level of 150mg/kg, atherogenic indices were lowered and extract levels of 150mg/kg and 300mg/kg reduced atherogenic index value after 6 weeks of administration while after 9 weeks of treatment, extract at 150mg/kg concentration reduced atherogenic indices.

The atherogenic indices of the diabetic animals under treatment were dose-and time dependently reduced by POE treatment as observed in this study. Atherogenic indices have been revealed as powerful indicators of the risk of heart disease. When the value is high there is a higher risk of developing CVD but when the value is low the risk decreases [2]. It can therefore be inferred that treatment of diabetics with *Pleurotus ostreatus* extracts may, reduce the level of bad cholesterol in their blood in a dose and time dependent manner.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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