



Effects of Dietary Supplement Oyster Mushroom on Lipid Profile, Liver Function, Serum Electrolytes and Kidney Functions of Hyperlipidemic Wistar Rats

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Abstract

Mushrooms have been widely used for nutritional and medicinal purposes, but the possible regulation of serum electrolytes concentration and amelioration of kidney functions as well as lipid profile and liver function enzymes by oyster mushroom (*Pleurotus ostreatus*) has not been verified. Twenty adult male Wistar rats were used for this study and were randomly distributed into five (1-5) groups. Hyperlipidemia was induced in groups 1-4 with prednisolone (0.1ml/200g b.wt of rats) orally while group 5 animals served as positive control. This was done repeatedly for 7 days. Then, the rats in Group 1 and 3 received dried oyster mushroom (20g and 30g) respectively per day and rat chow with water, Group 2 received pioglitazone, (0.1ml/200g), while Group 4 and 5 received rat feed for 4 weeks. At the end of the experiment, blood samples and tissues were harvested for the determination of lipid profile, serum electrolytes, kidney function test and liver function enzymes by standard methods. From the result obtained, there were non-significant ($P>0.05$) decreases in the levels of serum creatinine, urea and uric acid. However, serum levels of AST of groups 1 and 3 were significantly reduced ($p<0.05$) when compared to the negative control. There were also non-significant reduction in the lipid profiles of the *Pleurotus ostreatus* (group 1 and 3) and pioglitazone (group 2) treated groups compared to the negative control. The present study suggests that dietary supplement *P. ostreatus* provides health benefits by positively modulating serum electrolytes imbalance, lipid profile, and liver and kidney functions of Wistar rats.

Keywords: Oyster Mushroom, Liver function, Kidney function, lipid profile and serum electrolytes

INTRODUCTION

Since ancient times, mushrooms have been used as a taste enhancer, shrub, flavouring agent as well as condiments and ingredients. However, edible mushrooms have much more to offer than these. They have immense health benefits due to their content of phytochemicals, vitamins, minerals, primary and secondary metabolites as

well as other bioactive compounds some of which have proven antioxidant, anti-inflammatory, antidiabetic and antihypertensive potentials etc. and as such, mushrooms are considered a functional food (Chorváthová et al., 1993; Freeney et al., 2014; Knop et al., 2015).

Mushroom contains a wide variety of bioactive molecules including terpenoids, phenols and steroids (Ma et al., 2013).

Several important compounds including polysaccharides, ergosterol, alpha-tocopherol, beta-carotene, lovastatin and unspecified bioactive properties (Bindhu et al., 2013) have been isolated from mushrooms.

The oyster mushroom, *Pleurotus ostreatus* is an edible fungus of the genus *pleurotus*. It is one of the widely cultivated mushrooms in the world and it is also the most consumed (Valverde et al., 2019).

This is because of its nutritional contents and medicinal properties in addition its taste and flavour. Oyster mushroom has low caloric value; low sodium/potassium ratio; low starch and fat value hence, can be consumed by patients with hyperlipidemia, high blood pressure and diabetes (Alam et al., 2011; Abdulazim et al., 2013).

P.ostreatus deserves special attention because of its remarkable ability to break down lignocellulose and other lignin contents of organic wastes and residues and because of this, it is about the fastest growing edible mushrooms (Sánchez, 2010). It also contains B-glucans, a highly viscous dietary fibre that gives bulkiness to food particles as well as reduces transit time in the GIT. Because of this, B-glucan has been researched for its potentials to prevent dyslipidemia, hypertension, obesity and other cardiovascular diseases (Oloke, 2017).

It has recently been found that *P. ostreatus* also contain mevinolin, a statin, which are group of drugs which are known to reduce cholesterol biosynthesis by inhibiting beta hydroxyl beta Meta glutaryl coA, (HMG-CoA), an enzyme that catalyses the committed step in cholesterol biosynthesis. These show that *P.Ostreatus* may have cardio protective and hepato-protective potentials. (Piskov et al., 2020; Lavelli et al., 2018)

Despite these myriads health benefits derivable from oyster mushroom, information on its cardio-protective and hepato-protective effects as well as its ability to modulate kidney functions is scarce. These are emerging health issues that constitute a major health and public health concern. Therefore, this study is poised to investigate the effects of oyster mushroom on lipid profile, liver function enzymes, kidney functions as well as serum electrolytes in pioglitazone induced hyperlipidemia in Wistar rats.

MATERIALS AND METHODS

Study Location

Department of Biochemistry laboratory, Nasarawa State University and Innovative Biotechnology Ltd. keffi, Nasarawa state, Nigeria.

Reagents/Chemicals

Prednisolone tablet (Laborate pharmaceutical Limited, India), Pioglitazone (Micro LABS Limited, India)

Plant material (sample collection)

Oyster mushroom (*Pleurotus ostreatus*) was purchased from vegetable market, museum street, Jos Plateau state Nigeria. The plants were authenticated by a Taxonomist at the Botany Department, Nasarawa state University, Keffi. They were thoroughly cleaned and shade dried for six days. After which, they were ground to powder with a blender. The dried ground mushroom was then transferred into an air tight container for storage prior to commencement of the experiment.

Experimental Animal

Male Wistar rats ((105-230g) were bought from National Veterinary Research Institute (NVRI) Vom, Plateau State. They were housed in a well-lit, ventilated, fine wooden cage at the Laboratory of the Department of Biochemistry and molecular biology, Nasarawa State University, Keffi. They were allowed access to clean water and feed (Vital Feeds, Browsers Finisher, Nigeria Limited. Jos) ad libitum and then allowed to acclimatize to laboratory conditions for two weeks prior to commencement of study.

Experimental Design

Overall, 20 male Wistar rats were used. The animals were randomly distributed into five groups of four animals. Hyperlipidemia was induced in groups 1-4 with prednisolone (0.1ml/200g b.wt of rats) orally. Equal volume of normal saline was injected to control rats (Group 5). All rats were observed for about two hours and thirty minutes before feeding. This was done repeatedly for seven days (a week). The rats in group 1 and 3 received dried oyster mushroom (10g and 30g/day respectively) and rat chow with water, group 2 received pioglitazone (0.1ml/200g b.wt of rats), a standard anti-lipidemic drug while group 4 (negative control), and group 5 (positive control) were given normal saline and rat chow for four weeks.

Biochemical investigation

At the end of the dosing period, the animals were sacrificed; incision was made into the animal's cervical region with the aid of a sterile blade. Blood and tissue samples were collected into plain bottles from all the rats under ether induced anaesthesia for enzyme and biochemical analysis.

Determination of enzymatic liver function parameters:

Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities were assayed using the method of Reitman and Frankel (Reitman S et al., 1957) as described in Randox kits while Alkaline Phosphatase (ALP) activities were assayed based on the methods of Kind and King (Kind PRN et al., 1954).

Determination of lipid Profile:

Triacylglycerols, Total cholesterol and HDL-cholesterol concentration were determined according to the method of Trinder in plasma were enzymatically using commercially available test kits (Boehringer, Mannheim, Germany) (Tinder et al., 1952). LDL-cholesterol was calculated by the Friedewald formula:

LDL cholesterol = total cholesterol – HDLcholesterol – triglyceride/5 (Tietz, 1986).

Determination of kidney function indices: Total bilirubin and direct bilirubin were determined according to the method of Jandrasik and Garber (1981). Total protein concentration was determined by Biuret method (Umemoto, 1966) while albumin concentration was determined through their binding method as reported by Spencer and Price (Spencer K et al., 1977). Urea concentration was determined by the diacetyl monoxime method using assay kit from Randox laboratories UK while creatinine concentration was determined by Picrate method (Tietz et al., 1986).

Determination of serum electrolyte: Sodium and potassium concentration were determined using reagent kit (Garber 1981; Tietz et al., 1986). Sodium bicarbonate concentration was determined trimetrically while mercuric nitrate method was used to determine the concentration of chloride (Schales et al., 1969).

Statistical analysis

The experimental results were expressed as mean \pm SD. Intergroup differences were analysed by a one way analysis of variance followed by Duncan's new multiple range tests. The SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used for the analysis. Values of $p < 0.05$ was considered statistically significant.

RESULTS

(Table 1) shows the effects of dietary supplement of *Pleurotus ostreatus* on liver function enzymes. The results show that dietary supplement of *Pleurotus ostreatus* significantly ($P < 0.05$) reduced the levels of Aspartate transaminase (AST) in the treated groups (1 and 3) compared to the untreated

group 4. There were non-significant reductions in the activities of Alanine transaminase ALT, Alkaline phosphatase ALP, and levels of total bilirubin T-bil and Albumin in the treated groups compared to the negative control. The levels of these enzymes and molecules were previously increased by prednisolone induced hyperlipidaemia.

All values are expressed as mean \pm standard division ($n=4$.)

Reference value: $p < 0.05$ was considered statistically significant

A: was considered statistically significant ($P < 0.05$) when compared with the negative control (group 4)

B: was considered statistically non-significant ($P > 0.05$) when compared with the negative control (group 4). M = Oyster mushroom), (P= Prednisolone), (PZ= Pioglitazone) and (F=Rat chow).

(Table 2) Shows the effects of dietary supplement of *Pleurotus ostreatus* on lipid profile of hyperlipidaemic wistar rats. The result shows a non-significant reduction in the levels of triglycerides, TG, Total cholesterol TC, High density lipoproteins HDL, Low density lipoproteins and very low Density lipoproteins VLDL in the treated groups compared to the negative control (group 4).

All values are expressed as mean \pm standard division ($n=4$.)

Reference value: $p < 0.05$ was considered statistically significant;

B: was considered statistically non-significant when compared with control (group 4);

(M = Oyster mushroom), (P= Prednisolone), (PZ= Pioglitazone) and (F=Rat chow)

(Table 3) shows the effects of dietary supplementation of

Table 1. Effects of *Pleurotus ostreatus* on liver enzymes.

Parameters	Group 1 (10g M+P)	Group 2 (F+PZ)	Group 3 (30g M+P)	Group 4 (F+P)	Group 5 (F)
AST (μ /L)	23.05 \pm 5.47a	40.77 \pm 19.26a	44.07 \pm 22.35a	71.7 \pm 17.65b	55.23 \pm 10.87b
ALT (μ /L)	20.58 \pm 7.27b	26.17 \pm 18.13b	23.60 \pm 13.55b	31.33 \pm 20.93b	34.30 \pm 10.96b
ALP(μ /L)	88.25 \pm 87.44b	87.33 \pm 45.39b	90.00 \pm 32.60b	189 \pm 73.01b	124 \pm 111.39b
T.BiL(μ mol/L)	1.70 \pm 1.01b	3.97 \pm 4.19b	1.13 \pm 0.06b	2.13 \pm 0.73b	1.66 \pm 1.62b
ALB (g/Dl)	1.80 \pm 0.63b	1.90 \pm 1.31b	1.63 \pm 0.29b	2.37 \pm 0.50b	1.47 \pm 0.55b

Table 2. Effects of *Pleurotus ostreatus* on lipid profile.

Parameters	Group 1 (10g M+P)	Group 2 (F+PZ)	Group 3 (20g M+P)	Group 4 (F+P)	Group 5 (F)
T.CHO (mmol/L)	1.45 \pm 0.27b	1.45 \pm 0.69b	1.24 \pm 0.08b	2.00 \pm 0.60b	1.41 \pm 0.39b
TG (mmol/L)	0.98 \pm 0.39b	0.91 \pm 0.35b	0.69 \pm 0.27b	1.71 \pm 0.37b	0.66 \pm 0.17b
HDL (mmol/L)	0.28 \pm 0.06b	0.24 \pm 0.33b	0.23 \pm 0.02b	0.38 \pm 0.13b	0.32 \pm 0.05b
LDL (mmol/L)	1.06 \pm 0.39b	0.80 \pm 0.61b	0.70 \pm 0.09b	1.37 \pm 0.57b	0.80 \pm 0.34b
VLDL (mmol/L)	0.45 \pm 0.18b	0.42 \pm 0.1	0.32 \pm 0.13b	1.32 \pm 0.17b	0.30 \pm 0.08b

Table 3. Effects of *Pleurotus ostreatus* on serum electrolytes.

Groups	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ₃ ⁻ (mmol/L)
1	130 ± 26.77 a	5.68 ± 0.93a	94 ± 11.16a	20.5 ± 3.41a
2	144.6 ± 2.51a	7.6 ± 0.34b	121.3 ± 11.50a	25.0 ± 3.60a
3	125 ± 7.54a	5.17 ± 1.23a	92 ± 20a	21.66 ± 0.57a
4	150.6 ± 7.57b	9.63 ± 1.75b	139.3 ± 8.50b	40.33 ± 4.72b
5	139 ± 15.62b	7.83 ± 2.25b	100 ± 40b	24.66 ± 1.15b

Table 4. Effects of *Pleurotus ostreatus* on serum kidney function test.

Group	Urea (mmol/L)	Creatinine(μmol/L)	Uric acid(mg/dl)
1	13.02 ± 3.261b	50.32 ± 6.061b	4.6 ± 0.5099b
2	12.27 ± 3.021b	51.9 ± 2.930 b	5.0 ± 2.821b
3	10.68 ± 1.633b	59.9 ± 17.20 b	4.9 ± 0.8165b
4	14.05 ± 2.982b	71.46 ± 25.97b	7.8 ± 2.910 b
5	13.52 ± 3.988b	52.13 ± 17.52b	5.8 ± 1.769 b

Pleurotus ostreatus on serum electrolytes of hyperlipidemic rats. The result obtained shows that *Pleurotus ostreatus* significantly reduced the levels of serum electrolytes, sodium, potassium, chloride and bicarbonate in groups (1 and 3) that was previously increased by prednisolone induced hyperlipidemia I wistar rats when compared with negative control (group 4).

All values are expressed as mean ± standard deviation (n=4,)

Reference value: p<0.05 was considered statistically significant

A: was considered statistically significant (P<0.05) when compared with the negative control (group 4) b: was considered statistically non-significant (P>0.05) when compared with the negative control (group 4). M = Oyster mushroom), (P= Prednisolone), (PZ= Pioglitazone) and (F=Rat chow)

(**Table 4**) shows the effects of dietary supplementation of *Pleurotus ostreatus* on serum kidney functions of hyperlipidemic rats. The result shows that *Pleurotus ostreatus* supplementation caused a non-significant reduction in the levels of urea, creatinine and uric acids of the treated animals when compared to the negative control.

Values were expressed in mean ± standard deviation (n=4)

Reference value: p<0.05 was considered statistically significant;

B: was considered statistically non-significant when compared with control (group 4);

(M = Oyster mushroom), (P= Prednisolone), (PZ= Pioglitazone) and (F=Rat chow)

DISCUSSION

Cardiovascular diseases CVD, a spectrum of diseases that

affects among other cells and tissues the lymphatic system, is the leading cause of mortality in all human populations (Mc Namara et al., 2019). And recently, the incidence of disorders of metabolism and metabolic syndrome such as hyperlipidemia and obesity constitute a major unmet health needs and public health concern (Hruby et al., 2016).

Disorders of metabolism results from breakdown of metabolic processes. This often leads to deficiency of certain metabolic products and accumulation of certain other metabolites some of which constitute toxicological indices in disorders of metabolism.

Decades of research into the etiology of cardiovascular and metabolic syndrome had led to the development of drugs that have been the main-stay of therapeutic intervention of these disorders. But these drugs have side effects which are undesirable. This is why the quest for more effective pharmaceutical intervention with lesser effects is on the increase. Scientists have recently focused their attention on mushrooms for its numerous health benefits and potentials.

Pleurotus Ostreatus is one of the widely cultivated and most consumed species of mushroom. It has been reported to have myriad health potentials and therapeutic uses.

This research was designed to investigate the effects of *Pleurotus ostreatus* on lipid profile, liver enzymes, serum electrolytes and kidney functions in prednisolone induced hyperlipidemia in male wistar rats.

Hyperlipidemia is a spectrum of disorders that is characterized by abnormal high levels of lipids in the body tissues. Other words commonly used in lieu of hyperlipidemia are hypercholesterolemia, dyslipidemia etc (Wang F et al., 2015) Hyperlipidemia has been implicated in the etio-pathogenesis of cardiovascular diseases and related disorders such as peripheral vascular diseases, Ischemic and atherosclerosis and other coronary heart diseases. It is the leading cause of death in human populations. (Minicozzi et al., 2021)

These spectrum of disorders also induces myriad other physiological disturbances in the body, which complicates the hyperlipidemic state and may eventually lead to death if left untreated.

Some of these include altered liver function enzymes, impaired kidney functions and altered electrolyte balance. (Kundu et al., 2012). A potent anti-hyperlipidemic agent

should be able to positively modulate these parameters and return it to physiological levels in healthy state.

This research was designed to investigate the effects of *Pleurotus ostreatus* as a potential anti-hyperlipidemic agent on lipid profile, liver enzymes, serum electrolytes and kidney functions in prednisolone induced hyperlipidemia in male wistar rats. 25 animals were randomly divided into 5 groups of five animals each. Hyperlipidaemia was induced in groups 1-4 by prednisolone while group 5 served as a positive control. Group 1 and 3 animals received 10 and 20mg/Kg body weight of *Pleurotus Ostreatus* respectively before induction of hyperlipidemia. While group 2 animals received pioglitazone, a standard anti-hyperlipidemic drug and group 4 animals received normal rat chow.

Hyperlipidemia induces free radical, which have damaging effects on the membranes of the liver and as a result causes liver enzymes to leak into the serum. The level of these enzymes in the serum is an indication of high oxidative load or stress. (Singh et al., 2015). Levels of these liver enzymes AST, ALT, ALP, T.BIL and ALB have been used for decades to assess oxidative status in vivo. From the result obtained, Prednisolone successfully induced hyperlipidemia in groups 1-4 as evident in the increased levels of AST, ALT, ALP, TBIL and ALB of the test groups (1-4) compared to the control. However, administration of graded dosage of *pleurotus Ostreatus* (10&20mg) significantly reduced the levels of these enzymes and brought it closer to the levels observed for the positive control (group 5) and the pioglitazone (standard drug treated group). This is because *Pleurotus Ostreatus* contains antioxidant phytochemicals and other bioactive compounds such as phenolic components, flavonoids, terpenes etc (Rahimah et al., 2019) Some of which have been reported to have immense antioxidant potentials which can counter some of the free- radicals induced by hyperglycemia. (Waktola et al., 2020). Potent markers of liver damage were markedly and significantly ($P<0.05$) reduced by *Pleurotus Ostreatus* intake compared to the pioglitazone and the positive control.

(Table 2) Shows the effects of administration of oyster mushroom on lipid profiles of hyperlipidemic rats. From the result obtained, levels of T.CHOL, TG, LDL, and VLDL were markedly reduced in the oyster mushroom treated groups 1 and 3 compared to the control. High levels of T.chol, LDL and VLDL are indicative of hypercholesterolemia and its attendant adverse consequences. (Barret et al., 2019). High levels of these lipids profiles have been implicated in the hyperlipidemia and related diseases of lipid metabolism and metabolic syndrome (Feingold, 2012). Bad cholesterol is a commonly used term to depict high levels of LDL and VLDL. Because they are lipoproteins that are responsible for reverse transport of cholesterol and cholesterol esters from the liver into the cell and tissues where their accumulation and oxidation to lipid plaques can cause atherosclerosis and other diseases in the CVD spectrum. However, the presence of mevinolin in oyster mushroom makes it a good therapeutic

agent for the reversal of dyslipidemia in hyperlipidemia and related CVD. Mevanolin is a lovastatin which belongs to the statins. Statins are inhibitors of 3 hydroxyl, 3 methyl glutaryl coA (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, the rate limiting step of Cholesterol biosynthesis. Reduced levels of cholesterol and cholesterol esters is the goal of most hyperlipidemia regimen and intervention.

Altered and abnormally high levels of serum electrolytes is an indicator of diseased state and conditions. (Lamis et al., 2013). And a good therapeutic intervention would seek to ameliorate and reduce the levels by bringing it closer to physiological ranges in healthy subjects. Results obtained in table 3 shows that oyster mushroom significantly ($P<0.05$) reduced the levels of Na^+ , K^+ , Cl^- and HCO_3^- compared to the control group. High levels of Na^+ . High levels of Na^+ (Hypernatemia), K^+ (Hyperkalaemia), Cl^- and HCO_3^- are indicators of a diseased state and have been reported in diseases such as diabetes mellitus, diuretics, Chronic kidney disease and diuretics. (Lytvyn, et al., 2019),(Fried et al., 2017). Oyster mushroom contains phytochemicals such as flavonoids, and other bioactive substances that can reverse high levels of serum electrolytes and improve the disease conditions implied.

The Kidney is a vital organ in the body of human and it is used for filtration, screening of body fluids, absorption of vital minerals, ions as well as excretion of waste from the body. It excretes urea, Creatinine, uric acid and other waste from the body. However, impairment of kidney function during disease conditions leads to high levels of kidney function parameters and this has been used in the assessment of kidney health for decades (Kellum et al., 2021). Impaired renal function is a feature of most debilitating and life threatening disease conditions such as hyperlipidemia and other disorders of metabolism diseases.

High levels of these parameters such as urea, Creatinine and uric acid are indicative of a diseased state. However, Oyster Mushroom reversed the levels of these parameters and as such can be used in the treatment of diseases with impaired kidney functions.

Reduction in plasma potassium, sodium, and chloride concentrations through the effect of dried *Pleurotus ostreatus* as dietary supplement on serum electrolytes is related to one of the mechanisms of action of antihypertensive drugs, particularly diuretics (Jude et al., 2010). Diuretics act by diminishing sodium chloride re-absorption at different sites in the nephrons, thereby increasing urinary sodium chloride and water losses, consequently leading to decreased plasma levels of these electrolytes (Antonov et al., 1997) reported that plasma electrolyte contents increased significantly in hypertensive rats. Impaired function of Na^+ , K^+ -ATPase and the Na^+ -H antiport, which is typical of arterial hypertension, may promote an increase in plasma electrolytes

CONCLUSION

From the foregoing, we realize the immense health benefits that oyster mushroom possess and as such we recommend that it should be incorporated into our foods for optimum use and benefits. However, further research is encouraged to decipher the mechanism of action of oyster mushroom

DECLARATIONS

Ethics approval

This research was approved by the Research and Ethics Committee of Nasarawa State University, Keffi, Nigeria.

Competing Interests

The Authors declared no competing interests.

Authors' Contributions

This work was carried out in collaboration between all authors. Author CCN designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author NRO Performed statistical analysis, managed the analyses of the study and wrote the final draft. Author IPE managed the literature searches. All authors read and approved the final manuscript.

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