ANTI-DYSLIPIDEMIC EFFECTS OF PLEUROTUSOSTREATUS (OYSTER MUSHROOM) IN DIET-INDUCED HYPERLIPIDEMIC MICE.

Olusegun S. Ajala*, Nwamaka O. Chukwumah, Ogechi N. Nzomiwu and Titilope A. Adelekan Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, CMUL Campus, PMB 12003, Idiaraba-Surulere, Lagos, Nigeria.

*Corresponding author: olusegunajala@yahoo.co.uk, olajala@unilag.edu.ng

ABSTRACT

Background: Only a few antidyslipidemic drugs are currently available in clinical practice for the prophylactic control of ischemic stroke and coronary heart diseases. Fungal secondary metabolism remains a veritable repertoire for the discovery of new ones. This study was aimed at evaluating a methanol crude extract of the fungus, Pleurotusostreatus (oyster mushroom), and its fractions for their potential anti-dyslipidemic activities in high fat diet-induced models of dyslipidemia.

Methods: A methanol extract of Pleurotusostratus, obtained by cold maceration, was subjected to phytochemical screenings and triturated in succession into dichloromethane and methanol to obtain non-polar and polar fractions respectively. Each of the extract and the fractions was subjected to antidyslipidemic assay in vivo in mice by measuring their effects on diet-induced hyperlipidemic mice using plasma Total Cholesterol (TC), Very Low Density Lipoprotein cholesterol (VLDLC), Low Density Lipoprotein cholesterol (LDLC), High Density Lipoprotein cholesterol (HDLC) and triglycerides (TG) concentration changes as biomarkers, 2% tween 20 and 25 mg/Kg artovastatin being negative (i.e., dyslipidemic) and positive controls respectively.

Results: A NOVA comparison of the test groups mean values of these biomarkers with those of the dyslipidemic control group showed that the crude extract significantly reduced plasma TC ($p < 0.01$), LDLC ($p < 0.0001$), TG and VLDLC ($p < 0.05$), and significantly increased HDLC ($p < 0.05$). In addition, the non-polar fraction significantly reduced plasma TG and VLDLC ($p < 0.01$) and significantly elevated HDLC ($p < 0.0001$) while the polar fraction had significant reduction effects on the TC and LDLC plasma concentrations ($p < 0.01$).
Conclusion: The methanol crude extract of Pleurotusostratusde monstrated antidyslipidemic activity, reversing all the biomarkers of dyslipidemia in diet-induced hyperlipidemic mice. The effect on the biomarkers appeared separated, though, with fractionation, the non-polar fraction reversing the TG, VLDLC and HDLC and the polar fraction the TC and LDLC parameters. Pleurotusoestratus could therefore be explored for the discovery of new anti-dyslipidemic drugs.

Key words: Oyster mushrooms, Pleurotusostratus, dyslipidemia, hyperlipidemia

1. INTRODUCTION

Dyslipidemia refers to abnormal blood lipid profile manifest as at least one of the following: elevated plasma total cholesterol (TC), elevated Low Density Lipoprotein cholesterol (LDLC), elevated triglycerides (TG) and reduced High density Lipoprotein cholesterol (HDLC)[1,2], each of which is a risk factor of ischemic stroke and coronary heart disease with its attendant cardiac arrest complications[3-5]. Given the severity and devastating effects of these aforementioned dyslipidemia-associated diseases, very cheap, effective and highly tolerable anti-dyslipidemic drugs are required for routine prophylactic control of these diseases. Current antidyslipidemic drugs are however expensive, associated with intolerable side effects and are not effective in all patients[6-7], making the discovery of new antidyslipidemic drugs an urgently required task in clinical medicine.

The discovery of the anti-hypercholesterolemic HMG-CoA reductase-inhibiting statins from fungal cultures[8,9] remains an impetus for further exploitation of the unique secondary

metabolism of the kingdom fungi for new and possibly more effective antidyslipidemic agents. Mushrooms (or macrofungi), most of which have diverse degrees of ethnomedicinal anti-dyslipidemic claims[10-13], are suitable fungal subjects for this exploitation.

An example of such mushrooms is the oyster mushroom, Pleurotusostreatus. It is a higher fungus used in traditional medicine for the control of a number human ailments including diseases like stroke wherein abnormal blood lipid profile is implicated[14,15]. Past investigations have established the mushroom to have antioxidant, anticancer/cytotoxic, anti-cataract and blood lipid lowering activities, to mention a few[16-18]. Most antidyslipidemic data on Pleurotusostratus, however, have not included its potential effects on plasma HDLC concentration, a low value of which is considered a form of dyslipidemia[19]. Moreover, available data are largely on extracts. Obtaining data on fractions should therefore be highly desirable as it could chart a course for the isolation of pure antidyslipidemic agents from the mushroom.

In the light of the above, we subjected a methanol extract of a sample of Peurotusostreatus and its polar and non-polar fractions to anti-dyslipidemic evaluation in which elevated TC, LDLC, TG and VLDL as well as reduced HDLC served as biomarkers.

2. MATERIALS AND METHODS

2.1. Materials and Experimental Animals

The Pleurotusostreatus sample evaluated was from the laboratory of Dr. Nwaneka Ofordile, Head of Department of Biological Sciences, Yaba College of Technology, Yaba-Lagos, Nigeria. An ACUREX bichromatic analyzer was used for the assay of plasma total cholesterol, HDLC and TG concentrations. TC and TG assay kits were from Erba diagnostics, Mannheim, GmbH, Germany, while HDLC assay kit was from Accurex Biomedicals Pvt. Ltd, Mumbai, India. All other chemicals and solvents were obtained from Sigma-Aldrich (Taufkirchen, Germany) and were of at least analytical standards. Albino mice weighing 11-20g were used for this study; they were obtained from the animal house of the College of Medicine of the University of Lagos, acclimatized to the experimental environment for 7 days prior to the commencement of the experiment. They were sustained on standard mice feed (Pfizer) and water ad libitum. All animal procedures as stipulated by the animal and ethics committee of the College of Medicine, University of Lagos were followed.

2.2. Extraction and fractionation

The Pleurotusostreatus sample was dried for 14 days at room temperature and pulverized. The pulverized mushroom (2kg) was extracted by cold maceration in methanol (3L) two consecutive times. Extracts were combined, filtered and evaporated to dryness in vacuo at 45°C to obtain dried extract (30g). Part of the dried extract (20g) was triturated with dichloromethane (DCM) to obtain DCM soluble (2g) and DCM-insoluble (17.8g) fractions, herein referred to as non-polar and polar fractions respectively Both fractions and the remaining crude extract were kept under refrigeration (at 4°C) until use.

2.3. Phytochemical Screenings

The crude extract was qualitatively screened for the presence or otherwise of tannins (hydrolysable and condensed), anthraquinones, glycosides, saponins, flavonoids and alkaloids, using standard procedureswith slight modifications, where necessary[20,21]:

2.4. Preparation of high-fat diet

A reported method[22] was used to prepare the high-fat diet. This simply involved increasing the mineral/vitamin and fat contents of a commercially available mice chow as shown in the table Table 1 below:

Table 1: Composition of normal and high fat diets (g/1000 g of diet)

Ingredients	Normal diet	High fat diet
Casein	0	1
Corn Starch	0	39
Margarine	0	29
Vitamin mixture[1]	0	20
Lard fat	0	600
Mice chow	1000	300
Salt mixture[2]	0	1
Cellulose	0	10

[1] Composition (mg/g of mixture): vitamin A-4000IU, vitamin B1-1mg, vitamin B2-0.4mg, vitamin B6-0.5mg, vitamin C- 25mg, vitamin D2-400IU, Nicotinamide - 5mg.

[2] Composition (g/Kg of mixture): NaCl - 139.3 / KI - 0.79 / $MgSO_4.7H_2O$ - 57.3 / $CaCO_3$ - 381.4 / $MnSO_4.H_2O$ - 4.01 /$FeSO_4.7H_2O$ - 27.0 / $ZnSO_4.7H_2O$ - 0.548 / $CuSO_4.5H_2O$ - 0.477/$CoCl_2.6H_2O$ - 0.023/KH_2PO_4 - 389.0[22].

2.5. Evaluation of anti-dyslipidemic effects of Pleurotusostreatus.

Forty-five female mice were randomly sorted into nine groups (groups 1-9) of five mice each. Dyslipidaemia was induced in rats in groups 2 – 8 by feeding them with a high fat diet for 14 days with concurrent daily oral 0.5 mg/Kg carbimazole, setting group 1 as control.

Each mice group was subjected to 21 days daily oral therapeutic regimen as follows: Groups 1 and 2 were treated with 10 ml/kg 2% tween 20 (vehicle) and set as normal and dyslipidemic controls respectively. Each of groups 3-9 was treated with one of two doses (200 mg/kg, 400 mg/kg) of the extract, two doses (100 mg/kg, 200 mg/kg) each of the fractions, and 25 mg/kg artovastatin. The mice were afterwards sacrificed under diethyl ether anesthesia. Blood samples were obtained from their orbital sinus into heparinized bottles and centrifuged at 3000 rpm for 30 mins. The clear non-haemolysed supernatant plasma were removed and stored at -20°C until use for biochemical analysis.

2.6. Biochemical Analysis

Total cholesterol (TC), HDL-cholesterol (HDLC) and triglycerides (TG) concentrations in each plasma sample collected were determined in colorimetric enzymatic assays[23,24]with commercial kits (Erba diagnostics, Mannheim, GmbH, Germany; Accurex Biomedicals Pvt. Ltd., Mumbai, India) in an Accurexbichromatic analyser, monitoring colour change-dependent concentrations at 500 nm and 600 nm wavelengths for the TC/triglycerides and HDL-C assays respectively.

Plasma LDL-C concentration was calculated using the formula: LDLC=TC-HDLC-(TG÷5)and VLDL-C concentration using the formula: VLDLC=TG÷5[25]

2.7. Data Analysis

Results of biological assays were expressed as Mean ± SEM. The mean of each of the dyslipidemic parameter for the normal control was compared in a t-test to that of the corresponding dyslipidemic control group to ensure that dyslipidaemia has been established in the experimental animals. The mean of each of the dyslipidemic parameter for the dyslipidemic control group was correspondingly compared to those of extract-, polar fraction-, nonpolar fraction- and standard drug-treated groups by analysis of variance (ANOVA) followed by Tukey's Multiple Comparison using Graphpad Prisms software, version 6 (Graphpad Software Inc. La Jolla, CA, USA). p value < 0.05 was considered significant

3. Results

Phytochemical screening revealed the presence of condensed tannins, cardiac glycosides, flavonoids and alkaloids in the methanol extract of *Pleurotusostratus.* (**Table 2**).

Table 2: Phytochemical screening results

Phytochemicals	Observation
Hydrolysable tannins	-
Condensed Tannins	+
Free Anthraquinones	-
Cardiac glycosides	-
Glycosylated anthraquinones	+
Saponins	+
Flavonoid	+
Alkaloids	+

+ = Present - = Absent

While the crude extract showed reversal capability on each of the dyslipidemic biomarkers, the fractions showed rather selective activities, the non-polar fraction showing more tendency for reversing the HDL-C and TGs parameters than the polar which shows more activity in reversing the TC, and LDL-C parameters. These are well-detailed in Table 3.

Table 3: Blood lipid profiles of nine experimental mice groups after a 21-day treatment with various doses of a crude methanol extract and fractions of P. oestratus.

Mice group	Mean plasma concentration(mg/dl) ± SEM				
	TGs	TC	LDL-C	HDL-C	VLDL-C
Group 1	147.2 ± 26.2	99.0 ± 9.2	37.9 ± 8.7	31.7 ± 5.4	29.4±5.2
Group 2	215.1±8.4**	183.0±5.3**	124.1±4.0**	15.9±0.3**	43.0±1.7**
Group 3	178.1±25.6	101.2±12.8[c]	35.2±11.9[d]	30.4±1.8[b]	35.6±5.1
Group 4	144.2±26.3[a]	81.9±13.8[d]	25.9±16.2[d]	27.0±2.8[b]	29.0±5.3[a]
Group 5	138.8±10.9[b]	165.0±10.1	95.0±6.6	42.2±4.5[c]	27.8.8±2.2[b]
Group 6	140.3±11.2[b]	156.6±6.3	91.5±5.9	37.0±0.3[c]	28.1±2.2[b]
Group 7	171.2±3.9	136.0±11.4[b]	78.0±8.9[b]	23.8±4.9	34.2±0.8
Group 8	170.4±14.4	134.3±13.9[b]	80.3±16.3[b]	19.9±4.2	34.08.0±2.9
Group 9	129.4±0.7[c]	104.3±2.9[d]	10.3±3.3[d]	68.1±1.1[d]	25.9±0.1[c]

Groups 1 and 2 are normal and dyslipidemic controls respectively; groups 3 and 4 are 200 mg/kg and 400 mg/kg extract-treated groups respectively; groups 5 and 6 are 100 mg/kg and 200 mg/kg non-polar fraction-treated groups respectively; groups 7 and 8 are 100 mg/kg and 200 mg/kg polar fraction-treated respectively while group 9 is 25 mg/kg artovastatin-treated.

** $p < 0.001$ compared to group 1; [a] $p < 0.05$ compared to group 2, [b] $p < 0.01$ compared to group 2, [c] $p < 0.001$ compared to group 2, [d] $p < 0.0001$ compared to group 2. (n = 5).

4. DISCUSSIONS

This investigation was primarily the evaluation of the effects of the extract and fractions of Pleurotusoestratus on high fat diet induced dyslipidemiain mice using elevated TGs, elevated VLDL-C, elevated TC, elevated LDL-C and reduced HDL-C plasma concentrations as biomarkers. Dyslipidemia is implied if at least one of these biomarkers is present. Table 3 shows that there is a highly significant difference between the normal and the dyslipidemic control groups for each of the biomarkers ($p < 0.001$). This implies a successful induction of dyslipidemia in the experimental animals and, to some extent, justifies the oral carbimmazole-induced hypothyroidism augmentation of the dyslipidemia-inducing hyperlipidemia, given the latter's reports on resistance[26].

Each of the extract and the fractions showed similar effects on the plasma TGs and VLDL-C concentrations. This is expected as the VLDL is largely made up of TGs[28]. There is a significant reduction in each of plasma TG and VLDL concentrations of the 400 mg/Kg extract-treated group compared to those of the dyslipidemic group ($p < 0.05$), showing that the crude extract significantly reduced plasma TGs and VLDL concentrations at this dose. In the same vein, the two doses (200 mg/Kg and 400 mg/Kg) of the extract significantly reduced TC and LDL-C concentrations ($p < 0.01$) and significantly increased HDL-C concentration ($p < 0.001$). These results, more or less, provide a scientific basis for the earlier mentioned folkloric use of the mushroom, Pleurotusoestratus, in the treatment of diseases wherein abnormal blood fat profile is implicated.

While the crude extract had significant reversal effects on the five biomarkers of dyslipidemia monitored, these effects seemed to be separated by fractionation into polar and non-polar fractions. The non-polar fraction showed significant TG/VLDL-C lowering effects ($p < 0.05$) compared with dyslipidemic control, and HDL-C elevation effects ($p < 0.01$) compared with dyslipidemic control. Elevated plasma HDL-C has been considered desirable on the recognition of the fact that HDL is the lipoprotein saddled with the responsibility of transport of cholesterol from the body tissues to the liver for ultimate destruction. Since the cholesterol in HDL is destruction-bound, it is termed good cholesterol[27].

The polar fraction demonstrated significant TC and LDL-C plasma concentration- lowering effects ($p < 0.01$ compared with dyslipidemic control). Though this implies that the TC and LDL-C lowering principles in the mushroom are essentially polar, the synergistic and/or potentiation effects of its non-polar principles can, nevertheless, be safely inferred, given the smaller p- values ($p < 0.0001$) the crude extract demonstrated for these activities compared to the polar fraction ($p < 0.001$).

5. CONCLUSION

This work has established that the oyster mushroom, Pleurotusostreatus, has the capability to reverse the major parameters of dyslipidemia including low HDL-C plasma concentration, thereby providing further scientific basis for the anti-dyslipidemic folkloric use of the mushroom and for the possibility of discovery of new anti-dyslipidemic drugs from it.

REFERENCES

1. Davidson M (2008). A review of the current status of the management of mixed dyslipidemia associated with diabetes mellitus and metabolic syndrome. The American Journal of Cardiology102(12);19L-27L.
2. Dhaliya SA, SuryaAS, DawnVT, Betty C, Arun K and Sunil C (2013). A review of hyperlipidemia and medicinal plants. International Journal of Applied Pharmaceutical Sciences and Biological Sciences2(4); 219-237.
3. Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, Culebras A, DeGraba TJ, Gorelick PB, Guyton JR, Hart RG (2006). Primary prevention of ischemic stroke: A

guideline from the American heart association/American stroke association stroke council: Cosponsored by the atherosclerotic peripheral vascular disease interdisciplinary working group; cardiovascular nursing council; clinical cardiology council; nutrition, physical activity, and metabolism council; and the quality of care and outcomes research interdisciplinary working group: The American academy of neurology affirms the value of this guideline. Stroke; A Journal of Cerebral Circulation37(6); 1583-1633.

4. Anyfantakis ZA, Baron G, Aubry P, Himbert D, Feldman LJ, Juliard JM, Ricard-Hibon A, Burnod A, Cokkinos DV and Steg PG (2009). Acute coronary angiographic findings in survivors of out-of-hospital cardiac arrest. American Heart Journal157(2); 312-318.
5. Tziomalos K, AthyrosVG, Karagiannis A and Mikhailidis DP(2009). Dyslipidemia as a risk factor for ischemic stroke. Current topics in medicinal chemistry9(14);1291-1297.
6. Pollex RL, Joy TR and Hegele RA (2008). Emerging AntidyslipidemicDrugs. Expert Opinion on Emerging Drugs13(2); 363-381.
7. Sashidhara KV, Kumar A, Kumar M, Srivastava A and Puri A (2010). Synthesis and antihyperlipidemic activity of novel coumarin bisindole derivatives. Bioorganic and Medicinal Chemistry Letters20(22); 6504-6507.
8. Stossel TP (2008). The discovery of statins. Cell134(6); 903-905.
9. Endo A (2010). A historical perspective on the discovery of statins. Proceedings of the Japan Academy, Series B86(5); 484-493.
10. Liang B, Guo Z, Xie F, Zhao A (2013). Antihyperglycemic and antihyperlipidemic activities of aqueous extract of Hericiumerinaceus in experimental diabetic rats. BMC Complementary and Alternative Medicine13(1); 253-260.
11. de Miranda AM, Ribeiro GM, Cunha AC, Silva LS, dos Santos RC, Pedrosa ML and Silva ME (2014). Hypolipidemic effect of the edible mushroom Agaricusblazei in rats subjected to a hypercholesterolemic diet. Journal of Physiology and Biochemistry70(1); 215-224.
12. Yadav S, Satapathy T, Roy Aand Prasad P (2014). Antihyperlipidemic potential of herbals. Journal of Applied Pharmaceutical Research2(1); 7-17.
13. ChoiD, Piao Y, Yu SJ, LeeYW, Lim DH, Chang YC, Park SS, Lee MK, ChaWS, You DS and Cho H (2016). Antihyperglycemic and antioxidant activities of polysaccharide produced from Pleurotus ferulae. Korean Journal of Chemical Engineering33(6);1872-1882.
14. BobekP and Galbavý Š (1999). Hypocholesterolemic and antiatherogenic effect of oyster mushroom (Pleurotusostreatus) in rabbits. Molecular Nutrition and Food Research43(5); 339-342.
15. Schneider I, Kressel G, Meyer A, Krings U, Berger RG and Hahn A (2011). Lipid lowering effects of oyster mushroom (Pleurotusostreatus) in humans. Journal of Functional Foods3(1);17-24.
16. GuYH, Sivam G (2006). Cytotoxic effect of oyster mushroom Pleurotusostreatus on human androgen-independent prostate cancer PC-3 cells. Journal of medicinal food9(2);196-204.
17. Kim JH, Kim SJ, Park HR, Choi JI, JuYC, Nam KC, Lee SC (2009). The different antioxidant and anticancer activities depending on the color of oyster mushrooms. Journal of Medicinal Plants Research3(12); 1016-1020.
18. Ganeshpurkar A, Bhadoriya SS, Pardhi P, Jain AP and Rai G (2011). In vitro prevention of cataract by Oyster Mushroom Pleurotusflorida extract on isolated goat eye lens. Indian journal of pharmacology43(6); 667-.670
19. Rosenson RS (2005). Low HDL-C: a secondary target of dyslipidemia therapy. The American Journal of Medicine118(10);1067-1077.
20. Trease GE and Evans WC (2002). Pharmacognosy. 15th Ed. London, Saunders Publishers, Pp. 42–44, 221-229.
21. Edeoga HO, Okwu DE, Mbaeble BO (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. African Journal of Biotechnology 4(7); 685-688.
22. Matos SL, Paula HD, Pedrosa ML, Santos RCD, Oliveira ELD, Chianca Júnior DAand Silva ME (2005). Dietary models for inducing hypercholesterolemia in rats. Brazilian Archives of Biology and Technology48(2); 203-209.
23. Bacolo G, David H(1973). Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry 19; 476-482.
24. Allain CC, PooLS, Chan CSG, Richmond W, Fu PC (1974). Enzymatic determination of total serum Cholesterol. Clinical Chemistry 20; 470-475.
25. Friedewald W T, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18(6); 499–502.
26. Joris I, Zand T, Nunnari JJ, Krolikwoski FJ, Majno G (1983). Studies on the pathogenesis of atherosclerosis I; adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. American Journal of Pathology 113; 341-358.
27. Rosenthal MD and Glew RH(2009). Medical Biochemistry, Human metabolism in health and disease. John Wiley, Hoboken, NJ. USA. Pp 246-270.