

The Ameliorating Effect of Chloroform and Aqueous Ethanol Extracts from *Pleurotus tuber reguim* (Fr.) Sing. (Pleurotaceae) sclerotia on Triton-X Induced Hyperlipidemia in Wistar Rats

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Abstract

This report validates the anti-hyperlipidemic property of *Pleurotus tuber reguim* an edible mushroom widely used for weight loss among other ailments, using Triton X-100 induced hyperlipidemic rats. The lipophilic chloroform (CHE) and hydrophilic 70 % aqueous ethanol (AQE) extracts were obtained by successive cold maceration in increasing order of solvent polarity. Anti-hyperlipidemic activities of the extracts at 100 and 200 mg/kg body weight, was evaluated using Triton X-100 induced hyperlipidemic rats. Atrovastatin was used as the standard drug. Compared to the hyperlipidemic untreated control group, there was a significant ($p < 0.05$) dose dependent reduction in the plasma total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL), low density lipoprotein (LDL), and increase in the high density lipoprotein (HDL) after 7 days treatment with the extracts. The trend in the ameliorative activities of the extracts and atrovastatin were in the following rank order: atrovastatin (43.5%) > CHE-200mg/kgbw (41.4%) > CHE-100mg/kgbw (38.4%) > AQE-200 mg/kgbw (35.9%) > AQE-100 mg/kgbw (27.6%) for TC; atrovastatin (73.5%) > CHE-200mg/kgbw (68.0%) > CHE-100mg/kgbw (64.5%) > AQE-200 mg/kgbw (62.3%) > AQE-100 mg/kgbw (62.2%) for TG; CHE-200mg/kgbw (114.4 %) > AQE-200mg/kgbw (87.3%) > atrovastatin (68.7%) > CHE-100mg/kgbw (38.5%) > AQE-100mg/kgbw (8.8%) for HDL; atrovastatin (73.5%) > CHE-200mg/kgbw (68.0%) > CHE-100mg/kgbw (64.5%) > AQE-200 mg/kgbw (62.3%) > AQE-100 mg/kgbw (62.2%) for VLDL; and CHE-200mg/kgbw (78.5%) > atrovastatin (66.8%) > AQE-200mg/kgbw (63.5%) > AQE-100mg/kgbw (26.8%) for LDL. Triterpenoids (phytosteroids), fatty acids, and cardenolides are present in the CHE while, amino acids, saponins and carbohydrate were present in the AQE as metabolites with alkaloids and anthraquinones were absent. *P. tuber reguim* possesses anti-hyperlipidemic effects which could be due to the phyto-constituents present thus justifying its traditional use for weight loss.

Keywords: *Pleurotus tuber reguim*, dyslipidemia, cardiovascular health, nutraceuticals.

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INTRODUCTION

Hyperlipidemia assists significantly in the development of atherosclerosis and coronary hearts disease. Atherosclerosis and related coronary heart diseases are among the most common causes of mortality and morbidity worldwide. They are responsible each year for 30% of mortality in the world [1]. Hyperlipidemia is a condition in which there is abnormal high levels of lipids in serum largely cholesterol and triglyceride [2]. These lipids are transported in the blood as part of large molecules called lipoproteins, and the density of the lipids and the type of protein determines the fate of the particle and its influence on metabolism [2, 3]. They are either low density lipoprotein (LDL), or high density lipoprotein (HDL) [4] of which the LDL when elevated,

pre-dispose one to cardiovascular ailments unlike the HDL. Hyperlipidaemia leads to various cardiovascular disorders like angina pectoris, myocardial infarction, hypertension, atherosclerosis, and congestive heart failure [5]. Treatment of hyperlipidaemia is geared towards a reduction in the elevated LDL, triglyceride (TG) and total cholesterol (TC), alongside an enhancement in the diminished HDL values since hyperlipidemia is usually an elevated level of TC, TG and LDL, and decreased level of HDL [6, 7]. The major side effects of anti-hyperlipidemic agents include muscle toxicity, rhabdomyolysis, and psychiatric adverse reactions which include depression, memory loss, confusion and aggressive reactions [5, 8, 9]. These drawbacks have made researchers to continue in the search for safer and effective anti-hyperlipidaemic agents

that could be developed into clinically useful drugs. Recourse to botanical derived bioactive metabolites have continued to drive this strata of the drug discovery pipeline. The edible mushroom species *Pleurotus tuber regium* (Fr.) Sing. is found in tropical and sub-tropical regions of the World [10]. It is an edible mushroom that produces the edible sclerotia or underground tubers as well as fruit bodies belonging to the family Pleurotaceae. Several pharmacological properties have been reported for extracts of *Pleurotus* species such as antigenotoxic, bioantimutagenic [11], anti-inflammatory, anti-hyperlipidemic, antihypertensive, and antihyperglycaemic [12] antibacterial and antifungal [13] activities. There is also research evidence that extracts from medicinal mushrooms can function as immune-modulators [14]. The folklore use of *Pleurotus tuber-regium* in Nigeria against weight loss reduction has been documented [15]. This present study is hence aimed at validating the anti-hyperlipidemic activities of the chloroform (non-polar) and aqueous ethanol (polar) extracts of the sclerotia of *Pleurotus tuber regium* using Triton X-100 induced hyperlipidemic rats as a preliminary step towards the isolation of the anti-hyperlipidemic constituents from this edible mushroom acclaimed for weight loss and the treatment of heart ailments. Triton x-100 is a non-ionic detergent that causes an increment of total cholesterol and triglycerides in the blood altering hepatic lipid metabolism [16]. It has been widely used in *in vivo* studies to produce acute hyperlipidemia in animal models in order to study the lipid-lowering effect of natural or synthetic drugs [17]. Triton x-100 has been found to prevent the catabolism of triglycerol-rich lipoprotein by lipoprotein lipase [18].

MATERIAL AND METHODS

Sample Collection and Identification

Pleurotus tuber regium sclerotia was purchased from Oil Mill Market, Port-Harcourt, Rivers State, Southern Nigeria and authenticated by a Mycologist at the International Centre for Ethnomedicine and Drug Development, Nsukka, Nigeria with Voucher specimen number: Inter CEDD/971.

Chemicals/Drugs/Reagents

Analytical grade chemicals (Merck, Germany) which includes absolute ethanol, and chloroform were used. Others include: Triton X-100 (Sigma-Aldrich, Germany), Atorvastatin, and distilled water,

Animals

One hundred and twenty (120) Wistar rats 170- 200g were used for the study. The rats were purchased from the Faculty of Pharmaceutical Sciences animal house, University of Port-Harcourt. The animals were provided ad libitum access to tap water throughout the experimental duration and maintained in a 12 hours light-dark cycle under standard laboratory conditions (22 ± 2 °C). All experiments were approved by the

Research Ethics Committee of the University of Port Harcourt, Nigeria (approval Ref No.: UPH/CEREMADREC/MM67/016).

Extraction Method

Chloroform (CHE) and 70% aqueous ethanol (AQE) extracts of *Pleurotus tuber regium* were obtained by successive cold maceration in order of solvent polarity. Briefly, The sample (10 kg) was first extracted by cold maceration in chloroform as solvent for three consecutive days each with agitation at interval of 6hrs and fresh replacement of solvent every 24hrs. The combined chloroform filtrate was, after concentration using a rotary evaporator and drying, kept as the chloroform (non-polar) extract CHE. The marc obtained after extraction with chloroform was then further extracted with 70 % aqueous ethanol in a similar manner and the aqueous ethanol extracts obtained were pooled together, concentrated using a rotary evaporator and further dried in a vacuum desiccator to obtain the aqueous ethanol (Polar) extract (AQE). The CHE and AQE were used for the study.

Acute Toxicity

This was done using the method reported by Lorke [19] using Wistar albino rats. Briefly, A total of eighteen (18) Wistar albino rats selected for this studies were divided into six groups of three animals each. The animals were fasted overnight before oral administration of the extract. The first three groups were treated with 10, 100, and 1000 mg/kg body weight of the extract. The animals were kept under observation for 24 hours for signs of toxicity and death. After 24 hours, none of the animals died or adverse effect was recorded. Then the remaining three groups of the animals were given doses of the extract at 1600, 2900, and 5000 mg/kg, respectively and observed for further 24 hours.

Induction of Hyperlipidemia

Acute hyperlipidemia was induced in animals by intraperitoneal administration of Triton X-100 (Tyloxapol, Sigma-Aldrich) to the rats at a dose of 150 mg/kg body weight. Induced rats were allowed to stabilize for 72 hours before commencing treatment.

Antihyperlipidemic Assay

The extracts were evaluated for *in vivo* anti-hyperlipidemic activities using method described by Kothiyal and Gupta[20] with modification. The study was carried out in the animal house of Faculty of Pharmaceutical Sciences, University of Port Harcourt. Briefly, Thirty Wistar rats were randomly divided into seven groups each having 5 animals each (A-G). Group A was not given any treatment (normal control group), while the other six groups (B-G) were treated with single dose triton X-100 (150 mg/kg bw, ip) to induce hyperlipidemia. After hyperlipidemia induction, group B received no other treatment (hyperlipidemic untreated coontrol group), while group C received

standard antilipidemic drug atorvastatin (10 mg/kgbw, orally, groups D and E received 200 and 100 mg/kgbw AQE orally, respectively, and groups F and G were administered 100 and 200 mg/kgbw CHE orally, respectively. The agents were administered daily for 7 days. On the 8th day after the treatment, blood samples were collected by retro-orbital puncture in dry EDTA bottles and the serum lipid levels; total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were determined using standard methods with commercially available kits.

Phytochemical Screening

This was done using standard phytochemical screening methods [21, 22].

Data presentation and Statistical Analysis

All data were expressed as mean \pm SEM. One way analysis of variance (ANOVA) and student's t-test were used to analyze the data and test for significance between experimental and control groups (p values $<$ 0.05 were considered significant).

RESULTS AND DISCUSSION

The extracts of *P. tuber reguim* were found to be safe at 5000 mg/kg bw as there were neither deaths nor observed visible signs of toxicity in all the doses that were used in the LD₅₀ study. TC, TG, LDL, and VLDL levels were significantly ($p <$ 0.05) increased, while HDL was significantly ($p <$ 0.05) decreased in the hyperlipidemic untreated control group compared to the normal control group (Table-1). Hyperlipidemic groups treated with Atorvastatin (10 mg/kg), chloroform, CHE (100, 200 mg/kg) or aqueous ethanol, AQE (100, 200 mg/kg) extracts of *Pleurotus tuber reguim* showed significant ($p <$ 0.05) ameliorating effect on Triton-X-induced hyperlipidemia by decreasing the elevated levels of TC, TG, LDL, and VLDL in hyperlipidemic untreated control group, and increasing the reduced levels of HDL (Table-1). There was significant and dose-dependent decrease in TC level in AQE, CHE, and atorvastatin treated groups compared to hyperlipidemic control group. Also, except for the group that received 100 mg/kg bw of AQE, where the observed TC value remained significantly increased, all other extract and atorvastatin treated groups were not significantly different when compared with the normal control group (Table-1). Also, except for the group that received 100 mg/kg bw of AQE, where the observed TC value remained significantly increased, all other extract and atorvastatin treated groups were not significantly different when compared with the normal control group (Table-1). When atorvastatin was compared with extract groups, only the low dose AQE (100 mg/kg) group showed significant difference with higher TC level than atorvastatin (Table-1). The trend in the rank for TC was observed to be: atorvastatin (43.5%)>CHE-200mg/kgbw (41.4%)>CHE-100mg/kgbw(38.4%)>AQE-200 mg/kgbw (35.9%)>AQE-100 mg/kgbw (27.6%).

Thus it could be inferred that the lipophilic extract(CHE) showed a better ameliorating effect on the TC elevation compared to the hydrophilic AQE.

Ameliorating effect on Total triglyceride (TG)

There is a significant ($p <$ 0.05) reduction in TG level in extract and atorvastatin treated groups compared to the hyperlipidemic untreated control (Table 1).The observed TG values of the extracts and atorvastatin hyperlipidemic treated groups were not significantly different ($p >$ 0.05) compared to that of the normal control group. Similar to TC result, the ameliorative effects on TG were dose dependent with the non-polar CHE exhibiting a better (though not significant ($p >$ 0.05) effect compared to the polar AQE. The rank order of ameliorative effect on TG elevation by the agents was: atorvastatin (73.5%)>CHE-200mg/kgbw (68.0%)>CHE-100mg/kgbw(64.5%)>AQE-200 mg/kgbw (62.3%)>AQE-100 mg/kgbw (62.2%).

Ameliorating effect on High Density Lipoproteins (HDL)- cholesterol

At the different dose levels, the non polar CHE increased HDL level more than the polar AQE, and HDL levels in 200 mg/kg bw AQE or CHE groups were higher compared to the atorvastatin, although values were not significant (Table-1). The general trend in the calculated ameliorating effect for HDL-cholesterol was observed to be: CHE-200mg/kgbw (114.4 %) > AQE-200mg/kgbw(87.3%) > atorvastatin (68.7%)>CHE-100mg/kgbw (38.5%)>AQE-100mg/kgbw(8.8%). The effects of HDL is to make the transportation of cholesterol easy from peripheral tissues such as arteries to the liver for catabolism [23]. This results in the reduction of cholesterol and triglycerides and hence a reduction in cardiovascular risk. HDL has been recognised as the protective cholesterol fraction because there is an inverse relationship between the concentration of HDL-cholesterol and the agent of cardiovascular complications.

Ameliorating effect on Very Low Density Lipoproteins (VLDL)- cholesterol

VLDL is a proportion of the TG and its plasma elevation just like TC and TG, is a risk factor in the progression of arteriosclerosis, and related cardiovascular diseases. In this investigation, the elevated VLDL cholesterol peaked in the hyperlipidemic untreated controls group and for the hyperlipidemic groups treated with the various doses of the test extracts and atorvastatin, there was a significant ($p <$ 0.05) amelioration effect. Just as observed for the other parameters, the CHE extract showed relatively better reduction in VLDL-cholesterol compared to the AQE though not significant ($p >$ 0.05).The ranked order in amelioration of the elevated VLDL-cholesterol was observed to be: atorvastatin (73.5%)>CHE-200mg/kgbw (68.0%)>CHE-100mg/kgbw(64.5%)>AQE-200 mg/kgbw (62.3%)>AQE-100 mg/kgbw (62.2%).

Ameliorating effect on Low Density Lipoproteins (LDL)- cholesterol

Unlike HDL-cholesterol which is good, elevated LDL- cholesterol is a risk to cardiovascular health. From the result, the elevation of LDL-cholesterol seen in the hyperlipidemic untreated group was significantly ($p < 0.05$) reduced in a dose dependent manner after the 7 days treatment with the CHE and AQE extracts as well as the standard drug atorvastatin. Just as observed for the other parameters, the non-polar CHE extract showed a better (though not significant $p > 0.05$) ameliorating effect compared to the polar AQE extract. The general trend in the calculated ameliorating effect for LDL-cholesterol was observed to be: CHE-200mg/kgbw (78.5%) > atorvastatin (66.8%) > AQE-200mg/kgbw (63.5%) > AQE-100mg/kgbw (26.8%).

Phytochemical Screening

The result of the phytochemical screening showed the presence of triterpenoids, saponins, cardenolide, amino acids, fatty acids and carbohydrates derivatives with the absence of phenolics, alkaloids and anthraquinones. Whereas the non-polar CHE is found to contain triterpenoids (phytosteroids), fatty acids and cardenolide, the polar AQE was amino acids, saponins and carbohydrate. In an earlier phytochemical study

[24], ergosterol and poly-unsaturated fatty acids derivatives have been characterized from this mushroom using Gas-Chromatography-Mass Spectrometry techniques. Ergosterol derivatives and related phytosterols have been reported to be antihyperlipidemic [12, 25, 26]. Poly unsaturated fatty acids such as the omega-3 derivatives are also good in the management of obesity and cardiovascular ailments unlike the saturated fatty acids. Essential amino acids like methionine, threonine and tryptophan have also been found to reduce cholesterol level and prevent fatty liver [27]. Saponins have been reported to act as cholesterol reduction agents by binding with cholesterol in the intestinal lumen [28] and also as surface active/fat emulsifying agents. Water soluble polysaccharides such as Lecithin found in mushrooms have been reported to play a major role in the incorporation of free cholesterol in HDL and VLDL which turn them back to the liver cells for catabolism [20]. The observed antihyperlipidemic activity of the extracts from the sclerotia of this edible mushroom *P. tuber regium* could therefore be attributed to the presence of one or more of these phytochemical. The lower potency and ameliorative effect of AQE compared to CHE might be due to difference in polarity and bioactive phytochemicals present in the extracts.

Table-1: Anti-hyperlipidemic parameters results

Test groups	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	VLDL = TG/5 (mmol/L)	LD=TC-VLDL – HDL (mmol/L)
Normal control	2.200±0.212	1.240±0.114	1.500±0.173	0.248±0.023	0.452±0.353
Hyperlipidemic untreated control	4.180±0.098	4.300±0.141	0.758±0.325	0.860±0.032	2.562±0.228
Atorvastatin 10 mg/kg bw	2.360±0.167 (43.5%)	1.140±0.207 (73.5%)	1.280±0.130 (68.7%)	0.228±0.041 (73.5%)	0.852±0.058 (66.8%)
AQE 200 mg/Kg bw	2.680±0.192 (35.9 %)	1.620±0.164 (62.3%)	1.420±0.130 (87.3%)	0.324±0.033 (62.2%)	0.936±0.129 (63.5%)
AQE 100 mg/Kg bw	3.025±0.096 ^{a,b} (27.6%)	1.625±0.25 (62.2%)	0.825±0.171 (8.8%)	0.325±0.034 (62.2%)	1.875±0.170 ^{a,b} (26.8%)
CHE 200 mg/Kg bw	2.450±0.100 (41.4%)	1.375±0.075 (68.0%)	1.625±0.206 (114.4%)	0.275±0.010 (68.0%)	0.550±0.390 ^a (78.5%)
CHE 100 mg/ Kg bw	2.575±0.096 (38.4%)	1.525±0.189 (64.5%)	1.050±0.129 (38.5%)	0.305±0.038 (64.5%)	1.220±0.016 ^a (52.4%)

Note: AQE and CHE are the hydrophilic 70% aqueous ethanol and lipophilic chloroform extract respectively of *P. tuber regium* sclerotia. All the treated groups exhibited significant ($p < 0.05$) ameliorating effect compared to the Triton-x100 induced hyperlipidemic untreated group. ^asignificantly different ($p < 0.05$) compared to standard drug atorvastatin, ^bsignificantly different ($p < 0.05$) compared to CHE at dose of 100 mg/kgbw. Values in parenthesis are the percentage ameliorating effect

CONCLUSIONS

This work demonstrated that both aqueous extract (AQE) and chloroform extract (CHE) of *Pleurotus tuber-regium* possess lipid lowering effect which suggests that the sclerotium has anti-hyperlipidemic properties. Further investigation is ongoing to isolate in the pure form the anti-hyperlipidemic chemical constituents that could serve as leads in the development of novel and useful drugs for the management of obesity and related complications due to diabetes and cardiovascular ailments.

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