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Pleurotus florida Aqueous Extracts and Powder Influence Lipid Profile and Suppress Weight Gain in Rats Fed High Cholesterol Diet

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Abstract

Pleurotus florida mushroom is a nutritious food with therapeutic potential. This study investigates the effects of *P. florida* aqueous extracts and powder on serum lipid profile and weight gain in rats fed high cholesterol (HC) diets. Twenty five male albino rats were partitioned into five groups (n = 5): BD: basal diet; HC: High Cholesterol diet; HC-PFP5: HC plus 5% *P. florida* powder; HC-PFE5: HC plus 5% *P. florida* powder extract; HC-PFE7.5: HC plus 7.5% *P. florida* powder extract. Animals had free access to diets and water for 4 weeks. Results showed that weight gain was significantly ($P < 0.05$) suppressed in rats fed high cholesterol diet supplemented with *P. florida* powder or its extracts, compared to rats fed high cholesterol diet alone. Additionally, supplementation with *P. florida* increased fecal lipid excretion, while serum triglyceride, LDL, VLDL and total cholesterol decreased. HDL-C increased and LDL-C/HDL-C ratio decreased in rats fed *P. florida*. These results show that *P. florida* extracts like powders possess antihypercholesterolemic effects and prevent weight gain, thus reducing the risk for cardiovascular diseases. Mechanisms for these effects are suggested.

Keywords: Antihypercholesterolemic effect; *P. florida* aqueous extract; *P. florida* powder; LDL-C/HDL-C ratio; Serum lipid profile

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death, accounting for 31% of deaths worldwide [1]. In 2008, more than 17 million deaths were attributed to CVDs alone, with more of these deaths occurring in low income countries [1]. Premature deaths attributed to CVDs ranged from 4% in high income countries to 42% in low income countries [1]. Hypercholesterolemia is a major risk factor associated with CVDs, such as atherosclerosis and its related complications [1-4]. Keeping blood serum cholesterol levels at desirable low levels therefore is one of the major preventive strategies for these diseases. In this regard, the search for natural substances (functional foods, nutraceuticals), with antihypercholesterolemic effects is desirable; as they provide a safe and natural means to combat the rising incidence of hypercholesterolemia and cardiovascular diseases especially noticeable in low income countries.

Edible mushrooms have for long been appreciated for their flavor and texture. They are recognized as nutritious foods as well as an important source of biologically active compounds of medicinal value [5-7]. They are rich in fiber, protein and micronutrients, and low in caloric value [8,9], making them a natural food for the prevention of cardiovascular diseases as first suggested by Traditional Chinese Medicine [10,11]. To this effect, the cholesterol lowering properties of some edible mushrooms have been reported [12-14].

The oyster mushroom, Pleurotus species, is a highly nutritious, edible mushroom and a common species in tropical West Africa and Southern parts of Asia [15]. They are the main species of mushroom cultivated in Cameroon (*P. ostreatus, P. pulmonarius, P. florida* and *P. sajou-cajou*) [16], where they are mostly consumed fresh after cooking, or dried and used in the preparation of stews and soups as substitute for fish or meat [17]. Oyster mushrooms are interesting as they have demonstrated immunoregulatory [18], antioxidant [5-7,19], and anti-inflammatory [19,20] properties. These beneficial effects have been attributed to their water soluble polysaccharide component (β-glucans), as well as their phytochemical composition. In particular, the antihypercholesterolemic properties of water soluble and ethanol extracts of *P. ostreatus* [21] as well as 5% powder incorporations of *P. ostreatus* [22] and *P. ferulae* [13] have been demonstrated in animals. With respect to *P. florida*, the hypocholesterolemic properties of its powder has been demonstrated in rats fed cholesterol enriched diets [23]. It's hot water extracts on the other hand have been shown to possess antioxidant effects [5,19,24]. The beneficial effect of antioxidants in regulating lipid metabolism in hypercholesterolemic rats has been reported [25]. These studies suggest that the oyster mushrooms *P. florida*, like others of the Pleurotus family can be an important food in the management of hypercholesterolemia. Given that the powder form of *P. florida*, and extracts of other species, has been shown to possess hypocholesterolemic effects, this study set out to investigate the antihypercholesterolemic properties of water extracts of *P. florida* in comparison with the powder. With increasing emphasis on functional and convenience foods, and the increased production and consumption of *P. florida* mushroom and mushroom juice in Cameroon and beyond, information on the antihypercholesterolemic property of *P. florida* mushroom and its juice, is necessary to promote its consumption as a health food. This study therefore had as objective to determine and compare the antihypercholesterolemic effect of aqueous extracts of *P. florida* mushroom with the powder form, in experimental animals, through analyses of weight gain and serum lipid profile.

Materials and Method

Materials

The mushroom (*Pleurotus florida*) used in this study was purchased from a mushroom production center in Akak, at the outskirts of Yaoundé, Center Region-Cameroon, and transported to the Food Biochemistry and Biophysics laboratory of the University of Ngaoundere.

Preparation of *Pleurotus florida* powder and aqueous extract

The mushroom samples were sorted to remove spoils after which they were washed twice and rinsed with distilled water, sliced and allowed to drain for 30 minutes. Samples were then dried in an electric dryer (Riviera & Bar QD105A, Paris, France) at 50°C for 24 hr. The dried samples were ground into powder (1 mm) using an electric grinder (Cullati Polymix, France). The powder obtained was stored under refrigeration at 4°C until needed for analysis. Given that previous studies had shown hypocholesterolemic effects of 5% *P. florida* mushroom powder supplementation in rat models [23], aqueous extracts were prepared using 5 g and 7.5 g mushroom powder, and the extracts incorporated into feed to correspond to 5% and 7.5% levels of powder incorporation. Extraction conditions were as previously determined in an optimization study to maximize the extraction of soluble fibers [26]. Powders samples were extracted in distilled water at 100°C for 30 min using a 1/10 solute / solvent ratio, and thereafter centrifuged for 10 min at 2500 g.

Determination of soluble fiber

Soluble fiber content in the powder and extracts was determined using a gravimetric method following the procedure described by [27]. The Mushroom powder was first extracted by mixing with distilled water 1:10 (w/v) and the mixture boiled for 1.5 hr to extract soluble fiber. After cooling, the mixture was centrifuged at 2500 g for 10 min. The supernatant was decanted and saved, and the residue extracted twice more in boiling water for 30 min. The supernatants were pooled and analyzed for soluble fiber. Extracts from 5 and 7.5 g mushroom powder were analyzed directly for their soluble fiber content without any further extraction. For each of the extracts, a 5 ml aliquot was slowly added to three volumes of 96% ethanol and stored at 4°C overnight to precipitate the soluble fiber fraction. The samples were centrifuged anew at 2500 g for 10 min and the supernatant discarded. The precipitates obtained were washed with a mixture of water and 96% ethanol (1:3) and centrifuged as previously indicated. The precipitates were then re-suspended in distilled water to dissolve completely. Proteins were removed by adding an equal volume of 10% trichloroacetic acid and allowed to stand for 2 hr. The protein precipitates were removed by centrifugation at 2500 g for 20 min. The supernatant was collected and slowly added to three volumes of 96% ethanol and stored at 4°C overnight to precipitate the soluble fiber. The solution was centrifuged at 2500 g for 10 min, and the supernatant discarded. The precipitate was then dried at 100°C for 30 min and the soluble fiber content expressed as (mg/g).

Total polyphenols

Total polyphenols were determined according to [28] with some modification. To 0.5 g of mushroom powder, 10 ml ethanol (70%) was added and the mixture stirred for 2 hr using a Prolabo 54 433 agitator, Paris, France at 220 rpm, to extract total polyphenols. After centrifuging (DL 6000 mark, rotor 15 cm, Japan) at 3000 g for 20 min, 20 µl of the supernatant was mixed with 0.2 ml of Folin-Ciocalteu reagent diluted (1/16), 0.4 ml of sodium carbonate (20%) and 1.38 ml distilled water. The mixture was vortexed and incubated in a water bath at 40°C for 20 minutes in the dark. Mushroom extracts were analysed as such without any further extraction. Gallic acid (0.2 g/l) was used as standard and absorbance was read at 725 nm.

Total proteins

Proteins (N × 6.25) were determined using the microkjeldahl method of [29]. Samples were first mineralized using Kjeldahl method and the nitrogen content of the mineralisate was evaluated after a reaction with ammonia (NH_3) and acetyl acetone / formaldehyde. The resulting yellow complex (3,5-diacetyl-1,4-dihydrolutidin) had a maximum absorption at 412 nm.

Soluble sugar contents

Soluble sugar contents of the flours and extracts were determined according to the method of Fischer and Stein [30]. Mushroom flours were previously extracted with distilled water (1:5 w/v) for 1h at 100°C and centrifuged at 2500 g for 30 min before quantification.

Determination of antihypercholesterolemic effect of *Pleurotus florida* powder and extracts in rat models

Feeding experiments: Twenty five adult male albino rats (2-3 months old) weighing 204-366 g were purchased from the University of Yaoundé I animal house and transported to the National School of Agro-Industrial Sciences (ENSAI), of the University of Ngaoundere. Animals were housed individually in semi-metabolic cages in the animal house at ENSAI, on a 12 / 12 cycle of light and darkness. The average temperature and relative humidity of the animal house were 25 ± 2°C and 60-70% respectively. The animals were distributed into five groups of five animals each and acclimatized for one week on a basal diet (Corn starch 50%; Rice powder 11.25%; Vegetable oil 1%; Egg white 10%; Dried fish 8%; Cellulose 19%; Mineral mix 0.125%; Vitamin mix 0.125%; Table salt 0.5%) formulated according to Alam et al. [13]. Thereafter the rats were fed the treatment diets, and consisted of a negative control group that received the basal diet (BD); a positive control group fed a cholesterol enriched diet (HC) and 3 experimental groups that were fed the cholesterol enriched diet supplemented with 5% mushroom powder (HC-PFP); extract from 5 g (5%) mushroom powder (HC-PFE5) and extract from 7.5 g (7.5%) mushroom powder (HC-PFE7.5) respectively. The 5% mushroom powder was used as a standard to compare the effects of substituting mushroom powder with extracts from an equivalent amount of powder, given that previous studies had demonstrated the hypocholesterolemic effects of 5% mushroom powder supplementation. The different formulations are presented in Table 1.

The animals were allowed free access to food and water for 4 weeks. Food intake and body weight were measured daily and weekly respectively. Fecal matter was collected from the animals during the last four days of the experiment for estimation of fecal lipid excreted. At the end of the experimental period rats were fasted overnight, anesthetized using chloroform and sacrificed. Blood samples were collected from the carotid artery in dry tubes; and various organs (liver, kidney and heart) were rapidly excised and weighed.

Table 1: Treatment groups and Diet formulations (g/100 g of diet).

Diet Treatment Group	Diet code	Diet formulae
Basal diet (BD)	Negative control	100 g basal diet*
High Cholesterol diet (HC)	Positive control	100 g basal diet + 1% cholesterol
HC + 5% *P. florida* powder (PFP)	HC-PFP	100 g basal diet + 1% cholesterol + 5% PFP
HC + 5% *P. florida* powder extract (PFE5)	HC-PFE5	100 g basal diet + 1% cholesterol + 5% PFE5
HC + 7.5% *P. florida* powder extract (PFE7.5)	HC-PFE7.5	100 g basal diet + 1% cholesterol + 5% PFE7.5

*Nutrient content of basal diet (g/100 g DM): carbohydrates (35 ± 2.3), Total lipids (19.70 ± 0.28), protein (32.95 ± 2.4), ash (0.02 ± 0.005), and fiber (12.33 ± 1.50).

Determination of food intake and body mass gain: Daily food intake was evaluated as the difference between the quantity of food given to animals and that left unconsumed after a 24 hr period. Rats were weighed at the start of the experiment and then weekly. Body weight gain of rats was calculated as the difference between the initial and final weights and results expressed as a percentage (%) of the initial weight.

Determination of fecal lipid content: The fecal matter collected from the animals was dried and ground into powder. Total lipid was extracted with hexane using a Soxhlet apparatus and quantified [31].

Determination of serum lipids: Total serum cholesterol (TC) was determined using the Olympus OSR6516 kit (Olympus Diagnostica Gmbh, Ireland) fixed onto an OLYMPUS AU2700 analyzer. Low density lipoprotein (LDL) and high density lipoprotein (HDL) was determined using BioMerieux 61534 (BioMerieux France) directly connected to an automated analyzer (OLYMPUS 2700 analyzer), and total triglyceride (TG) were determined using automated method. Very low density lipoprotein cholesterol VLDL-C was obtained by difference:

VLDL-C = [TC - (HDL – C + LDL - C)]

Statistical analysis

Results were subjected to Analysis of variance (ANOVA) to determine variations between treatment groups and mean separation done using the Least Significant Difference test at the 5% level. The statistical software, Statgraphic Plus 5.0 (Manugistics, Rockville, Maryland, USA) was used for analysis.

Results and Discussion

Composition of mushroom (*P. florida*) powder and extracts

The chemical composition of the mushroom (*P. florida*) powder and extracts are presented in Table 2.

Soluble fiber, proteins, sugar and polyphenols were significantly ($p < 0.05$) higher in powder samples compared to extracts. The quantities in extracts increased with the amount of powder extracted. Soluble sugars were the major constituent in the extracts. This is in part explained by their higher initial concentrations in the powder. The low recovery of soluble fiber in extracts compared to powder, maybe attributed to the rigorous extraction conditions employed in the extraction of powder for determination of soluble fiber (100°C for 1.5 hr) compared to conditions for the experimental extracts (100°C for 30 min).

Table 2: Chemical composition of *Pleurotus florida* powder and extract (g/100 g DM).

Composition	Powder sample	5 g powder extract	7.5 g powder extract
Soluble fiber (g)	28.77 ± 1.20^c	4.0 ± 0.2^a	7.2 ± 0.1^b
Protein (g)	20.35 ± 0.20^b	2.2 ± 0.2^a	3.1 ± 0.2^a
Soluble Sugars (mg)	532 ± 27^c	72.1 ± 5.5^a	103.0 ± 7.8^b
Polyphenols (mg)	534 ± 7^c	9.7 ± 1.3^a	14.0 ± 2.0^b

Values are means ± standard deviations of three repetitions. Values on the same row with different superscripts are significantly different ($p < 0.05$).

Polyphenols also, though in high concentrations in powders were found in smaller quantities in the extracts. The poor solubility of polyphenols in water [32] could be responsible for the low quantities in the water soluble extracts, compared to the powders that were extracted with ethanol. In addition, the high extraction temperature (100°C) for the experimental extracts could cause oxidation of some polyphenols [33]. High extraction temperature (100°C) and prolonged heating times (30 min), could lead to Maillard type reactions with precipitation of some components (sugars and proteins). These may account in part for the poor extractability of these components and hence the smaller amounts in extracts.

Effect of *Pleurotus florida* powder and extracts on weight gain and lipid profile in rat models

In order to understand the effects of *Pleurotus florida* mushroom powder and extract on lipid metabolism and total weight gain in rats, the amount of lipids excreted in feces was determined and serum lipid profile analyzed. Weight of rats was measured and that of some organs such as the heart, liver, and kidney.

Food intake and body weight gain

Results indicate that there was no statistically significant difference ($P > 0.05$) in food intake amongst the groups throughout the experimental period (Table 3).

However, at the end of the four week experimental period, animals fed a HC diet had a significantly ($p < 0.05$) higher body weight (390 ± 2.1 g) compared to animals fed the BD, HC-PFP, HC-PFE 5 and HC-PFE 7.5 diets (Table 3).

Addition of 1% cholesterol to the basal diet favored weight gain in rats with a final increase in weight of 7.4% above rats fed a basal diet. Incorporation of mushroom powder and extracts in high cholesterol diet, on their part significantly ($p < 0.05$) prevented body weight gain and the animals had lower final body weights than those fed HC and the basal diets.

Table 3: Average weekly food intake and body weight gain of rats fed experimental diets for four weeks, values are means ± standard deviations of five animals.

Treatment	Average weekly food intake (g)	Initial weights (g)	Final weights (g)	Weight gain (g %)
BD	134.1 ± 20.1^{a}	286 ± 2^{a}	363.5 ± 5^{c}	77.5 ± 1.7^{b} (27.1)
HC	122.4 ± 11.6^{a}	290 ± 3^{a}	390 ± 2^{d}	100 ± 9.0^{c} (34.5)
HC-PFP5	120.8 ± 14.3^{a}	284 ± 3^{a}	338 ± 3^{a}	54.0 ± 3.2^{a} (19)
HC-PFE5	121.0 ± 18.8^{a}	284 ± 2^{a}	346 ± 4^{b}	62.3 ± 1.5^{a} (21.9)
HC-PFE7.5	114.0 ± 10.6^{a}	287 ± 4^{a}	342 ± 4^{ab}	55.5 ± 1.4^{a} (19.3)

Values in the same column with different superscripts are significantly different ($p < 0.05$). BD: Basal Diet; HC: High Cholesterol Diet; HC-PFP: HC + *P. florida* 5% Powder; HC-PFE 5: HC + *P. florida* 5% Extract; HC-PFE 7.5: HC + *P. florida* 7.5% Extract.

Addition of 5% *P. florida* powder in the HC diet prevented body weight gain by 15.5%, whereas extracts from an equivalent 5% and 7.5% *P. florida* powder prevented weight gain by 12.7% and 15.3% respectively compared to those fed HC diets. Compared to the basal diet, addition of 5% mushroom powder and extracts from 5% and 7.5% powders significantly ($p < 0.05$) prevented weight gain by 8.1%, 5.2% and 7.8% respectively. These results suggest that *P. florida* powder and extracts could influence lipid metabolism and consequently be beneficial in controlling weight gain. However, no statistically significant differences ($p > 0.05$) were observed in the effect of powder and extracts in preventing weight gain in rats.

Obesity is characterized by the presence of excess adipose tissue and an increased percentage of body fat [34]. Worthy of note is the fact that *P. florida* showed positive effects in suppressing weight gain with no significant difference between the powder form and the extracts. This finding is important as obesity is a chronic disorder associated with complications in the body such as diabetes, cardiovascular diseases, respiratory abnormalities and cancer [35-37]. We observed that *P. florida* powder and extracts had similar effects in preventing weight gain suggesting that the components most active in preventing weight gain are extractable by water. Powders and extracts contain soluble fiber which is said to bind lipids in the intestinal tract resulting in their elimination in feces [36], thereby preventing weight gain. The efficacy of extracts is important as it means more versatility and convenience in the use of mushrooms for controlling obesity and its related diseases.

Mass of organs and fecal lipid excreted

With the exception of the liver of HC fed rats that was significantly larger ($p < 0.05$), there was no significant ($P > 0.05$) difference in the kidney, heart and liver masses of rats fed the BD, HC-PFP, HC-PFE5, and HC-PFE7.5. Similar findings have been reported by [13] while investigating the antihypercholesterolemic effect of *P. ferulae*. The authors observed lipid droplets in liver tissues of animals fed high cholesterol diet indicating accumulation of lipids in hepatocytes. This is possible given that lipid metabolism takes place in the liver and adipose tissues and in the event of excesses; lipids accumulate in these tissues [34]. In this respect, it can be suggested that *P. florida* powder as well as its extracts, like *P. ferulae*, are capable of preventing liver steatosis.

In the present study, no significant differences ($P > 0.05$) were observed in total fecal lipids excreted by rats fed HC diets alone, or incorporated with mushroom powder and extracts, although the quantities of lipid excreted increased with incorporation of mushroom powder and extracts (Table 4).

Table 4: Effects of consumption of *P. florida* powder and extracts on organ weights (g) and fecal lipid excretion (g/day/100 g dry fecal matter),

Treatment	Liver	Heart	Kidney	Fecal lipid excreted
BD	11.31 ± 1.06^{a}	1.15 ± 0.13^{a}	2.14 ± 0.42^{a}	2.97 ± 0.3^{a}
HC	15.47 ± 1.04^{b}	1.25 ± 0.08^{a}	2.17 ± 0.06^{a}	3. 82 ± 0.61^{ab}
HC-PFP	11.08 ± 1.11^{a}	1.15 ± 0.19^{a}	2.13 ± 0.12^{a}	4.13 ± 0.33^{ab}
HC-PFE5	11.12 ± 1.01^{a}	1.16 ± 0.13^{a}	2.13 ± 0.07^{a}	4.54 ± 0.4^{b}
HC-PFE7.5	11.18 ± 1.00^{a}	1.20 ± 0.16^{a}	2.13 ± 0.29^{a}	4.57 ± 0.45^{b}

Values are means ± SD of five animals. Values in the same column with different superscripts are significantly different ($p < 0.05$). BD: Basal Diet; HC: High Cholesterol Diet; HC - PFP: HC + *P. florida* powder 5%; HC - PFE 5: HC + *P. florida* 5% extract; HC-PFE 7.5: HC + *P. florida* 7.5% extract.

However, fecal lipids of rats fed HC diet coupled with mushroom extracts was significantly ($P < 0.05$) higher than those of rats fed the BD. The enhanced fecal lipid excretion observed with the consumption of mushroom powder and extracts in the treatment groups indicates a reduction in absorption of lipids at intestinal level. Similar findings by [38], had reported increased fecal lipid excretion in hamsters fed straw mushroom diets, indicating that this effect is common with mushroom consumption. Soluble fiber is said to bind lipids in the intestinal tract resulting in their elimination in feces [36]. The presence of soluble fiber

in mushroom powder and extracts, suggests this could be one of the mechanisms by which *P. florida* powder and extracts control weight gain in rats fed high cholesterol diets.

Analyses of serum lipid profile

Blood serum lipid profile and especially cholesterol which is the major component of atherogenic fatty plaque can be used to measure the risk for the development of cardiovascular diseases (CVDs) and other metabolic syndromes [36,39,40].

Total serum cholesterol and total triglycerides

Addition of 1% cholesterol to the basal diet induced hypercholesterolemia in HC fed animals since the total cholesterol level rose by 26% and total triglycerides by 40% with respect to the group fed the basal diet (Table 5).

Table 5: Effect of *P. florida* mushroom on serum lipid profile (mg/dl). Values are means ± SD of five animals.

Treatment	TG	TC	HDL-C	LDL-C	VLDL-C
BD	54.0 ± 2.3^{b}	46.9 ± 2.1^{a}	24.1 ± 1.5^{a}	17 ± 1.8^{a}	5.2 ± 1.2^{a}
HC	75.0 ± 5.8^{c}	58.9 ± 5.9^{b}	19.3 ± 1.2^{b}	26.1 ± 4.2^{b}	13.4 ± 1.6^{b}
HC + PFP	47.2 ± 2.5^{a}	50.6 ± 2.4^{a}	25.2 ± 1.7^{a}	18.3 ± 1.6^{a}	7.1 ± 1.8^{a}
HC + PFE 5	46.6 ± 3.4^{a}	45.4 ± 3.1^{a}	24.4 ± 1.2^{a}	14.9 ± 1.1^{a}	6.1 ± 1.6^{a}
HC + PFE 7.5	48.0 ± 4.4^{ab}	46.2 ± 1.8^{a}	22.5 ± 2.9^{a}	14.7 ± 2.1^{a}	9.0 ± 1.7^{a}

Values in the same column with different superscripts are significantly different $p < 0.05$. TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; BD: Basal Diet; HC: High Cholesterol Diet; HC-PFP: HC + *P. florida* Powder 5%; HC-PFE 5: HC + *P. florida* 5% Extract; HC-PFE 7.5: HC + *P. florida* 7.5% Extract.

These levels were significantly ($P < 0.05$) lower in the treatment groups with no significant differences among treatments. *P. florida* powder and extracts maintained total cholesterol and triglyceride levels at those found in the control BD group. Similar observations were made by [21] after feeding whole fungus (*Pleurotus ostreatus*), its water and ethanol extracts to rats for 6 weeks. According to [41], mushrooms contain statins, which have been shown to inhibit the activity of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) thereby suppressing hepatic biosynthesis of cholesterol. Decrease in HMG-CoA reductase activity has been reported in rats fed high cholesterol diets with 5% oyster mushroom (*Pleurotus ostreatus*) [42]. All these point to the cholesterol lowering effects of mushroom. In addition, mushrooms have been shown to contain soluble fiber which binds bile acids resulting in their elimination in feces. Consequently more cholesterol from the liver will have to be used in the synthesis of bile acids thus lowering cholesterol levels [34]. The high lipid excretion observed in the treatment groups (Table 4) supports this assertion. Thus binding of cholesterol and reduction of HMG CoA reductase activity suggests underlying mechanisms by which mushrooms lower cholesterol levels.

HDL cholesterol (HDL-C)

Consumption of the cholesterol rich diet decreased the amount of HDL-C in blood serum compared to animals fed the basal diet. However, supplementation of HC diet with *P. florida* powder and extracts maintained HDL-C at levels initially present in the control group fed the basal diet (Table 5). High levels of plasma HDL-C in blood implies that more cholesterol from peripheral tissues is being returned to the liver for catabolism and subsequent excretion resulting in its reduction in blood [36]. The increase in HDL-C may be attributed to the inhibition of Apo D activity which is responsible for transforming cholesteryl ester (CE) into VLDL [43]. High HDL-C levels are generally associated with a protective effect against atherosclerosis and cardiovascular diseases, whereas high levels of LDL constitute a risk factor. Consequently, *P florida* powder and extracts can be considered as having protective effects against CVDs.

LDL and VLDL Cholesterol

Serum concentrations of LDL and VLDL cholesterol in rats fed *P florida* powder and extracts were significantly ($p < 0.05$) lower than those of rats fed the HC diet alone, with no significant differences ($P > 0.05$) between treatment groups. VLDL-C is the major transport form in which TG produced in the liver are transferred to peripheral tissues, and during this time it is hydrolyzed with the partial removal of TG by lipoprotein lipase forming LDL-C [14,44]. Previous work [45] had shown that consumption of *P. ostreatus* mushroom reduces VLDL entry into circulation and accelerates fractional turnover rate of VLDL, which possibly explains the lower serum concentration of VLDL-C compared to LDL-C levels in all the treatment groups. In addition, the low serum VLDL and LDL-cholesterol levels in animals fed HC-PFP, HC-PFE5 and HC-PFE7.5 diets may be due to elevation of hepatic LDL receptor levels allowing for greater amounts of VLDL remnants and LDL to be removed from circulation. Higher hepatic LDL receptor mRNA levels had previously been reported in rats fed mushroom (*Agaricus bisporus*) fiber compared to those in rats fed cellulose, and hepatic LDL receptor level correlated negatively with serum VLDL and LDL cholesterol concentrations [46]. Thus mushrooms favor the removal of LDL-C and VLDL-C from circulation, thereby reducing their concentration in serum, and consequently removing the risk for atherosclerosis and other CVDs associated with them.

Polyphenols present in mushrooms could equally constitute an important factor contributing to this decrease in cholesterol, as it has previously been shown with green tea that their polyphenols stimulate LDL receptors and reduce LDL and VLDL cholesterol levels [47,48]. Studies with polyphenols of grape seeds have shown that these lower cholesterol by inhibiting pancreatic cholesterol esterase, binding bile acids, and reducing solubility of cholesterol in micelles which delay cholesterol absorption and enhance their excretion [49]. As such, polyphenols prevent dyslipidemia and cardiovascular diseases. Polyphenols of mushroom could have similar effects.

Atherosclerosis index

The ratio HDL-C / Total cholesterol and LDL-C / HDL-C (also called atherosclerosis-index) measures the risk for developing cardiovascular diseases given the relationship between serum lipids and artherogenesis [36,50]. Results of serum LDL-C / HDL-C ratio measured in this study are shown in Figure 1. In rats fed the high cholesterol diet, this ratio increased by 85% compared to the ratio for rats fed the basal diet.

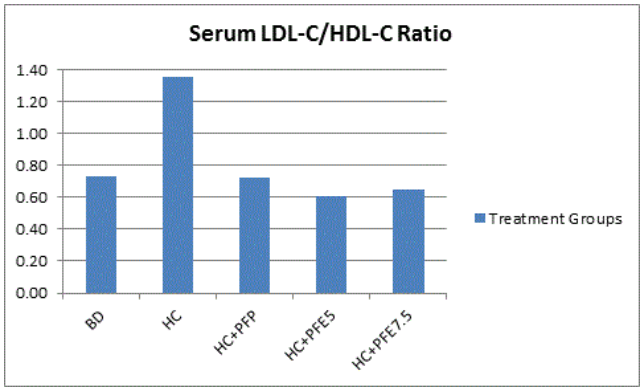

Figure 1: Influence of *Pleurotus florida* on artherosclerosis index (LDL-C / HDL-C) ratio in rats fed hypercholesterol diet. The consumption of a high cholesterol (HC) diet by rats led to an increase in the LDL-C / HDL-C ratio, whereas incorporation of *Pleurotus florida* powder and water extracts into this high cholesterol diet led to a reduction in the LDL-C / HDL-C ratio, the higher the LDL-C / HDL-C ratio, the greater the chances of developing cardiovascular diseases. Values are means of five animals. BD: Basal Diet; HC: High Cholesterol Diet; PFP: HC + *P. florida* Powder 5%; PFE 5%: HC + *P. florida* 5% Extract; PFE 7.5%: HC + *P. florida* 7.5% Extract; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein Cholesterol.

On the other hand, the LDL-C / HDL-C ratio was significantly lowered by 46%, 54% and 52%, in rats fed HC-PFP, HC- PFE5 and HC-PFE7.5 diets respectively compared to rats fed the HC diet. No significant difference was observed between animals fed the BD and the treatment diets. This decrease in LDL-C / HDL-C ratio in *P. florida* treated animals strongly supports the anti-atherogenic activity of this mushroom previously reported by [23]. The present study further reveals that the anti-atherogenic potential of the extracts is equally important in the reduction of cardiovascular disease risk. In the light of growing consumer interest in convenience and functional foods, and the increased consumption of mushroom juice, the present findings are important in promoting mushroom juice as a functional food and provide more versatility in the consumption and use of mushrooms.

Conclusion

The present study has demonstrated that the inclusion of *Pleurotus florida* extracts like powder in the diets of rats fed high cholesterol diets suppresses weight gain, through increased fecal lipid excretion and a reduction in total cholesterol and total triglycerides. Other mechanisms are suggested. Consumption of *P. florida* increased HDL-C, and lowered HDL-C / LDL-C ratio. *P. florida* extracts were as effective as the powders in their effects in stimulating lipid metabolism, and reducing CVD risk. Thus *P. florida* extracts like powder are potential convenient functional foods in the prevention and management of hypercholesterolemia, obesity and its related complications.

References

1. Mendis S, Puska P, Norrving B (2011) Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization, Geneva, (Section A).
2. Omboni S, Giorgia C, Edoardo G, Stefano C (2013) Awareness, treatment, and control of major cardiovascular risk factors in a small-scale Italian community: results of a screening campaign. VHRM 9: 177-185.
3. Stapleton PA, Goodwill AG, James ME, Brock RW, Frisbee JC (2010) Hypercholesterolemia and microvascular dysfunction: interventional strategies. J Inflamm (Lond) 7: 54.
4. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ; Comparative Risk Assessment Collaborating Group (2002) Selected major risk factors and global and regional burden of disease. Lancet 360: 1347-1360.
5. Thillaimaharani KA, Sharmila K, Thangaraju P, Karthick M, Kalaiselvam M (2013) Studies on antimicrobial and antioxidant properties of oyster mushroom *Pleurotus florida*. IJPSR 4: 1540-1545.
6. Wandati TW, Kenji GM, Onguso JM (2013) Phytochemicals in edible wild mushrooms from selected areas in Kenya. J Food Rese 2: 137-144.
7. Ramkumar L, Ramanathan T, Thirunavukkarasu P (2010) Antioxidant and radical scavenging activity of nine edible mushrooms extract. IJP 6: 950-953.
8. Pushpa H, Purushothama KB (2010) Nutritional analysis of wild and cultivated edible medicinal mushrooms. WJDFS 5: 140-144.
9. Mattila P, Suonpaa K, Piironen V (2000) Functional properties of edible mushrooms. Nutrition 16: 694-696.
10. Hobbs C (1995) Medicinal Mushrooms: An exploration of Traditional, Healing and Culture. Santa Cruz, CA: Botanica Press.
11. Breene WM (1990) Nutritional and medicinal value of specialty mushrooms. J Food Protec 53: 883-894.
12. Priya G, Chellaram C (2011) *In vivo* Anti–hyperlipidemic effects of edible mushroom, *Agaricus biosporus*. J Advanc Biotech 10: 38-40.
13. Alam N, Yoon KN, Lee TS (2011) Antihyperlipidemic activities of *Pleurotus ferulae* on biochemical and histological function in hypercholesterolemic rats. J Res Med Sci 16: 776-786.
14. Alam N, Amin R, Khan A, Ara I, Shim MJ, et al. (2009) Comparative effects of oyster mushrooms on lipid profile, liver and kidney function in hypercholesterolemic rats. Mycobiology 37: 37-42.
15. Chang ST (1991) Cultivated mushrooms. In: Arora DK, Mukerji KG, Marth EH (eds) Handbook of Applied Mycology, Foods and Feed. Boca Raton: CRC Press 3: 221-240.
16. Yongabi K, Agho M, Carrera D (2004) Ethnomycological study of wild mushrooms in Cameroon, Central Africa. MAI 16: 34-36.
17. van Dijk H, Onguene NA, Kuyper TW (2003) Knowledge and utilization of edible mushrooms by local populations of the rain forest of south Cameroon. Ambio 32: 19-23.
18. Yu S, Weaver V, Martin K, Cantorna MT (2009) The effects of whole mushrooms during inflammation. BMC Immunol 10: 12.
19. Im KH, Nguyen TK, Shin do B, Lee KR, Lee TS (2014) Appraisal of antioxidant and anti-inflammatory activities of various extracts from the fruiting bodies of *Pleurotus florida*. Molecules 19: 3310-3326.
20. Bobek P, Nosálová V, Cerná S (2001) Effect of pleuran (beta-glucan from *Pleurotus ostreatus*) in diet or drinking fluid on colitis in rats. Nahrung 45: 360-363.
21. Bobek P, Ozdin L, Kuniak L (1993) Influence of water and ethanol extracts of the oyster mushroom (*Pleurotus ostreatus*) on serum and liver lipids of the Syrian hamsters. Nahrung 37: 571-575.
22. Bobek P, Ozdín L, Kajaba I (1997) Dose-dependent hypocholesterolaemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats. Physiol Res 46: 327-329.
23. Bajaj M, Vadhera S, Brar AP, Soni GL (1997) Role of oyster mushroom (*Pleurotus florida*) as hypocholesterolemic / antiatherogenic agent. Indian J Exp Biol 35: 1070-1075.

24. Khan MA, Rahman M, Mousumi T, Uddin MN, Ahmed S (2011) *Pleurotus sajor-caju* and *Pleurotus florida* Mushrooms improve some extent of the antioxidant systems in the liver of hypercholesterolemic rats. ONJ 4: 20-24.
25. Sengupta A, Ghosh M (2013) Protective role of phytosterol esters in combating oxidative hepatocellular injury in hypercholesterolemic rats. Pak J Biol Sci 16: 59-66.
26. Lobe EE (2012) Antihypercholesterolemic Effects of *Pleurotus florida* (*pleurotaceace*) aqueous extracts in hypercholesterolemic rats. MSc. Thesis, ENSAI, University of Ngaoundere, pp: 94.
27. Guo FC, Williams BA, Kwakkel RP, Verstegen MW (2003) *In vitro* fermentation characteristics of two mushroom species, an herb, and their polysaccharide fractions, using chicken cecal contents as inoculum. Poult Sci 82: 1608-1615.
28. Makkar HPS, Blummel M, Borowy NK, Becker K (1993) Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agri 6: 161-165.
29. Devani MB, Shishoo C, Shah AS, Suhagia BN (1989) Spectrophotometric method for micro determination of Nitrogen in Kjeldahl Digest. JAOAC 72: 953-956.
30. Fischer E, Stein EA (1961) DNS colorimetric determination of available carbohydrates in foods. Biochem Prepar 8: 30-37.
31. Bourely J (1982) Observation sur le dosage de l'huile des graines de cotonnier. Coton et Fibre Tropicales 27: 183-196.
32. Mariod AA, Ibrahim RM, Ismail M, Ismail N (2012) Antioxidant activity of phenolic extracts from kenaf (*Hibiscus cannabinus*) seedcake. Grasas Y Aceites, 63: 167-174.
33. Kårlund A, Ulvi M, Mari S, Karjalainen RO (2014) The impact of harvesting, storage and processing factors on health-promoting phytochemicals in berries and fruits. Processes 2: 596-624.
34. Whitney E, Sharon RR (2011) Understanding Nutrition. (12thedn) Belmont, CA: Wadsworth, (Chapters 4,5,7,9).
35. Tyrovolas S, Lionis C, Zeimbekis A, Bountziouka V, Micheli M, et al. (2009) Increased body mass and depressive symptomatology are associated with hypercholesterolemia among elderly individuals; results from the MEDIS study. LHD 8: 10.
36. Gropper SS, Smith JL, Groff JL (2009) Advanced Nutrition and Human Metabolism, (5th edn), Belmont, USA: Wadsworth, (Section II).
37. Khairunnuur FA, Zulkhairi A, Hairuszah I, Azrina A, Nursakinah I, et al. (2010) Hypolipidemic and weight reducing properties from *Tamarindus indica L.* pulp extract in diet induced obese rats. IJP 6: 216-223.WE
38. Cheung PC (1998) Plasma and hepatic cholesterol levels and fecal neutral sterol excretion are altered in hamsters fed straw mushroom diets. J Nutr 128: 1512-1516.
39. Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365: 1415-1428.
40. Ahmed N, Dawson M, Smith C, Wood E (2007) Diet and disease. In: Owen E (edn) Biology of disease. New York: Taylor & Francis pp. 239-276.
41. Keim NL, Marlett JA, Amundson CH, Hagemann LD (1982) Comparison of rat hepatic cholesterol biosynthesis during skim milk versus whey permeate ingestion. J Dairy Sci 65: 2274-2280.
42. Bobek P, Hromadová M, Ozdín L (1995) Oyster mushroom (*Pleurotus ostreatus*) reduces the activity of 3-hydroxy-3-methylglutaryl CoA reductase in rat liver microsomes. Experientia 51: 589-591.
43. Park HS, Choi JS, Kim KH (2000) Docosahexaenoic acid-rich fish oil and pectin have a hypolipidemic effect. JNR 20: 1783-1794.
44. Mayes PA (1997) Metabolism of lipids. In: Harper HA, Rodwell VW, Mayes PA (eds). Review of physiological chemistry. Los Altos: Lange Publications, pp: 280-321.
45. Bobek P, Kuniak L, Ozdín L (1993) The mushroom *Pleurotus ostreatus* reduces secretion and accelerates the fractional turnover rate of very-low-density lipoproteins in the rat. Ann Nutr Metab 37: 142-145.
46. Fukushima M, Nakano M, Morii Y, Ohashi T, Fujiwara Y, et al. (2000) Hepatic LDL receptor mRNA in rats is increased by dietary mushroom (*Agaricus bisporus*) fiber and sugar beet fiber. J Nutr 130: 2151-2156.
47. Kuhn DJ, Burns AC, Kazi A, Dou QP (2004) Direct inhibition of the ubiquitin-proteasome pathway by ester bond-containing green tea polyphenols is associated with increased expression of sterol regulatory element-binding protein 2 and LDL receptor. Biochim Biophys Acta 1682: 1-10.
48. Bursill C, Roach P, Bottema C, Pal S (2001) Green tea up regulates the low-density lipoprotein receptor through the sterol-regulated element binding protein in HepG2 liver cell. J Agri Food Chem 49: 5639-5645.
49. Ngamukote S, Mäkynen K, Thilawech T, Adisakwattana S (2011) Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules 16: 5054-5061.
50. Mertz DP (1980) "Atherosclerosis-index" (LDL/HDL): risk indicator in lipid metabolism disorders. Med Klin 75: 159-161.