ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY

Research Article

Bioactive Constituents and Hypocholesterolemic Effect of Pleurotus ostreatus (Jacq.: Fr.), a Potential for the Reduction of Cardiovascular Risk

Duru Majesty Kelechi[1]*, Nwadike Constance Nnedimma[2], Ezekwe Ahamefula Sunday[3], Aaron Chinomso Friday[4], Akataobi Kelechi Uzoma[5], Alugbuo Stanley Uzoma[6] and Eboagwu Ijeoma[7]

[1]*Department of Chemical Sciences (Biochemistry Unit), Rhema University, Nigeria*

[2]*Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria*

[3]*Department of Medical Biochemistry, College of Medical Sciences, River State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria*

[4]*Department of Biochemistry, Abia State University, Uturu, Nigeria*

[5]*Department of Medical Biochemistry, Abia State University, Uturu, Nigeria*

[6]*Department of Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo Ebonyi State, Nigeria*

[7]*Department of Food Technology, Federal Institute of Industrial Research Oshodi, Nigeria*

***Corresponding Author:** Duru Majesty Kelechi, Department of Chemical Sciences (Biochemistry Unit), Rhema University, Nigeria.

Received: March 28, 2019; **Published:** January 31, 2022

Abstract

Objectives: This study identified the bioactive constituents of *Pleurotus ostreatus* and analyzed the potential effect of a diet rich in *P. ostreatus* on reducing the cardiovascular risk on rats.

Methods: The bioactive constituents of *P. ostreatus* were evaluated using standard methods whereas for hypocholesterolemic study, forty-five male Wistar albino rats weighing between 50g-70g were procured and separated into four groups of control (standard growers mesh + 10.00g of cholesterol diet +potable water), reference (nutrend (known commercial baby food) + potable water) and three test groups I (5% of *P. ostreatus* incorporated diet+10.00g of cholesterol diet + potable water), II (15% of *P. ostreatus* incorporated diet +10.00g of cholesterol diet + potable water), and III (25% of *P. ostreatus* incorporated diet +10.00g of cholesterol diet + potable water).

Results: Results of bioactive constituents of *P. ostreatus* revealed the presence of saponins, tannins and flavonoids as phytochemicals as well as proteins, peptides, and some individual amino acids. Total food intake and weight gained reduced significantly ($p < 0.05$) in test rats against the control. Apart from weight of heart and liver of test group II rats and spleen of test group III; the weight of other visceral organs of test rats were insignificantly affected ($p > 0.05$) against the control. Results obtained for lipid profiles reveled significant reduction ($p < 0.05$) in cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol, and non-cholesterol levels in test groups against those of the control. The lipid profile ratios in test rats showed significant improvement against those of the control but compared favourably with those of the reference in some lipid profile ratio parameters.

Conclusion: The observed hypocholesterolemic property and the lipidaemic strength could be linked to the presence of bioactive constituents in *P. ostreatus* incorporated in the diet given to test rats.

***Keywords**: Bioactive Constituents; Lipid Profile; Diet; Pleurotus ostreatus; Visceral Organ*

Introduction

Aside serving as food materials [1-3], most edible plants also serve as therapeutic materials against disease conditions [4-6]. It has been noted that foods of plant origin contain bioactive constituents which are effective against certain disease causing pathogens [7-9]. Okwu [10] noted that bioactive constituents exert physiological effects against disease causing organisms. Diseases such as cancer, ulcers, malaria, hepatitis, pile, liver cirrhosis and others have been reported as being among those whose pathogens are influenced by the physiological effects of some bioactive constituents found in plants [11-14]. According to Amadi., *et al.* [1], different researchers have noted that the ability of plant to have medicinal property lies in certain plant`s chemical compounds that are bioactive in nature.

Research using plant therapy on dyslipidemia has been on the increase in recent years [15,16]. Dyslipidemia is a condition of high or low lipid blood stream level [17]. The condition has been noted as major risk factor of cardiovascular diseases [17]. Dyslipidemia can be primary or secondary, and can accompany diseases such as hypertension, diabetes mellitus or obesity [18]. Dyslipidemia usually involves a deviation from the normal levels of plasma triglyceride, total-LDL-cholesterol, VLDL-cholesterol and HDL-cholesterol [19]. According to Gordon and William [16], the most common types of dyslipidemia are high levels of low-density lipoprotein (LDL or "bad") cholesterol; low levels of high-density lipoprotein (HDL or "good") cholesterol and high levels of triglycerides. Secondary causes have been reported as causative agents for many cases of dyslipidemia in adults. Robert [18] noted that the secondary causes of dyslipidemia are type 2 diabetes mellitus, excessive alcohol consumption, cholestatic liver diseases, and nephrotic syndrome. Sedentary lifestyle with excessive dietary intake of saturated fats, cholesterol and trans-fats has also been implicated as one of the causes of dyslipidemia in adult [20]. According to Anne [20], trans-fats are polyunsaturated or monounsaturated fatty acids to which hydrogen atoms have been added; they are used in many processed foods and are as atherogenic as saturated fat.

In recent times, research on the medicinal potential of mushroom has been on the increase [21-25]. According to María [26], more than 100 medicinal functions are produced by mushrooms and fungi; and the key medicinal uses are antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects. Yu., *et al*. [27] noted that mushrooms are effective against tumor development and inflammatory processes. It has also been reported that numerous molecules synthesized by macrofungi are bioactive. These bioactive compounds are found in fruit bodies, cultured mycelium, and cultured broth [28]. They are important for modern medicine and include; polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes, ascorbic, and organic acids [29,30].

Studies on *Pleurotus specie* mushrooms have been reported by different authors [31,32]. *Pleurotus species* are among the edible mushrooms and are popularly or generally known as oyster mushrooms due to their shape [33]. They are known for their pleasant taste and excellent flavor and are recognized for their nutritional importance [31]. Members of the species have also been reported to contain important bioactive compounds that can facilitate physiological and pharmacological activities [26]. *Pleurotus ostreatus* is among the known *Pleurotus specie* mushrooms, characterized by a white spore print attached to decurrent gills, often with an eccentric stipe, or no stipe at all. It grows on wood in nature, usually on dead standing trees or fallen logs. *P. ostreatus* along with other members of *Pleurotus species* have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects [33]. Evaluation of medicinal potentials of oyster mushroom on modulating immune system, inhibition of tumour growth and inflammation, hypoglycemic and antithrombotic activities, prevention of high blood pressure and atherosclerosis as well as microbial and other activities of the mushroom have been reported by different authors [34]. However, these studies were mostly centered on *Pleurotus tuber-regium*, which is relatively known and forms part of staple foods than other members of the *Pleurotus species*.

There is need to extend the study on evaluation of medicinal potentials of *P. species* to accommodate the lesser known members other than *P. tuber-regium.* The present study evaluated the bioactive constituents and anti-cholesterolemic effect of *P. ostreatus* (Jacq.: Fr.), as a potential for the reduction of cardiovascular risk.

Materials and Methods

Collection of mushrooms

Matured *P. ostreatus* samples used in this study were purchased from a village market of Emekuku town in Owerri North Local Government of Imo State, South-Eastern Nigeria. The purchased samples were properly identified and transported to the laboratory where they were prepared for usage.

Bioactive constituents

Bioactive constituents such as flavonoids and the variety such as chalcones, aurones, flavones, flavonols, and leukoanthocyanins were screened using the methods as described by AOAC [35]. Saponins, tannins and alkaloids were qualitatively determined as described by described by Odika., *et al*. [36], and Ezekwe., *et al*. [37]. Proteins, amino acids, aromatic amino acids and the individual amino acids such as tyrosine, phenylalanine, tryptophan, arginine, cysteine, cystine, and proline were qualitatively analyzed following the methods described Ojiako and Akubugwo [38].

Feed preparation for experimental animals

Milled *P. ostreatus* was mixed with standard growers mash to obtain a 5%, 15%, and 25% dietary incorporation of the mushroom. The method as described by Ijeh*., et al.* [39], was used to transform the mixtures obtained into pelletized form.

Experimental animals

Forty-five male wistar albino rats weighing between 50g - 70g obtained from the animal colony of Department of Biochemistry, Abia State University, Nigeria were procured for this study. The animals were housed in clean and dry plastic cages with good ventilation, and were given pelletized commercial rat feed (Pfizer Livestock Co., Ltd, Aba, Nigeria), and potable water *ad libitum* for four weeks to enable them acclimatize to their new environment. After acclimatization, the rats were divided into five groups of nine rats each with equalized weights. Three groups served as the test groups and received the prepared standard growers mesh incorporated with the mushroom *P. ostreatus*. The remaining two groups served as experimental control and reference. Apart from the reference, experimental control and all the test groups were placed on 10.00g of cholesterol diet.

Treatments given to the animals are designated below.

Control groups

Reference =Nutrend (known commercial baby food) + potable water.

Experimental control=Standard growers mesh + 10.00g of cholesterol diet +potable water.

Test groups

Test group I = 5% of *P. ostreatus* incorporated diet+10.00g of cholesterol diet + potable water; Test group II = 15% of *P. ostreatus* incorporated diet +10.00g of cholesterol diet + potable water; Group III = 25% of *P. ostreatus* incorporated diet +10.00g of cholesterol diet + potable water.

The cholesterol diet was introduced on more than 2.5% per kilogram body weight of animal as reported by other studies. The food intake and weight of the animals were taken on weekly basis throughout the period of the study. The treatments of experimental animals were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [40] and the treatment lasted 28 days.

Blood sample and tissue collection

The experimental animals were sacrificed under light chloroform anesthesia after 4 weeks of feeding and overnight fasting of 10 - 14 hours. Blood samples were collected by cardiac puncture into EDTA-tubes for lipid and lipoproteins profiles. The plasma samples were separated from cells by centrifugation. The plasma samples were prepared under refrigeration until analyzed. The heart, kidney, liver and spleen organs of each animal were also excised, blotted on filter paper to remove blood and the wet weight were measured. The final weights of the experimental animals were also taken before sacrifice.

Plasma lipid profiles determination

Assay of plasma total cholesterol, HDL-cholesterol, and triglyceride were assayed enzymatically with Randox test kits (Randox Laboratories, England). The methods as described by Friedwald*., et al.* [41]; [LDL-cholesterol (mg/dl) = Total cholesterol (mg/d) - (HDL-cholesterol (mg/dl) - TG/5)]; and VLDL-cholesterol = [Triglyceride/5] were used to estimate the LDL-cholesterol and VLDL-cholesterol respectively. Plasma non-HDL-cholesterol concentration was determined as reported by Bruzell*., et al* [42]. The established relationship for Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC), Atherogenic Index of Plasma (AIP), LDL-cholesterol/HDL-cholesterol ratio, and triglyceride/HDL-cholesterol ratio were used for their estimation.

Statistical analysis

Values were presented as mean and standard error of mean. + (presence) and - (absence) signs were used for the bioactive constituents (Table 1), whereas bar charts were used for values in figure 1-5. Significant difference between bars was established at 5% levels using least significant difference (LSD).

Results and Discussion

Constituents	Status
Saponins	+
Flavonoids	+
*Chalcones	-
*Flavones	+
*Flavonol	+
*Leukoanthocyanins	-
Tannins	++
Alkaloids	+

Table 1: *Screening of phytochemical constituents in P. ostreatus*

Key: (+) = presence; and (-) = absence.

The bioactive constituents found in the present study include saponins, flavonoids and the variety such as flavones and flavonols, tannins and alkaloids. The importance of these constituents cannot be overemphasized. They have been reported by different authors to have prominent effects on animals and plants. According to Seigler [43], saponins are glycoside components often referred to as natural detergents due to their foamy nature. Saponins have been known to possess both beneficial and deleterious properties, based on their concentration in a sample. Seigler [43] implicated saponins against cholesterol increase as well as inhibition of growth of cancer cells. The multiple biological effects of flavonoids, which include antibacterial, antiviral, antitoxic and anti-inflammatory activities, have been reported. Many of these effects according to Jimoh and Oladiji [44], have been liked to their known functions as strong antioxidants, free radical scavengers and metal chelators. Also, the medicinal contributions of tannins and alkaloids are well established amongst researchers. The presence of flavonoids in the present study is in line with the earlier study of Obadai., *et al*. [45]. Afiukwa., *et al*. [46] also reported the presence of alkaloid and saponin in an earlier study on *P. ostreatus*.

Constituents	Status
Proteins	+++
Amino acid	++
Peptide	+
Aromatic amino acid	++
Tyrosine	++
Phenylalanine	++
Tryptophan	++
Arginine	+
Cysteine	+
Cystine	+
Proline	++

Table 2: *Qualitative tests for proteins and amino acids in P. ostreatus*

Key: (+) = present; and (-) = absence.

The physiological activities of proteins and amino acids have been noted [47]. The qualitative tests for proteins and amino acids of the studied sample (Table 2) revealed the presence of proteins, amino acids, peptide, aromatic compound and individual amino acids such as tyrosine, phenylalanine, tryptophan, arginine, cysteine, and proline. The observed proteins, amino acids, and individual amino acids such as tyrosine, phenylalanine, tryptophan, arginine, cystine, and proline agree with earlier work of Patil., *et al*. [48] on *P. ostreatus*. By definition, amino acid is known as any of a class of organic compounds containing the amino (NH_2) and the carboxyl (COOH) groups, occurring naturally in plant and animal tissues and forming the chief constituents of protein. Peptide, a compound consisting of two or more amino acids linked in a chain, with the carboxyl group of each acid being joined to the amino group of the next by a bond of the type -OC-NH- is normally found in proteins [1,49]. Functionally, dietary aromatic amino acids are need for the synthesis of phenylalanine and tyrosine [49]. Tyrosine is the precursor of catecholamines and thyroid hormones [47]. Phenylalanine is an essential amino acid and the precursor of tyrosine amino acid in the liver and kidney [50]. Tryptophan gives rise to serotonin, a neurotransmitter of the brain, platelet clothing factor and neurohormones. Arginine has been implicated against cancer, and tumour. Cystine is a dimer of cysteine, and cysteine is a known precursor of glutathione, a known detoxifying agent found in the liver. Proline aids in the healing of cartilage and the strengthening of joints, tendons, and heart muscle. It also plays important role in the production of collagen and improves the skin by reducing the loss of collagen through aging process [51,52].

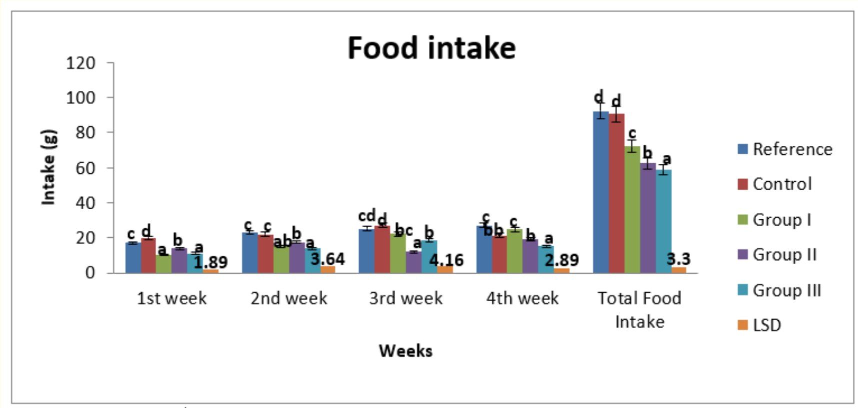

Figure 1: *Weekly/ total food intake of rats placed on diet incorporated with P.ostreatus*

The weekly and total food intake of the rats place on *P. ostreatus* as shown in figure 1, revealed increased consumption with increase in number of weeks except in group III and the control where the weekly food intake reduced in 4th week. However, total food intake for the rats in test groups (I, II, and III) significantly ($p < 0.05$) reduced against those of control and reference (Figure 1). Palatability of the incorporated diet could be behind the observed reduction in food consumption. Weight gained by the rats progressively increased in weeks for all the groups as observed in the present study (Figure 2). However, on comparison, weight gained by test groups I, II, II rats reduced significantly ($p < 0.05$) against those of the control and reference. Test group III rats gained the least weight while test group I rats gained the highest weight among test group rats. Weight loss has been reported to help improve certain disease conditions. Weight loss helps to improve and control coronary risk incidence, diabetes mellitus, dyslipidemia, hypertension, obesity, and physical functioning [42,53].

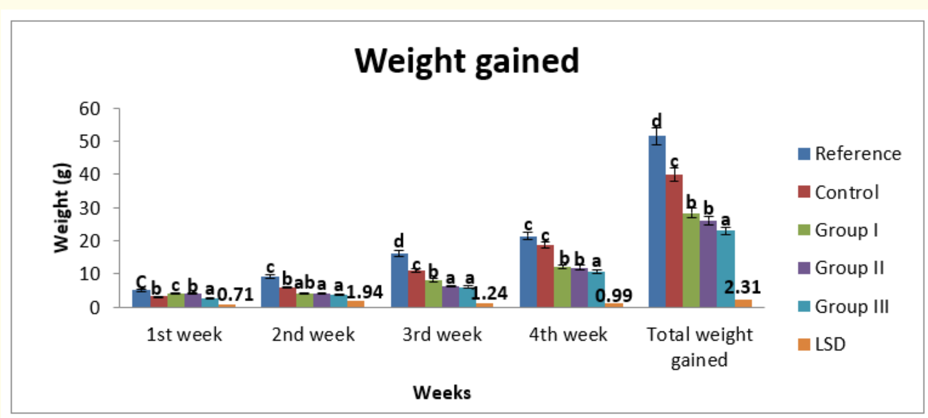

Figure 2: *Weekly/total weight gained by rats placed on diet incorporated with P. ostreatus.*

It has been reported that expansion of organs adds to body weight [19]. Expansion and injury of the organs could be linked to disease condition [9, 19]. No death was recorded among the test rats in the present study. Apart from weight of heart and spleen in test group III and liver in test groups II and III, weight of other visceral organs of test groups in this study were insignificantly ($p > 0.05$) affected when compared to those of the control and reference (Figure 3).

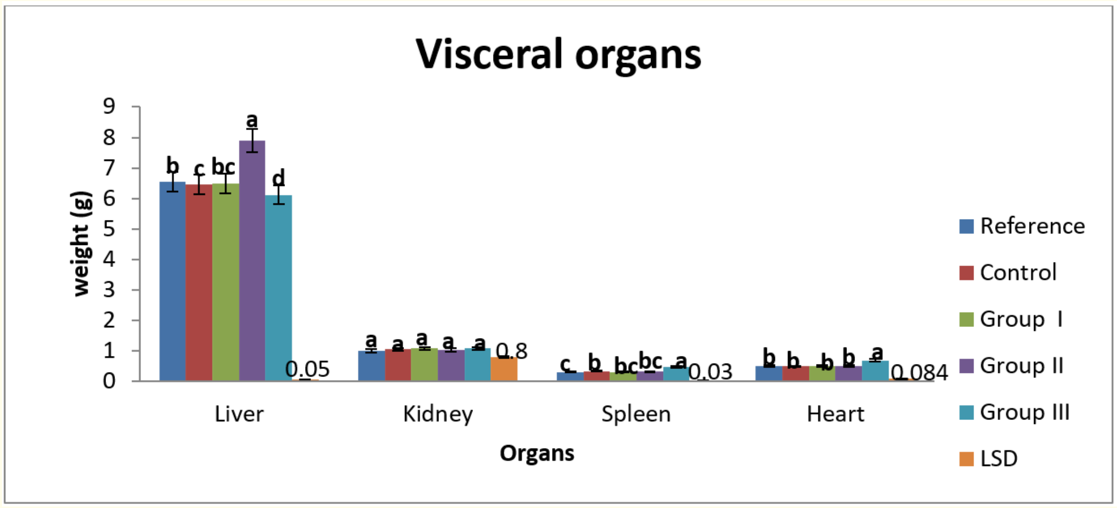

Figure 3: *Weight of visceral organs in rats placed on diet incorporated with P. ostreatus.*

Lipid profile indices (Figure 4) are used to determine or predict the status of the body in relation to heart diseases such as atherosclerosis, heart attack; stroke, etc [54]. It is also one of the strategies for increasing low HDL cholesterol levels [55]. Cholesterol is an essential substance involved in many cellular functions which include the maintenance of membrane fluidity, production of vitamin D on the surface of the skin, production of hormones, and possibly helping cell connections in the brain. However, rise in cholesterol levels in blood has been known for its causative role in arthrosclerosis. Arthrosclerosis is among the leading causes of death in most developed nations of the world. Cholesterol levels of test groups I, II, and III in the present study reduced significantly ($p < 0.05$) against the control and increased significantly ($p < 0.05$) when compared to the reference. The reduced levels of cholesterol in test groups against the control could be due to the inhibition of the absorption of dietary cholesterol in the intestine or stimulation of the biliary secretion of cholesterol and cholesterol excretion in faeces by the *P. ostreatus*. Triglyceride are partly taken up with diet and partly synthesized in the liver, and dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn, increases the levels of hepatic and plasma triglyceride [56]. Considerable evidence has shown that triglycerides are associated with coronary atherosclerosis [57]. Observed triglyceride levels of test groups in the present study reduced significantly ($p < 0.05$) against the control and increased significantly ($p < 0.05$) when compared to the reference. High density lipoprotein cholesterol (HDL-cholesterol) has been implicated in cardiovascular disease (CVD). Mora., *et al.* [58] noted that low HDL-cholesterol has been described in various forms of familial lipid disorders as part of metabolic syndrome. The HDL-cholesterol levels increased significantly ($p < 0.05$) in test rats when compared to those of the control and reference. The incontrovertible nature of arthrosclerosis and increase in levels of low density lipoproteins-cholesterol are (LDL-cholesterol) in the blood are well established [57]. LDL-cholesterol does not always get to its destination, but accumulates in artery walls causing atherosclerosis, which is among the leading cause of death as well as disability in the developed world [59]. It has been noted that LDL-cholesterol has been the primary focus of most lipid lowering therapies because it is a well-known risk factor for heart disease. LDL-cholesterol levels of test rats reduced significantly ($p < 0.05$) against those of the control and increased significantly ($p < 0.05$) when compared to the reference.

The production of very-low-density lipoprotein (VLDL-cholesterol) cholesterol has been associated with the liver, and development of plague deposit on artery walls [60]. Observed VLDL-cholesterol levels of test rats reduced significantly ($p < 0.05$) against the control and increased insignificantly ($p > 0.05$) when compared to the reference (Figure 4). Measuring non-HDL cholesterol levels gives a better assessment of the risk for heart diseases than measuring only LDL or even total cholesterol [9]. Non-HDL-cholesterol is a better predictor of cardiac death than LDL-cholesterol, and should be used as a treatment target for cholesterol [61]. According to Millian *et al*. [62], non-HDL-cholesterol has therefore been recommended as a secondary therapeutic target in individuals with high triglyceride concentration. The same authors suggested that non-HDL-cholesterol could be a surrogate marker of serum apoB concentration in clinical practice. Non-cholesterol levels of the test groups in the present study reduced significantly ($p < 0.05$) when compare to the control, but increased significantly ($p < 0.05$) against the reference. A large variety of mushrooms have been utilized traditionally in the last decade, the interest for pharmaceutical potential of mushrooms has been increased rapidly, and it has been suggested that many mushrooms are like mini-pharmaceutical factories producing compounds with miraculous biological properties.

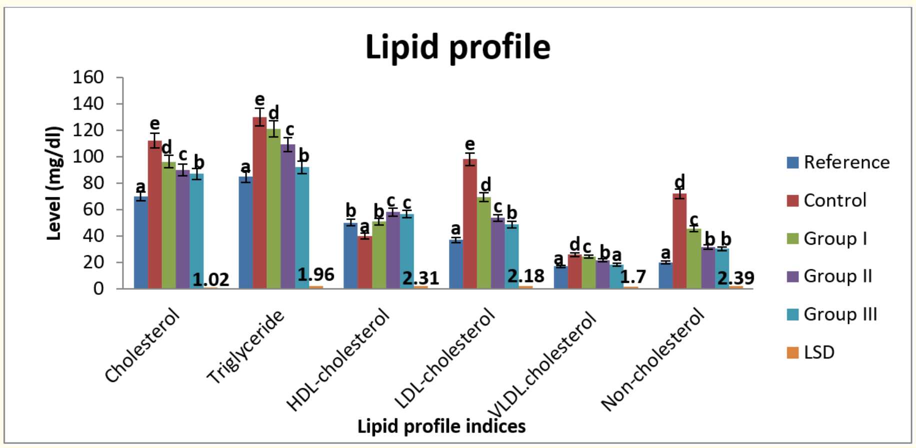

Figure 4: *Lipid profile indices of rats placed on diet incorporated with P. ostreatus.*

Lipid profile ratios are powerful indicators of the risk of heart disease [63-67]. Millian., *et al*. [62], noted total/HDL cholesterol (Cardiac Risk Ratio) and LDL/HDL cholesterol ratios are also good predictors of the degree of clinical benefit to be derived from lipid-lowering intervention. The observed levels of Cardiac Risk Ratio of the present study as present in figure 5, reduced significantly ($p < 0.05$) in test groups against the control. Cardiac Risk Ratio levels of test groups II and III increased insignificantly ($p > 0.05$) when compared to reference. LDL/HDL cholesterol ratio reduced significantly ($p < 0.05$) against the control and increased significantly ($p < 0.05$) when compared to reference. Nunes., *et al*. (2015) noted that atherogenic index of plasma (AIP) and the atherogenic coefficient (AC) are important atherogenic indexes. The levels of atherogenic coefficient and atherogenic index of plasma reduced significantly ($p < 0.05$) in test rats against those of the control. Atherogenic coefficient in test groups increased significantly ($p < 0.05$) against the reference. Atherogenic index of plasma increased significantly ($p < 0.05$) in test groups I and II against the reference and increased insignificantly ($p > 0.05$) in test group III when compared to the reference (Figure 5). Triglyceride/HDL-cholesterol ratio levels reduced significantly ($p < 0.05$) test groups when compared to the control and increased significantly ($p < 0.05$) against the reference. The observed significant reduction in lipid profile ratios of the test groups against those of the control and some cases compared favourably with the reference in the present study could be indication that *P. ostreatus* may lower atherogenic potential of the plasma.

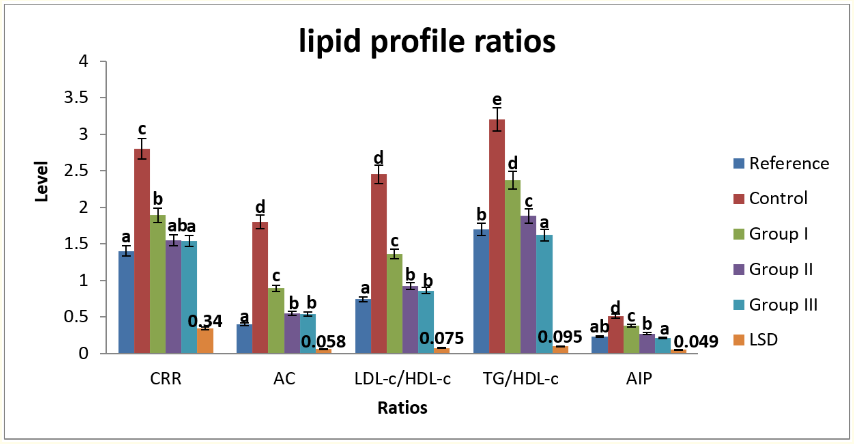

Figure 5: *Lipid profile ratios of rats placed on diet incorporated with P. ostreatus.*

Conclusion

The therapeutic benefits of many plant foods have been the focus of many extensive dietary studies and for centuries they have been used for treatment of illness, without subjecting many of them to scientific evaluation. The evaluation of diets incorporated with *P. ostreatus* influenced the lipid profiles and the lipid profile ratios of rats placed on high cholesterol diet positively. The findings of the present study may have pinched the consumption of *P. ostreatus* against high cholesterol level, thereby showing its anti-cholestrolemic property. The lipidaemic strength of the studied mushroom increased with increase in concentration. There is no doubt that the observed anti-cholestrolemic property and the lipidaemic strength could be linked to the presence of bioactive constituents of the studied mushroom specie. The present study has revealed the bioactive constituents and anti-cholesterolemic effect of *P. ostreatus* (Jacq.: Fr.), as a potential for the reduction of cardiovascular risk.

Bibliography

1. Amadi B., *et al*. “Elemental, amino acid and phytochemical constituents of three different species of eggplant”. *International Journal of Medicinal and Aromatic Plants* 3.2 (2013): 200-203.

2. Duru MKC., *et al*. “In vivo studies of Solanum aethiopicum fruit on some biochemical parameters using rats”. *Journal of Chemical and Pharmaceutical Research* 5.2 (2013): 1-4.

3. Duru M., *et al*. “Evaluation of some chemical constituents of “ji-otor” and “ntubiri ikpa” traditional foods of Ikwerre ethnic national in Nigeria”. *Proceedings of the 36th Annual International Conference, Workshop and Exhibition of Chemical Society of Nigeria* 1 (2013): 38-44.

4. Prasad B., *et al*. “Compositional characterization of traditional medicinal plants: Chemo-metric approach”. *Archives of Applied Science Research* 2.5 (2010): 1-10.

5. Arukwe U., *et al*. “Chemical composition of Persea americana leaf, fruit and seed”. *International Journal of Research and Reviews in Applied Sciences* 11.2 (2012): 355-358.

6. MKC., *et al*. "Biochemical studies on "Udu" an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria". *Continental Journal of Pharmacology and Toxicology Research* 5.2 (2012): 28-34.

7. Okigbo RN and Mmeka EC. "An appraisal of phytomedicine in Africa". *KMITL Science and Technology Journal* 6.2 (2006): 83-94.

8. Duru M., *et al*. "Nutritional evaluation and some biochemical studies on Ageratum conyzoides using its different parts". *Proceedings of the 35th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria* 1 (2012): 372-379.

9. Duru M., *et al*. "Bioactive constituents of Pleurotus squarrosulus (Mont) Singer and Effects of its dietary incorporation on body/organ weights and lipid profile levels of rats placed on high cholesterol diet". *Academic Journal of Chemistry* 2.4 (2017): 28-37.

10. Okwu DE. "Phytochemical, vitamin and mineral contents of two Nigeria medicinal plants". *International Journal of Molecular Medicine and Advance Sciences* 1.4 (2005): 375-381.

11. Sofowora A. "Medicinal plants and traditional medicine in Africa". 2nd edition., Spectrum Books Ltd. Ibdadan (2006).

12. Duru MKC., *et al*. "Bioactive constituents and macronutrients composition of anti-malarial concoction used in Umunchi village in Isiala Mbano L.G.A of Imo State, Nigeria". *International Science Research Journal* 3 (2011): 61-64.

13. Duru M., *et al*. "Chemical constituents of Buchholzia coriacea seed." *Proceedings of the 35th Annual International Conference, Workshop and Exhibition of Chemical Society of Nigeria* 2 (2012): 39-45.

14. Ugbogu AE., *et al*. "Toxicity evaluation of the aqueous stem extracts of Senna alata in Wistar Rats". *American Journal of Biomedical Research* 4.4 (2016): 80-86.

15. Ikewuchi JC and Ikewuchi CC. "Anti-cholesterolemic effect of aqueous extract of the leaves of Chromolaena odor (L) king and robinson (asteraceae): Potential for the reduction of cardiovascular risk". *Pacific Journal of Science and Technology* 12 (2011): 385-391.

16. Gordon HW and William FY. 'Dyslipidemia". hormone health network. International Resource Center. (2013).

17. Vodnala D., *et al*. "Secondary causes of dyslipidemia". *American Journal of Cardiology* 110 (2012): 823.

18. Robert SR. "Secondary causes of dyslipidemia". (2016).

19. Duru M., *et al*. "Effect of "udu", an antimalarial herbal preparation on visceral organ weight and blood lipid profiles in wistar rats". *JPCS* 8 (2014): 1-7.

20. Anne CG. "Dyslipidemia (Hyperlipidemia). Msdmanual professional version". (2015).

21. Chen H., *et al*. "Antioxidant activities of polysaccharides from Lentinus edodes and their significance for disease prevention". *International Journal of Biological Macromolecules* 50.1 (2012): 214-218.

22. Finimundy TC., *et al*. "Aqueous extracts of Lentinula edodes and Pleurotus sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity". *Nutrition Research* 33.1 (2013): 76-84.

23. Khan MA., *et al*. "Hericium erinaceus: an edible mushroom with medicinal values". *Journal of Complementary and Integrative Medicine* 10.1 (2013): 253-258.

24. Duru M., *et al*. "Evaluation of nutritional, anti-nutritional and some biochemical studies on Pleurotus squarrosulus (Mont.) singer using rats". *African Journal of Biochemistry Research* 12.2 (2018): 7-27.

25. Duru M., *et al*. “Nutritional, anti-nutritional and biochemical studies on the oyster mushroom, Pleurotus ostreatus”. *EC Nutrition* 14.1 (2019): 36-59.

26. María E., *et al*. “Edible mushrooms: improving human health and promoting quality life”. *International Journal of Microbiology* (2015): 1-14.

27. Yu S., *et al*. “The effects of whole mushrooms during inflammation”. *BMC Immunology* 10.12 (2009).

28. Wasser S. “Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides”. *Applied Microbiology and Biotechnology* 60.3 (2002): 258-274.

29. Chiejina NV and Olufokunbi JO. “Effects of different substrates on the yield and protein content of Pleurotus tuberregium”. *African Journal of Biotechnology* 9.11 (2010): 1573-1577.

30. Xu X., *et al*. “Bioactive proteins from mushrooms”. *Biotechnology Advances* 29.6 (2011): 667-674.

31. Adejumo T.O., *et al*. “Identification and Evaluation of Nutritional Status of some Edible and Medicinal Mushrooms in Akoko Area, Ondo State, Nigeria”. *International Journal of Current Microbiology and Applied Sciences* 4.4 (2015): 1011-1028.

32. Modi HA., *et al*. “Preliminary phytochemical screening and antibacterial activity of wild edible mushrooms collected from mahal forest of dang district, Gujarat, India”. *World Journal of Pharmacy and Pharmaceutical Sciences* 3.8 (2014): 1164-1174.

33. Alemu F. “Cultivation of Pleurotus ostreatus on Grevillea robusta leaves at Dilla University, Ethiopia”. *Journal of Yeast and Fungal Research* 5.6(2014): 74-83.

34. Egwim EC., *et al*. “Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms”. *American Journal of Food and Nutrition* 1.2 (2011): 89-94.

35. AOAC. “Official methods of analysis of association of official analytical chemists”. 18^{th} edition. Arlington, Virgina, USA: AOAC (2006).

36. Odika P., *et al*. “Bioactive profiling of Vitex doniana leaf and its effect on rat erythrocyte membrane stabilization activity”. *Pacific Journal of Science and Technology* 22.2 (2012): 167-181.

37. Ezekwe AS., *et al*. “Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis and antioxidant activity of Synsepalum dulcificum (Schumach and Thonn.Daneill) fruit”. *International Journal of Green and Herbal Chemistry* 1.1 (2021): 001-014.

38. Ojiako OA and Akubugwo EI. “An introductory approach to practical biochemistry”. CRC Publishers: Owerri, Imo State, Nigeria (1997).

39. Ijeh FI., *et al*. “Phytochemical composition of pleurotus tuber regium and effect of its dietary incorporation on body/organ weights and serum triacylglycerols in albino mice”. *Journal of Medicinal Plants Research* 3 (2009): 939-943.

40. National Institute of Health. “Guide for the care and use of laboratory animals”. U.S. Department of health education and welfare. Washington, D.C.: NIH Publication (1985).

41. Friedwald TW., *et al*. “LDL cholesterol estimation”. *Clinical Chemistry* 18 (1972): 499-501.

42. Bruzell JD., *et al*. “Lipoprotein management in patients with cardiometabolic risk”. *Journal of the American College of Cardiology* 51 (2008): 1512-1524.

43. Seigler DS. “Plants with saponins and cardiac glycosdies”. (1998).

44. Jimoh FO and Oladiji AT. "Preliminary studies on Piliostigma thonningii Seeds: proximate analysis, mineral composition and phytochemical screening". *African Journal of Biotechnology* 4 (2005): 1439-1442.

45. Obadai M., *et al*. "Phytochemical and mineral analysis of 12 cultivated oyster mushroom (Pleurotus species)". *Advances in Life Science and Technology* 26 (2014): 35-42.

46. Afiukwu CA., *et al*. "Phytochemical analysis of two wild edible mushrooms, Auricularia polytrichia and Pleurotus ostreatus, common in Ohaukwu Area of Ebonyi State, Nigeria". *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4.2 (2013): 1065-1070.

47. Matthews DE. "Proteins and amino acids". In: ME Shils, M Shike, AC Ross, B Caballero, RJ Cousins, editors. Modern nutrition in health and disease". 10th edition. Philadelphia: Lippincott, Williams and Wilkins (2006).

48. Patil SS., *et al*. "The nutritional value of Pleurotus ostreatus (Jacq.:fr.) kumm cultivated on different lignocellulosic agrowastes". *Innovative Romanian Food Biotechnology* 7 (2010): 66-67.

49. Uwakwe AA and Ayalogu EO. "Biochemistry, A tropical approach". *Port Harcourt, Nigeria: FIUS Publishers* 1 (1998).

50. Tessari P., *et al*. "Phenylalanine hydroxylation across the kidney in humans; rapid communication". *Kidney International* 56 (1999): 2168-2172.

51. Duru M., *et al*. "Evaluation of nutritional potential of selected floras of lower phylum". *45th Annual General Meeting and Scientific Conference of Nutritional Society of Nigeria Book of Proceedings* (2015): 131-147.

52. Olusanya JO. "Essential of food and nutrition". 1st edition. Apex book limited (2008).

53. Agomuo E., *et al*. "Effect of caffeine on some selected biochemical parameters using rat model" *Advances in Biology* (2017): 9.

54. Daniels TF., *et al*. "Lipoproteins, cholesterol homeostasis and cardiac hearth". *International Journal of Biological Sciences* 5 (2009): 474-488.

55. Assmann G and Getto JAM. "HDL cholesterol and protective factors in atheroscelerosis". *Circulation* 109 (2004): 111-118.

56. Fungwe TW., *et al*. "Dietary cholesterol stimulation biosynthesis of triglyceride and reduces oxidation of fatty acids in rats". *Journal of Lipid Research* 34 (1993): 933-941.

57. Cullen P. "Triacylglycerol-rich lipoproteins and atherosclerosis-where is the link?" *Biological Society Transaction* 315 (2003): 1080-1083.

58. Mora S., *et al*. "High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy". *Circulation* 128.11 (2013): 1189-1197.

59. Yusuf S., *et al*. "Global burden of cardiovascular diseases part 1: general consideration, epidemiological transition, risk factors and impact of urbanization". *Circulation* 104 (2001): 2746-2753.

60. Shen GX. "Lipid disorders in diabetes mellitus and current management". *Current Pharmaceutical Analysis* 3 (2007): 17-24.

61. Walker S and McMahon D. "Biochemistry demystified, a self- teaching guide". McGraw Hill USA (2008).

62. Millian J., *et al*. "Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention". *Vascula Health Risk Management* 5 (2009): 757-765.

63. Amadi BA., *et al*. "The chemical profiles of leaf, stem, and flower of Ageratum conyzoides". *Asian Journal of Plant Sciences and Research* 2.2 (2012): 428-443.

64. Duru M., *et al*. "Chemical profile of an anti-malarial concoction "Udu" used in Umunchi autonomous community in Isiala Mbano L.G.A of Imo State, Nigeria". *Journal of Emerging Trends in Engineering and Applied Science* 3.3 (2012): 444-447.

65. Criqui MH and Golom BA. "Epidemiologic aspects of lipid abnormalities". *American Journal of Medicine* 105.1 (1998): 48S-57S.

66. Martirosyan DM., *et al*. "Amaranth oil application for heart disease and hypertension". *Lipid in Health and Disease* 6 (2007).

67. Lipid Research Clinics Programme. "The lipid research clinics coronary primary prevention trial results. 1: Reduction in the incidence of heart disease". *Journal of American Medical Association* 251 (1984): 351-364.

Volume 10 Issue 2 February 2022