Hypolipidemic Activity of *Pleurotus florida* against Triton WR 1339 Induced Hyperlipidemia

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Abstract: *Pleurotus florida* (Oyster mushroom) belongs to the family *Pleurotaceae* and widely consumed all over the world. The aqueous ethanolic extract of the fruiting body of *P. florida* was evaluated for hypolipidemic activity. Hyperlipidemia was induced with the help of Triton WR 1339 (100mg/Kg b.w.) administered via intraperitoneal injection. Atorvastatin (2.5 mg/Kg b.w) was used as the standard drug. Different concentrations of aqueous ethanolic extract of *P. florida* (500, 250, and 100 mg/Kg b.w) were given orally before triton administration. The serum lipid profile was assayed and showed significant hypolipidemic activity compared to the control group (Triton alone). The activity of HMG CoA reductase was assayed using hydroxylamine hydrochloride. Hepatic HMG CoA reductase activity was significantly decreased in the treated group as compared to the control. The inhibition of lipid peroxidation was also assayed. A significant reduction in lipid peroxidation was seen in groups treated with extract compared to control. Lovastatin was also isolated from fruiting bodies and culture filtrate and screened using Thin Layer Chromatography. Lovastatin (sigma) was used as the standard. The finding suggests the significant hypolipidemic activity of the aqueous ethanolic extract of *P. florida*.

Keywords: hypolipidemia; Triton WR 1339; HMG CoA Reductase; *Pleurotus florida*.

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1. Introduction

Metabolites are the intermediates and products of metabolism. They have various functions as fuel, play an important role in biosynthesis, signaling process, stimulation and inhibition of enzymes, the catalytic activity of their own, and interactions with other organisms. The plant produces a vast and diverse assortment of organic compounds, which are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials [1]. From the early 90s, scientists began to produce secondary metabolites on a large scale with the help of various microorganisms such as bacteria, fungi, etc. using various methods.

Microbial secondary metabolites are mainly produced by fungi and actinomycetes, usually late in the growth cycle (idiophase). There are many best known secondary metabolites, with an enormous range of biological activities, mainly in the fields such as pharmaceutics, cosmetics, food, agriculture, and farming. These include compounds with anti-inflammatory, hypertensive, anti-tumor, anti-cholesterolemic, antioxidant, antiviral activities, etc. Biosynthesis of these products is a part of microbial metabolism, which is still being exploited by scientists to produce commercially important secondary metabolites on a large scale [2].
Among fungi, the mushroom is more prominent due to its distinctive fruiting body, which can be hypogeous or epigeous. They are characterized under the group-Macro fungus. Mushrooms include 14,000 to 22,000 species, while the real number may be much higher associated with the un-description of species and the non-differentiation associated with overlapping morphological characters [3]. There are over 2000 species of mushrooms that are edible; however, a dozen of them are commercially cultivated; a few of them are highly poisonous if consumed. Edible mushrooms are increasingly being recognized as an important food for their significant role in human health, nutrition, and disease [4].

The genus Pleurotus consists of 40 different species that are commonly referred to as “Oyster mushroom”. Among several species of this genus, Pleurotus ostreatus is popularly consumed all over the world due to its taste, flavor, high nutritional values, and medicinal properties. Because of the presence of various active metabolites in Pleurotus spp., have been reported to have ant diabetic, antibacterial, anti-cholesterol, anti-arthritic, antioxidant, anticancer, and antiviral activities [5].

Many studies have been conducted to identify the main factors that contribute to the development of obesity. Hypercholesterolemia is one of the greatest risk factors contributing to the prevalence and severity of cardiovascular diseases [6]. It is also reported that more than 12 million people die of cardiovascular diseases each year all over the world. Therefore, it is very important to pay attention to early-stage prevention and control of hypercholesterolemia in a comprehensive way.

Oyster mushrooms are a potent source of sterol, proteins, dietary fiber, and microelements and hence are ideal dietary substances for the prevention of hypercholesterolemia [7]. The risk of atherosclerosis can be reduced by controlling the blood cholesterol level [8] has stimulated the investigation and study of natural substances with hypocholesterolemic activity.

Lovastatin and its analogs are inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis [9]—thus leading to a decline in the production of cholesterol. These compounds, the early members of the statin family, are secondary metabolites produced by several fungal genera such as Penicillium, Monascus, Aspergillus, and Trichoderma, and also by some edible mushrooms like variants of oyster mushrooms including Pleurotus species. Statins are the most effective lipid-lowering agents in the market, is commonly used to prevent coronary heart disease.

The lovastatin present in the oyster mushroom is an important food supplement for hypercholesterolemia. Lovastatin was found in edible mushrooms, especially in Pleurotus spp., and the contents of fruiting bodies were 700–2500 mg/kg and 4–980 mg/kg, respectively [10].

Research into the effect of oyster mushroom and its extracts on the lowering of cholesterol levels on laboratory animals has been extensive, although the effect has been demonstrated in a very limited number of human subjects. Thus, the present study is to generate awareness of the beneficial effects of edible oyster mushroom, Pleurotus florida, on hypercholesterolemia, which poses serious health problems in both developed and developing countries.
2. Materials and Methods

2.1. Animals.

Male Wistar rats weighing 200 ± 20g were purchased from Veterinary college Mannuthy, Thrissur. The animals were fed with standard food (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum* prior to the experiments. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) (Approval No: ACRC/IAEC/15/04 -2) and approval of the Institutional Animal Ethics Committee (IAEC).

2.2. Collection of the fruiting body of *P.florida*.

Fresh Fruiting bodies of *P.florida* were collected from a mushroom cultivating center, Thrissur. The fruiting bodies were dried under sunlight and powdered using a mixer grinder.

2.3. Preparation of the aqueous ethanolic extract of fruiting bodies of *P. florida*.

Fruiting bodies of *P.florida* were powdered and extracted using 70% aqueous ethanol on a Soxhlet apparatus for 10-12 hrs. The solution was filtered using Buchner’s funnel with Whatman filter paper, and the filtrate was then concentrated using a vacuum evaporator. The residue left after evaporation was then lyophilized and used for the experiment.

2.4. Determination of the hypolipidemic activity of *P.florida* in Triton WR 1339 induced hyperlipidemia.

Male Wistar rats weighing 200 ± 20 g were used for this experiment. Animals were divided into 6 groups of 6 animals each and treated as follows;

- **Group 1** - Vehicle (distilled water)
- **Group 2** - Triton alone (Hyperlipidemic control)
- **Group 3** - Atorvastatin 2.5mg/kg body weight+ Triton
- **Group 4** - Aqueous ethanolic extract of *P.florida* 500mg/kg bodyweight+ Triton
- **Group 5** - Aqueous ethanolic extract of *P.florida* 250mg/kg bodyweight+ Triton
- **Group 6** - Aqueous ethanolic extract of *P.florida* 100mg/kg bodyweight+ Triton

Group1 was treated with vehicle (distilled water) served as normal; in all other groups, hyperlipidemia was induced by a single intraperitoneal (ip) injection of Triton WR 1339 (tialxopol-Sigma Aldrich) (100mg/kg body weight) dissolved in normal saline (pH 7.4). Group was kept in control without any treatment. Group 3 was treated with atorvastatin (2.5mg/kg body wt.). Group 4, 5 and 6 were treated with aqueous ethanolic extract of *P.florida* with different concentrations- 500mg, 250mg and 100mg/kg body wt. respectively. The animals have fasted for 12 hours. The extract and atorvastatin were administered by oral gavage 1hour before the triton administration, and after 24 hours of treatment, the animals were sacrificed. The animals were weighed again before sacrifice to determine the weight loss. Blood was directly collected from the heart of each animal, and the serum was separated and used for the estimation of serum lipid profile. The liver was excised from each animal and stored at -20°C until analysis could be completed. The liver samples were collected in order to check the activity of HMG CoA reductase enzyme present in the liver [11].
2.4.1. Biochemical analysis.

The serum samples were assayed for serum lipid profile. Total cholesterol, triglycerides, and HDL-cholesterol were determined using the kit of Agape Diagnostic Ltd. Liver was dissected out for the determination of lipid peroxidation activity and the activity of HMG CoA reductase.

2.4.2. Preparation of liver homogenate.

The liver was removed and stored at -20°C until analysis could be completed. The liver was excised, washed thoroughly in ice-cold saline to remove the blood, and were blotted between. The liver was homogenized in 50mM phosphate buffer (pH 7) to give a 10% homogenate (w/v). 2ml of the homogenate was stored for assaying the activity of HMG CoA reductase. The remaining homogenate was centrifuged in a cooling centrifuge at 7000 rpm for 30 minutes. The supernatant was collected and stored for assaying lipid peroxidation activity.

2.5. Maintenance of stock culture.

P.florida culture was transferred aseptically in PDA slants and incubated at 37°C in an incubator for 7 days. In this way, stock cultures were maintained.

2.6. Inoculation of P. florida culture in Hagen’s medium for lovastatin production.

The sterilized Hagen’s media was inoculated with a culture of P. florida aseptically on a LAF. The inoculated flasks were then incubated on a rotating shaker at 150 rpm for 10 days.

2.7. Isolation of lovastatin from the culture filtrate of P. florida.

The culture flasks kept in the shaker were taken after 10 days. The mycelia were filtered. The culture filtrate was acidified, and the pH was made 3 using hydrochloric acid.

The culture filtrate was concentrated using a rotary evaporator. An equal volume of ethyl acetate was added to the concentrated broth and agitated vigorously in a separating funnel. After agitation, the ethyl acetate extract accumulated at the top of the funnel was carefully separated from the broth. This process was repeated thrice. The ethyl acetate was then recovered from the extract using a rotary evaporator. The left over-extract was then used for spotting in a TLC plate.

2.8. Extraction of lovastatin from P. florida fruiting body.

25g of the powdered fruiting body was weighed, and ethyl acetate was added and was kept in the cold for 24 hours. The mixture was then stirred vigorously using a magnetic stirrer for 2 hours. The solution was centrifuged at 5000 rpm for 10 minutes. The organic layer was collected and concentrated using a rotary evaporator and was used for spotting on a TLC plate.

2.9. Screening of lovastatin using thin-layer chromatography.

Thin layer Chromatography is an analytical and separation technique for complex hydrocarbons. Here, ethyl acetate extracts of both culture filtrate and fruiting bodies of P. florida, were used for screening the presence of lovastatin.

TLC was performed in the heat activated 20x20 cm Merck silica gel 60F254 TLC plates. dichloromethane:ethylacetate (70:30) was used as the mobile phase. Lovastatin(Sigma
Aldrich) was used as a standard. Extracts and standards were dissolved in 1ml solvent separately. They were spotted on the TLC plate using capillary tubes. The TLC plate was kept enclosed in a chamber saturated with the solvent, and the solvent was allowed to develop on the plate. The plate was then removed from the solvent chamber, and air-dried and then stained using iodine vapor.

2.10. Statistical analysis.

Experimental data are expressed as means ± SD. One-way analysis of variance followed by Dunnet’s test was applied for expressing the significance. P < 0.05 was considered significant.

3. Results and Discussion

3.1. Effect of P.florida on serum lipid profile of Triton WR 1339 induced hyperlipidemic rats.

Serum total cholesterol, triglycerides, and LDL cholesterol were elevated in Triton treated groups compared to normal. There were approximately 2.21, 6.694, and 2.44 fold increases in total cholesterol, triglycerides, and LDL cholesterol, respectively, in the case of the control group with respect to normal. The rises in values were accompanied by a decline of plasma HDL by 1.8 fold. The atherogenic index was significantly increased in hypercholesteremic control rats by 6.91 fold as compared to the normal group (Table 1).

The total serum cholesterol, triglycerides, and LDL cholesterol had a remarkable decline in each group. The fold decrease in the levels of total cholesterol, triglycerides, and LDL cholesterol in the case of 500mg/kg body wt. were 2.02, 2.43, and 7.739, respectively. The fold decrease in the levels of total cholesterol, triglycerides, and LDL cholesterol in the case of 250 mg/kg body wt. were 1.87, 2.27, and 4.55, respectively. The fold decrease in the levels of total cholesterol, triglycerides, and LDL cholesterol in the case of 100mg/kg body wt. 1.60, 2.18, and 2.03, respectively. All these were compared to the total cholesterol, triglycerides, and LDL cholesterol in the control hyperlipidemic group (Table 1).

The HDL cholesterol levels were improved, there were approximately 1.96, 1.87, and 1.70 fold increase for 500, 250, and 100mg/kg body wt groups, respectively, compared to the control group. The Atherogenic Index was declined after treating with P.florida, there were 7.438, 5.391, and 3.586 fold decrease for 500, 250, and 100mg/kg body wt groups, respectively, compared to the control group. The VLDL cholesterol had a remarkable decline after treatment; there were 2.432, 2.265, and 2.181 fold decrease for 500, 250, and 100mg/kg body wt groups, respectively, compared to the control group (Table 1).

The standard reference drug atorvastatin (2.5 mg/kg body wt.) showed 2.241, 2.97, 10.820, and 2.97 fold decreases for total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol, respectively, and 1.96 fold increases for HDL cholesterol with respect to hyperlipidemic control rats. The Atherogenic Index was declined to 8.424 times with atorvastatin. All these data show that P.florida possessed significant hypocholestremic activity (Table 1).

| Groups   | Total cholesterol | Triglycerides   | Hdl-cholesterol | Ldl-cholesterol | Vldl-cholesterol | Atherogenic index |
|----------|-------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| Normal   | 102.507±0.028*    | 85.214 ± 0.064**| 50.636 ±0.055*  | 34.82           | 17.04           | 1.024            |
| Control  | 227.37 ± 0.209    | 570.42 ± 0.23    | 28.15 ± 0.046   | 85.137          | 114.09          | 7.07             |
| Standard | 101.442 ± 0.041*  | 192.21 ± 0.19**  | 55.131 ± 0.11*  | 7.86            | 38.44           | 0.84             |

https://nanobioletters.com/
| Groups | Total cholesterol | Triglycerides | Hdl-cholesterol | Ldl-cholesterol | Vldl-cholesterol | Atherogenic index |
|--------|-------------------|--------------|-----------------|----------------|-----------------|-----------------|
| FB 500 | 112.463 ± 0.0869* | 234.47 ± 0.25** | 54.56 ± 0.05*   | 11             | 46.89           | 0.951           |
| FB 250 | 121.692 ± 0.103 ns| 251.75 ± 0.10**| 52.62 ± 0.07*   | 18.72          | 50.35           | 1.31            |
| FB 100 | 142.006 ± 0.09 ns | 261.478 ± 0.16** | 47.75± 0.074 ns | 41.95          | 52.29           | 1.973           |

All values are expressed as Mean ± SD, n=6, **p< 0.01; *p< 0.05; compared to control considered as significant, ns-non significant.

3.2. Effect of *P. florida* on HMG CoA/Mevalonate ratio in the liver of Triton WR 1339 induced hyperlipidemic rats.

There was an increase of HMG CoA to mevalonate ratio in each group. There were 1.63, 1.40, and 1.14 fold increase for 500mg/kg body wt., 250mg/kg body wt., and 100mg/kg body wt groups, respectively compared to the control group. In the case of standard atorvastatin, there was an increase of 1.74 fold compared to the control group (Figure 1).

3.3. Effect of *P. florida* on inhibiting lipid peroxidation activity in Triton WR 1339 induced hyperlipidemic rats.

Lipid peroxidation activity was calculated by determining the amount of malonaldehyde in nanomoles per mg protein produced as a result of the action of toxic hydroxyl radicals on transition metals and plasma glucose, leading to cell membrane damage.

The amount of MDA increased across the groups provided with decreasing concentrations of *P. floridea* extract. There was a decline in the amount of MDA in each group after treatment. There was 1.81, 1.67, and 1.51 fold decrease for 500mg/kg body wt., 250mg/kg body wt., and 100mg/kg body wt groups, respectively, compared to the control group. In the case of standard atorvastatin, there was a decrease of 1.92 fold compared to the control group (Figure 2).
3.4. Screening of Lovastatin in P.florida fruiting body and culture filtrate using Thin layer chromatography.

The chromatogram showing the separation of lovastatin from the other cometabolites in the extracts and standard is shown in (Figure 3).

The Rf value of the extracts of the fruiting body and culture filtrate of P. florida is 0.67, which is the same as that of the lovastatin (sigma) standard. So it is inferred that both the samples contain lovastatin. In this way, a significant amount of lovastatin was detected in the fruiting body and culture filtrate of P. florida.

Cardiovascular diseases, as a group, is a leading cause of death worldwide. In fact, since 1990, greater than 85,000,000 disability-adjusted life-years were lost worldwide due to coronary heart diseases (CHD) and stroke; this CHD disease burden is projected to rise to 143,000,000 disability-adjusted life-years by 2020 [12,13].

Hypertriglyceridemia, in combination with abnormally low concentrations of HDL cholesterol (High-Density Lipoprotein Cholesterol), is one of the most common and atherogenic profiles of lipid metabolism of high prevalence seen in the Indian population [14]. Hyperlipidemia and hypercholesterolemia are reportedly the major risk factors in lifestyle-related diseases such as atherosclerosis and related cardiovascular complications, including cerebral paralysis and myocardial infarction [15]. Prevention or treatment of such disorders can be achieved by targeting the causative hyperlipidemia and hypercholesterolemia through diet and/or drug administration [16]. Research on natural medicines is gaining ground, and the demand to use of such products in the treatment of various disorders is increasing worldwide.

In the current study, tritonised animals have been used to test the antitriglyceridemic and anticholesterolemic efficacy of aqueous ethanol extract of P. florida as such a model has been used for the induction of acute hyperlipidemia [17] as well as for testing the potential of natural/chemical hypolipidemic drugs. Our data show that the P. florida extract exerts a significant antihyperlipidemic effect marked by significantly lower plasma cholesterol and triglyceride levels in HL+ extract rats compared to HL rats. Our extract seems to have a potent antitriglyceridemic effect as it could protect against Triton induced hypertriglyceride. It is shown that Triton elevates plasma triglyceride levels essentially by preventing its uptake and clearance by inhibiting catabolizing enzymes like lipoprotein lipase (LPL) and lecithin cholesterol acetyltransferase (LCAT) [18]. Apparently, our mushroom extract is able to reduce the inhibition of LPL and LCAT activity, making triglycerides available for uptake and
metabolism by tissues. The antilipidemic drug atorvastatin seems more potent in preventing the elevation in triglyceride levels [19].

Triton Wr-1339 has been widely used to block the clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [20]. This model is widely used for a number of different aims, particularly in rats. It has been used for screening natural or chemical hypolipidemic drugs [21]. Triton WR 1339 induced hypercholesterolemia has been related to its ability to alter the physical and chemical properties of lipoproteins and thereby block their uptake by the liver for clearance [22]. In our present study, it is seen that the P. florida extract is effective in minimizing triton induced decrease in HDL-C as well as an increase in non-HDL-C (LDL+VLDL), suggesting promotion of increased catabolism of non-HDL-C by the hepatic tissue. The hypolipidemic efficacy of the P. florida extract is also substantiated by the calculated Atherogenic Index, and despite being a crude extract, it seems to be quite competitive to the hypolipidemic drug atorvastatin [23].

The result shows the ratios of HMG-CoA/mevalonate of the Six different groups; In extract provided groups, cholesterol biosynthesis in the liver was decreased while the HMG-CoA/mevalonate ratio was increased as compared to the hyperlipidemic control group. On the other hand, in Triton-treated, cholesterol synthesis was increased, and the HMG-CoA/mevalonate ratio was decreased. The results are statistically significant in all conditions as compared to the hyperlipidemic controls. Thus the HMG-CoA/mevalonate ratio agrees with the results that would be anticipated on the actual assay of the enzyme activity [24].

The level of malondialdehyde was significantly elevated in the liver tissue of rats in which hyperlipidemia was induced using Triton compared with control, thus confirming that lipid peroxidation increases in hyperlipidemic rats. The source of the lipid peroxides is postulated to be the end products from membrane damage, which are elevated in rats with induced hyperlipidemia. These elevated levels of peroxides could result from the hyperlipidemic state in relation to auto-oxidation of plasma glucose and other small autooxidizable molecules and are associated with poor metabolic control of plasma glucose [25].

Bioactive metabolites found in edible mushrooms play a role in enhancing the health conditions of human beings. Mushroom fruiting bodies in powder form increases the excretion of lipids and cholesterol through fecal matter. Mevinolin (a statin: polysaccharide), present in fruiting bodies of P. ostreatus and P. citrinopileatus exhibited anti-hypercholesterolemic activities [26]. P. florida is a nutritionally functional food with valuable therapeutic use. The best known therapeutic agent stated to be of potential use for correcting hypercholesterolemia is lovastatin and its analogs. Pleurotus species are reported to be the best-known source of this drug [27]. Lovastatin was extracted from P. florida fruiting body and concentrated culture filtrate, using ethyl acetate extraction. Then a TLC separation was carried out for the primary screening, which effectively separated lovastatin from other major cometabolites using lovastatin standard (sigma). Comparing to the chromatogram obtained after spotting and running the two samples and standard, all the three spots were parallel to each other, indicating the presence of lovastatin. The RF value calculated was 0.67, which was the same as that of the standard. Thus it was inferred that both the fruiting body and the culture filtrate of P. florida had a sufficient amount of lovastatin, which could inhibit HMG CoA reductase and decrease cholesterol biosynthesis.

In this scenario, research on natural medicines is gaining ground, and the demand to use of such products in the treatment of various disorders is increasing worldwide.
Investigations on natural products might lead to the development of alternative drugs and strategies. Such alternative strategies are required for the effective management of dyslipidemic disorders as; cost and poor availability of modern therapies make the rural populations, particularly in developing countries, vulnerable to such ailments. Finally, the result of the current study suggested that *P. florida* possessed a significant hypocholesterolemic activity.

4. Conclusions

In conclusion, fruiting bodies of *P. florida* possessed a significant hypolipidemic effect against Triton induced hyperlipidemia in rats. According to our study, the presence of lovastatin (a naturally occurring statin) in *P. florida* fruiting body was inferred to provide the anti hypercholesterolemic activity as it has the ability to inhibit the HMG CoA reductase enzyme involved in cholesterol biosynthesis. Hepatic lipid peroxidation was also reduced when treated with the mushroom extracts.

Hypolipidemic activity of *P. florida* can be attributed to its profound antioxidant activity. Since *P. florida* is an excellently edible mushroom, the findings have significant therapeutic use as consumption of this mushroom can impart a preventive or curative measure of treating hyperlipidemia and cardiovascular diseases. This would be a more healthy and reliable solution to get rid of harmful side effects created by synthetic drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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