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Hypolipidemic Effect of *Pleurotus djamor* var. *roseus* in Experimentally Induced Hypercholesteromic Rats.

Raman Jegadeesh[a,b], Nanjian Raaman[a], Lakshmanan Hariprasath[a], V Ramesh[c], and R Srikumar[c]*.

[a]Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai- 600 025, Tamil Nadu, India.
[b]Mushroom Research Centre, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia.
[c]Centre for Research, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, Affiliated to Bharath University, Chennai, India.

ABSTRACT

Hypercholesteremia is one of the risk factors for coronary artery disease. Mushrooms are low calorie food with very little fat and are highly suitable for obese persons. The present study highlights the efficacy of edible mushroom *Pleurotus djamor var. roseus* extracts on total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), High density lipoprotein (HDL) and free fatty acid in experimentally induced hypercholesteromic rats. Five group of rats were employed which includes control, mushroom extract, hypercholesterolemia rats (4 % cholesterol + 1% cholic acid + egg yolk), hypercholesterolemia rats treated with Mevinolin and hypercholesterolemia rats with pretreatment of mushroom extract and each group includes six animals. Results from the present study showed that significant increase in the total cholesterol, LDL, VLDL and free fatty acid observed in hypercholesteromic feed rats were significantly reduced in mushroom extract treated hypercholesteromic rats. These results suggest that mushroom supplement provided health benefits by acting on the lipid profile in hypercholesterolemic rats.

Kewwords: *P. djamor var. roseus*, Cholesterol, Lipid profile, Mevinolin

Corresponding author

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INTRODUCTION

Coronary artery disease (CAD) occurs when a substance called plaque builds up in the arteries that supply blood to the heart. Plaque is made up of cholesterol deposits, which can accumulate in the arteries. When this happens, arteries becomes narrow over time leads to atherosclerosis remains the most common cause of morbidity and mortality all over the world with more than 4.5 million deaths occurring in the developing countries [1].

It is projected that CAD mortality rates will be double in 2020, with approximately 82% of the increase attributable to the developing world. Due to rapid socioeconomic growth in developing countries is increasing exposure to risk factors for CAD, such as diabetes, genetic factors, hypercholesterolemia, hypertension, and smoking [2].

Hypercholesteremia is one of the risk factors for CAD which is characterized by elevated serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high-density lipoprotein levels. Currently available hypolipidemic drugs have been associated with a number of side effects [3]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, gastric irritation, and abnormal liver function.

Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [4]. Mushrooms are highly nutritive as they contain good quality proteins, vitamins and minerals [5, 6]. Mushrooms are low calorie food with very little fat and are highly suitable for obese persons. Edible mushrooms are the ideal materials for the dietetic prevention of atherosclerosis, due to their high content of fiber, proteins, microelements, and their low fat content [7]. The present study was aimed to investigate the hypolipidemic potential of *P. djamor var. roseus* in experimentally induced hypercholestremia in albino rats.

MATERIALS AND METHODS

Experimental Animal

Healthy adult Wistar strain male albino rats, weighing between 160 - 180g (3 months old), were used in this study. The rats were kept in the animal house with controlled ambient temperature (24 – 28^{o}C), humidity and light (14 hrs/10 hrs light/ dark cycles) with food and water *ad libitum*. The animal procedures were approved by the Institutional Animal Ethical Committee (IAEC No. 03/016/08), University of Madras, Chennai, India, and Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All efforts were made to minimise both the number of animals used and unwanted stress or discomfort to the animals throughout experimental procedures.

Mushroom

Mushrooms belonging to the family Pleurotaceae (Basidiocarps) were collected from the IIT campus, Chennai, India. Based on the morphological and microscopic characteristics, the mushroom strain was confirmed as an edible mushroom belonging to the variety

P*leurotus djamor* var. *roseus*. The Basidiomata were dried under shade and powdered before use.

Methanolic extract (Methanolic extract of Pleurotus djamor var. roseus)

The Basidiomata of *P. djamor var. roseus* were powdered (coarse, 1000 g) and soaked in methanol (1:10) for 72h at room temperature (Maceration method). The mixtures were then filtered. The filtrate was than concentrated on a rotary evaporator at 45^{o}C and yield was 65g/1000g (MEPDR).

Dosage

P. djamor var. roseus crude methanol extract was mixed in normal saline (0.9%) and administered to the rat orally at the dose of 500 mg/kg body weight for 48 days.

Induction of hypercholesterolemia in rats

Normal rats were fed with 4% cholesterol (Sigma), 1% cholic acid (Sigma) and egg yolk (mixed with feed) for 48 days to induce hypercholesterolemia (hypercholesterolemic feed rats) by the method of Deepa and Varalakshmi [8].

Dosage of Mevinolin

Mevinolin was administered orally at a dose of 7.2 mg/kg body weight form 33rd day of the regimen till 48th day (15 days) [9].

Detection of Mevinolin in P. djamor var. roseus extract by HPLC

The standard Mevinolin and crude methanolic extracts were detected by High Performance Liquid Chromatography, using a Merck LiChrospher 100 RP18 reverse phase column with a diode array detector eluted at a flow rate 2 mL/min and elution with gradient 90:10 water : methanol (v/v).

Experimental groups

All the experimental animals were divided into 5 groups and each group consists of 6 animals as follows

Group 1: Control: Animal received standard diet + saline (1 mL) intragastrically for 48 days.

Group 2: Drug control: Animal received standard diet + crude methanolic extract of P. djamor var roseus MEPDR (500 mg/kg b.w.) for 48 days.

Group 3: Animal received hypercholesterolemic feed (4% Cholesterol + 1% cholic acid + egg yolk) for 30 days.

Group 4: Animal received methanolic extract of mushroom (500 mg/kg b.w.) for 48 days along with cholesterol feed from 18th days of the regimen

Group 5: Animal received hypercholesterolemic feed for 48 days along with Mevinolin 7.2 mg/kg body weight for 38 – 48 days.

Statistical Analysis

All data were expressed as Mean ± S.D. The data were subjected to One-way Analysis of Variance (ANOVA) to determine the significance of individual differences at $p < 0.05$ level. Significant means were compared by the Duncan's multiple range test. All statistical analyses were carried out using SPSS statistical package (SPSS, Version 10.0 for Windows, SPSS Inc., Chicago, USA).

RESULTS

Animal Body weight

The crude methanolic extract of *P. djamor* var. *roseus* (MEPDR) (500 mg/kg body weight) was administrated orally for 48 days. During the experiment regime, animal body weight was recorded every week. The body weight of Group 2 (MEPDR alone) animals were not significantly different throughout the study period when compared to the control animals (Figure 1).

Total cholesterol

There was no significant increase in total cholesterol levels of group 2 animals (treated with MEPDR) when compared to group 1 animals (control). The level increased significantly in Group 3 animals (Hypercholesteremic food) (143 ± 6.5 mg/dL) when compared to control animals (71 ± 4.5 mg/dL), whereas the group 4 (Hypercholesteremic food + MEPDR) and group 5 (Hypercholesteremic food + statin) animals did not show any significant alterations in the total cholesterol level when compared to the control grouped animals. The total cholesterol level of group 4 and group 5 animals were 66 ± 3.5 and 67 ± 3.1 mg/dL, respectively (Table 1).

Triglyceride

The triglyceride level was found to be significantly decrease in group 2 animals when compared to group 1 animals, whereas a significant increase (217 ± 11.28 mg/dL) in triglyceride level was observed in group 3 animals. It was interesting to observe that the group 4 and group 5 animals showed a comparable value (90 ± 4.09 and 89 ± 3.62 mg/dL, respectively) to that of the control group. Triglyceride levels were not altered significantly in group 4 animals (90 ± 4.09 mg/dL) when compared with the group 5 animals (89 ± 3.62 mg/dL).

Free fatty acid

The free fatty acid level of group 2 animals showed no significant changes (22 ± 1.11 mg/dL) when compared with group 1 animals (21 ± 1.15 mg/dL), but a two-fold increase (53

± 2.41 mg/dL) was observed in group 3 animals. This increase in fatty acid level was not observed group 4 & 5 and comparable to that of the control group animals (Table 1).

Table 1: Effect of crude methanol extract of *P. djamor var. roseus* (MEPDR) on experimentally induced Hypercholesteremia in male albino rats.

S. No	Parameter	Control (Group 1)	MEPDR (Group 2)	Hypercholesteremic feed rats (Group 3)	Hypercholesteremic feed + MEPDR (Group 4)	Hypercholesteremic feed + mevinolin (Group 5)
1.	**Total Cholesterol (mg/dl)**	71 ± 4.5	69 ± 4.4	143 ± 6.5^{ab}	66 ± 3.5^{c}	67 ± 3.1^{c}
2.	**Triglycerides (mg/dl)**	88 ± 4.32	61 ± 3.66^{a}	217 ± 11.28^{ab}	90 ± 4.09^{c}	89 ± 3.62^{c}
3.	**Free fatty acid (mg/dl)**	21 ± 1.15	22 ± 1.11	53 ± 2.41^{ab}	19 ± 1.04^{abc}	17 ± 1.21^{abcd}
4.	**HDL (mg/dl)**	32 ± 2.53	36 ± 3.23^{a}	35 ± 2.39	39 ± 2.31^{abc}	38 ± 2.23^{a}
5.	**LDL (mg/dl)**	19 ± 1.15	21 ± 0.98	64 ± 2.15^{ab}	12 ± 0.72^{abc}	11 ± 0.49^{abc}
6.	**VLDL (mg/dl)**	17 ± 0.72	15 ± 0.60^{a}	44 ± 2.87^{ab}	17 ± 0.98^{bc}	16 ± 0.49^{bc}

Values are expressed as Mean ± SD of six animals. The symbol ($^{a, b, c \text{ and } d}$) represent statistical significance $p<0.05$; ^{a} Compared with control, ^{b} compared with mushroom MeOH extract alone, ^{c} compared with Hypercholesteremia, ^{d} compared hypercholesteremia + MEPDR.

High density lipoprotein (HDL)

The HDL level increased significantly in group 4 (39 ± 2.31 mg/dL) and group 5 animals (38 ± 2.23mg/dL) when compared to the control group (32 ± 2.53 mg/dL). No significant alterations in HDL level was observed in group 3 animals (35 ± 2.39 mg/dL) when compared to that of the group 2 animals (36 ± 3.23 mg/dL).

Low density lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL)

The LDL level observed in group 2 animals showed no significant increase when compared to group 1 animals (19 ± 1.15 mg/dL), whereas a three-fold increase in LDL level was observed in group 3 animals (64 ± 2.15 mg/dL). Surprisingly a decrease in LDL level was observed in group 4 (12 ± 0.72 mg/dL) and group 5 animals (11 ± 0.49 mg/dL) when compared to group 1 control animals. No significant difference in LDL level was observed between group 4 and group 5 animals. Same result was observed for very low density lipoprotein in the studied groups.

Screening of lovastatin in P. djamor var. roseus

The Lovastatin content was detected by HPLC, the crude methanolic extract was eluted with gradient 90:10 water: methanol (v/v) and authentic mevinolin was used as a reference. The authentic sample showed peaks at retention time 2.642 and 2.775. The methanolic crude extract showed peaks at retention time 2.633 and 2.770, similar to that of the authentic sample.(Figure 2).

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Figure 1. Effect of crude methanolic extract of *P. djamor* var. *roseus* on body weight of albino rats.

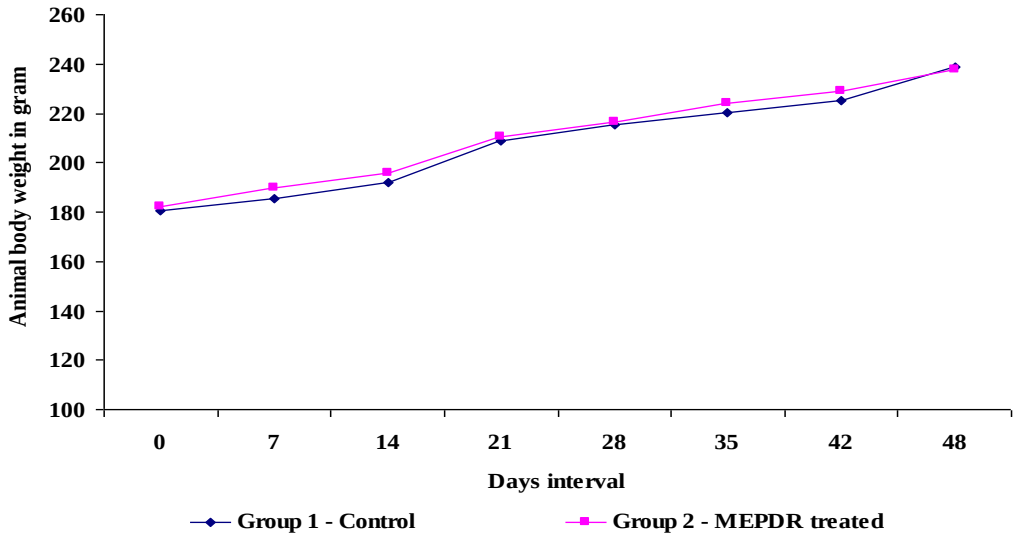
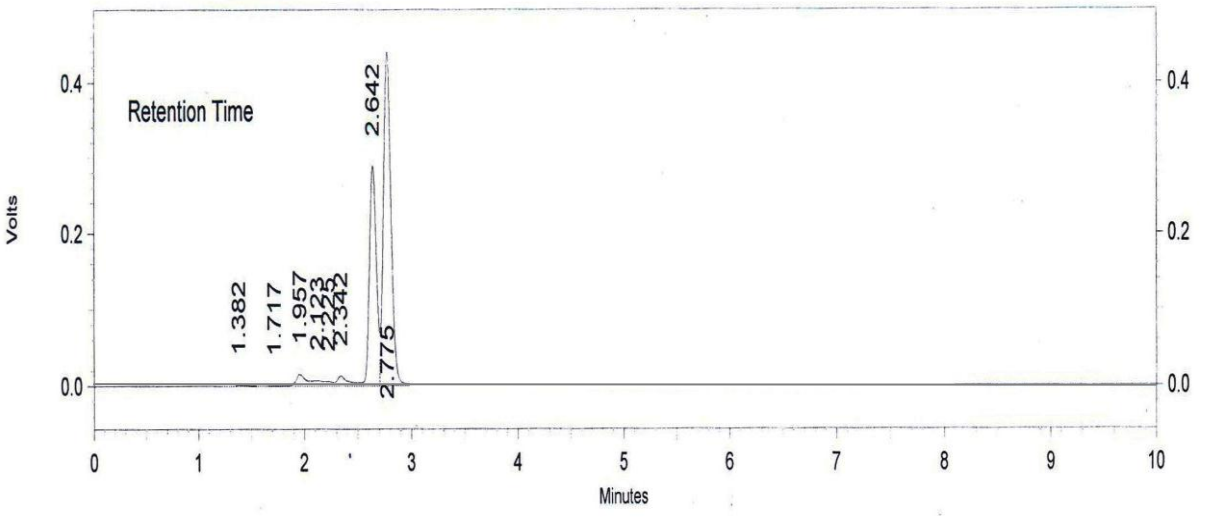
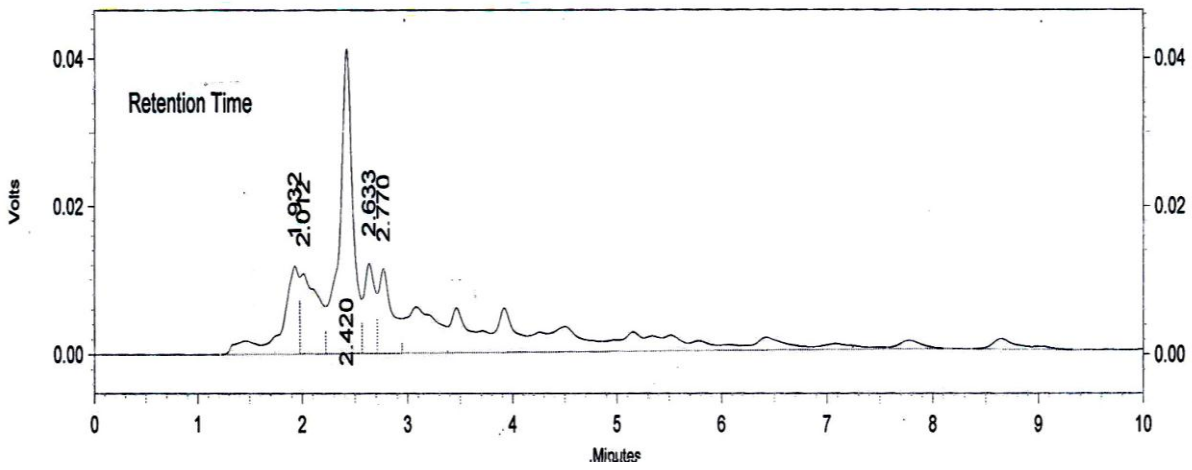


Figure 2: HPLC profile showing the Mevinolin

a. Authentic Mevinolin





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DISCUSSION

The cholesterol absorption and acceleration of cholesterol catabolism by mushroom in the diet is probably an accumulation effect of several compounds having the potential to effect these processes [10], such as sterols, water soluble β-glucan, pectin, other secondary metabolites and proteins which improve digestion without causing any laxative dependency and rich in antioxidant [11]. These metabolites can bind bile acids, therefore, reducing the formation of micelles [12] and cholesterol absorption. Higher excretion of bile acids [13] induces a decrease of their enterohepatic circulation and by a feedback mechanism, the stimulation of 7 alpha-hydroxylase [10], the rate-limiting enzyme in the catabolism of cholesterol to bile acids. Animal body weight remain unaltered significantly during forty eight days of administration with methanolic extract of P. djamor var. roseus, hence significant reduction in the studied lipid profile were not due to any overt toxicity by the studied drug (Figure. 1).

Controlling the blood cholesterol is an important for reducing the risk of developing atherosclerosis. Increasing frequency of anti-hyperlipidemic drugs use and their common side effects, make the researchers to search for natural products with few or no side effects. In the present study *P. djamor var. roseus* was selected for its hypolipidemic activity in experimentally induced hypercholestremia in albino rats. Rats fed with cholesterol (Group-3) showed significant increases in total cholesterol, triglycerides, free fatty acids, LDL and VLDL conforming that cholesterol fed in this study induced hypercholesteremia experimentally since cholesterol feeding can increase bile acid secretion by approximately three to four folds [14].

In the present study administration of crude methanolic extract of *P. djamor var. roseus* mushroom (500 mg/kg/b.w) for 48 days showed significant reduction in the Triglycerides and VLDL with concomitant increase in HDL levels. As the HDL plays a key role in protecting against the heart disease via their role in reverse cholesterol transport by peripheral tissues to the liver for catabolism and subsequent excretion might be one of the reason for the significant reduction in Triglycerides and VLDL observed in the present study [15]. In addition mushroom are rich in dietary fiber content which may increases small intestinal viscosity, resulting in reduced bile acid and cholesterol absorption might be the reason for the significant reduction in the studied lipid profile was observed in the *P. djamor var. roseus* administred groups [16].

Drugs like mevinolin administration results in decreased activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which is the rate-limiting enzyme of cholesterol biosynthesis [17]. In HPLC study, methanolic crude extract of *P. djamor var. roseus* showed peaks similar to that of the reference sample mevinolin. Reduction in the cholesterol level in hyperlipidimic rats treated with *P. djamor var. roseus* may involve suppression of endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

Overall results indicated that *P. djamor var. roseus* administration reduces the cholesterol levels and have greater significance in prevention of hyperlipidemia or cardiovascular disease.

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