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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Oyster Mushroom on Hypocholesterolemic study in the experimental animals (Male albino Wistar rats)

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ABSTRACT
The climate condition prevailing in Indian plains seems to be quite suitable for large-scale production of Oyster mushroom Pleurotus eous. Hence studies were taken up with an objective to find new technologies to test the hypocholesterolemic properties of P. eous .With regard to the studies on medicinal properties of P. eous, the serum enzyme profile of male Wistar rats revealed the total cholesterol level, LDL, TGL, and total lipids in both the serum and liver recorded a significant decline with an increase in the supplement of mushroom. Rats fed with diet co-administrated with P. eous recorded an increase in HDL (Good cholesterol) of all concentrations and durations of observation. Among the three levels of P.eous diet tested, the 10 per cent level recorded the maximum reduction in the cholesterol level when compared to 2.5 and 5 per cent level in the both serum and liver. The serum total cholesterol level, HDL, LDL, TGL are analysed by feeding rats with 10 per cent of P.eous diet (Group D) over a period of 90th day recorded a decline when compared with control (Group A) and therefore the study revealed the beneficial effects of P.eous on hypercholesterolaemia, which poses serious health problems in both developed and developing countries.
Key words: Pleurotus eous, Hypocholesterolemic , Wistar rats .

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INTRODUCTION

Several species of mushrooms are of great importance because of their medicinal importance. They are active against hypercholesterolemic conditions, hypertension, diabetes, cancer, infections etc. Mushrooms are known for their rich source of biologically active compound which offer protection to the human body in a natural way. Moreover, they contain proteins and nutritive fibers, as well as other vitamins necessary for the normal functioning of the human body. Mushrooms are rich in dietary fiber, minerals, vitamins and low in fat. Mushrooms are widely consumed as an edible and medicinal resource. Many of these mushrooms species are having therapeutic properties such as antioxidant, anticancer, antimicrobial, cholesterol lowering and immune stimulatory effect [14] .Cultivation of the oyster mushroom, *Pleurotus* spp has increased greatly throughout the world during the last few decades. In Tamil Nadu the farmers have developed preference to *Pleurotus* spp. because it can be cultivated in plains not only during the cooler months of the year but also during summer. There are about 38 species described under the genus Pleurotus from different parts of the world. The species like *P. ostreatus, P. flabellatus, P. citrinopileatus, P. eous, P. sapidus, P. sajor-caju, P. platypus* and *P.eous* are widely cultivated in India [10, 12]. *Pleurotus* is an efficient lignin- degrading mushroom and can grow well on different types of lignocellulolosic materials. New technologies and production techniques are being constantly developed as the number of required controllable environment parameters increases. Oyster mushrooms are food-stuff with a high therapeutically and nutritive value.

Incidence of heart disease in the world leads to search for natural substances capable of lowering blood cholesterol is ongoing in the field of nutrition. Excessive levels of blood cholesterol accelerate athreogenesis [13] and lowering of high blood cholesterol reduces the incidence of coronary heart disease. Mushrooms provide a wide variety of physiologically active components: *Pleurotus sajor-caju* inhibits hypertensive effects through its active ingredients, which affect the reninangiotensin system [3], *Tricholoma magnivelare* produces asorelaxation because of its lectin content, *P. ostreatus* possesses

antitumor or activity and hypoglycemic effects on experimentally induced diabetic rat [4], *Lentinus edodes* and *Grifola frondosa* have antihypertensive effects in spontaneously hypertensive rats and *Agaricus bisporus* decreases low -density lipoprotein-cholesterol (LDL-C) in serum by increasing the expression of LDL receptor at mRNA level and LDL receptor activity [8]. Hence, the objective of the present study was to generate awareness of the beneficial effects of edible mushrooms, particularly oyster mushrooms, on hypercholesterolaemia, which poses serious health problems in both developed and developing countries.

MATERIALS AND METHODS

Source and maintenance of culture

The culture of *Pleurotus eous* was obtained from Tamil Nadu Agricultural University, Coimbatore. The fungus was also cultured from fresh sporophore by tissue culture method. The mushroom cultures were maintained in PDA slants and used for the present investigation.

Isolation and Maintenance of Pure Cultures

A well-developed sporophore was selected and cut into two equal halves. A small piece of mushroom tissue was removed with a sterile forceps exactly form the center of junction of pileus and stipe from the half cut mushroom. The tissue bit was then sterilized with 70 per cent ethanol for 30 sec, washed thrice serially in sterile distilled water, air dried near a flame to remove excess moisture and placed aseptically into Petri dishes containing PDA or MA medium. The dishes were incubated at room temperature (25 ±2º C) for seven d.

Spawn Production

Pure cultures maintained in PDA medium were used for the preparation of sorghum grain spawn. Cleaned grains were thoroughly washed and soaked in water for 30 min and half cooked in an open vessel for 20 min. After draining the excess water, the grains were mixed with calcium carbonate at the rate of 20 g per kg of grains (dry weight), filled in autoclavable polypropylene bags (25 x 10 cm size) and sterilized at 1.42 kg cm^{-2} pressure in an autoclave for 1.5 h. After cooling, the bags were aseptically inoculated with the pure cultures of the respective mushroom fungus, incubated at room temperature (25 ±2^{0}C) for 15 d and used for spawning the substrate.

Bed preparation

Polypropylene bags of 60 × 30 cm size (100 G thickness) were used as containers for the preparation of cylindrical mushroom beds with layer spawning. For each bag 500 g of substrate (dry weight basis), respectively were used. Fifteen d old sorghum grain spawn was used to seed the substrate at the rate of 2 per cent (dry weight basis). The beds after spawning were provided with 4 - 8 holes of one cm dia. depending upon the size of the bed for air circulation.

Evaluation of medicinal values of *P. eous*

Experimental Animal : **Male albino Wistar rats**
Age : **5 weeks old**
Duration of Experiment : **Three months**

The rats were individually housed in wire mesh cages and kept in an isolated room at a controlled temperature (22-25^{o}C) and ambient relative humidity of 50-60% on a 12 hour light: dark cycle and air changes of 10-12 times per hour. Necessary ethical clearance was obtained from Institutional Animal Ethical Committee of Rajah Muthiah Medical College, Annamalai University to perform experimental studies on male wistar rats.

Preparation of rat feed

Normal feed : lab stock feed in pelleted form.

Normal plus mushroom feed: 100 g of lab stock feed in pelleted form was powdered. Then 2.5, 5, and 10 g of *P.eous* was powdered and mixed thoroughly with the lab stock diet with the help of a little amount of hot water, the moisture was made into a pelleted form and then air dried. Then it was stored in an air tight container at room temperature.

Cholesterol feed: feed rich in cholesterol, *viz*, groundnut, coconut scrapping, egg yolk, etc., were fed.

Cholesterol plus mushroom feed : 100 g of cholesterol feed was powdered, then 2.5, 5, and 10 g of *P.eous* was powdered and mixed thoroughly with the help of little amount of hot water, and made in to a pelleted form and then air dried. Then it was stored in an air tight container at room temperature.

Hypocholesterolemic studies in rats

Experimental Design:

The experimental rats were grouped as

Group A Rats fed with Normal Feed
Group B Rats fed with Normal Feed + 2.5% *P. eous*
Group C Rats fed with Normal Feed + 5% *P. eous*

Group D Rats fed with Normal Feed + 10 % *P. eous*
Group E Rats fed with Cholesterol feed
Group F Rats fed with Cholesterol feed + 2.5% *P. eous*
Group G Rats fed with Cholesterol feed + 5% *P. eous*
Group H Rats fed with Cholesterol feed + 10 % *P. eous*

Animals and diets.

Male wistar rats weighed 100 g and were 5-week-old were used for the study. The rats were individually housed in wire-mesh cages and kept in an isolated room at a controlled temperature of 22-25°C and ambient relative humidity of 50-60% on a 12-hour light: dark cycle (lights on from 0600 to 1800 h) and an air changes of 10 to 12 per hour. Animals were acclimated to the facility and given free access to water and the powdered laboratory stock diet. The animals belonging to experimental groups were given 5% powdered *P.eous* mixed with laboratory stock diet. For animals belonging to Cholesterol group, oils, egg yolk and ground nut were mixed with normal feed to increase the serum cholesterol level for experimental purpose. Necessary ethical clearance was obtained from Institutional Animal Ethical Committee of the Rajah Muthiah Medical College, Annamalai University to perform experimental studies on male wistar rats. The animals were reared in standard management practices and clinical as well as other parameters were recorded at 30 days, 60 days and 90 days duration.

Clinical Symptoms and Body Weight:

Both the controls as well as the experimental groups of rats were weighed at weekly intervals. The animals were observed daily for clinical symptoms if any and recorded.

Serum Chemistry

Serum enzyme assay was done by using ERBA CHEM semi auto analyzer. The values were taken on 30,60 and 90th day of experiment. The serum concentrations of total cholesterol, HDL cholesterol, free cholesterol, triacylglycerols and phospholipids were measured enzymatically with kits (Cholesterol C-Test, HDL Cholesterol-Test, Free Cholesterol C-Test, Triglyceride G-Test and Phospholipid B-Test, respectively, Wako). The difference between total cholesterol and HDL cholesterol or free cholesterol was assumed to be VLDL + HDL cholesterol or esterified cholesterol, respectively.

RESULTS AND DISSCUSSIN

Determination of serum total cholesterol (mg%)

Serum total cholesterol level in the test animals recorded a significant decline with an increase in the level of supplementation of *P.eous* and with an increase in the duration of the treatment when compared to their respective controls. Among the three levels of *P.eous* diet tested, the 10 per cent level recorded the maximum reduction in the cholesterol level when compared to 2.5 and 5 per cent level in the both serum and liver.

The serum total cholesterol level of rats fed with 10 per cent of *P.eous* diet (Group D) over a period of 30th, 60th and 90th days recorded 43.75, 48.63 and 49.09 units/L of cholesterol level respectively when compared to control (Group A) (56.13, 58.62 and 60.77 units/L respectively). Similarly, the animal fed with cholesterol feed plus 10 per cent *P.eous* diet (Group H) showed significantly decreased cholesterol level on the 30th, 60th and 90th day of observation (61.36, 50.45 and 49.93, respectively) when compared with their respective controls.

Table.1.Effect of *P.eous* on the total cholesterol (mg %) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	56.13	58.62	60.77
Group B (2.5% *P. eous*)	51.96	50.99	55.40
Group C (5% *P. eous*)	47.84	49.78	52.21
Group D (10% *P. eous*)	43.75	48.63	49.09
Group E (Cholesterol feed)	77.29	87.99	91.17
Group F (Cholesterol feed + 2.5% *P. eous)*	70.90	72.85	75.90
Group G (Cholesterol feed + 5% *P. eous)*	65.48	60.64	60.91
Group H (Cholesterol feed + 10% *P. eous)*	61.36	50.45	49.93
SE	1.46	3.16	3.73
CD (P=0.05)	4.40	9.50	11.20

Determination of liver total cholesterol (mmol/l)

The liver total cholesterol, the data clearly indicated that there was a significant reduction in the total cholesterol level in the liver of rats in the treatment Group D. 171.85, 168.90 and 166.95 mmol/l, respectively) on the 30th, 60th and 90th day of observation when compared to control (Group A. 211.13,

214.08 and 230.77 mmol/l, respectively) it is worthy to note that the cholesterol level of rats fed with cholesterol feed plus 2.5 per cent *P.eous* (Group F) diet (235.79, 218.07 and 213.14 mmol/l, respectively). Cholesterol feed plus 5 per cent *P.eous* (Group G) diet 219.51, 215.26 and 212.69 mmol/l, respectively). Cholesterol feed plus 10 per cent *P.eous* (Group H) diet 217.23, 213.48 and 210.97 mmol/l, respectively)was almost on par with control Group A at all the periods of observation thus, proving the cholesterol lowering properties of *P. eous*

Table.2. Effect of *P.eous* on the liver total cholesterol (mmol/l) level of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	211.13	214.08	230.77
Group B (2.5% *P. eous*)	213.09	204.25	202.29
Group C (5% *P. eous*)	189.19	186.24	184.62
Group D (10% *P. eous*)	171.85	168.90	166.95
Group E (Cholesterol feed)	220.08	226.04	241.58
Group F (Cholesterol feed + 2.5% *P. eous)*	235.79	218.07	213.14
Group G (Cholesterol feed + 5% *P. eous)*	219.51	215.26	212.69
Group H (Cholesterol feed + 10% *P. eous)*	217.23	213.48	210.97
SE	0.67	0.11	0.08
CD (P=0.05)	0.24	0.33	2.01

Determination of High Density Lipid (HDL)(mg%)

The data clearly indicated that the rats fed with 10 percent *P.eous* Group D diet recorded significant changes in the level of HDL over their respective controls. The test animal in Group D recorded the maximum level of HDL (28.06 mg%) on the 90th day of observation when compared to control (22.96 mg %) whereas, when 5 and 10 per cent *P.eous* is mixed with feed rich in cholesterol (Group G and H), the level of HDL is found increased further in all the durations of the experiment. Test animal in Group H recorded the maximum level of HDL (29.35 mg %) on the 90th day of observation when compared to control (22.96 mg %).

Table.3.Effect of *P.eous* on the HDL (mg %) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	20.13	21.29.	22.96
Group B (2.5% *P. eous*)	22.16	23.46	23.86
Group C (5% *P. eous*)	23.35	24.60	24.96
Group D (10% *P. eous*)	24.96	26.30	28.06
Group E (Cholesterol feed)	21.59	22.13	24.16
Group F (Cholesterol feed + 2.5% *P. eous)*	22.76	23.31	25.26
Group G (Cholesterol feed + 5% *P. eous)*	24.73	25.86	26.18
Group H (Cholesterol feed + 10% *P. eous)*	26.16	27.10	29.35
SE	0.43	0.06	0.36
CD (P=0.05)	0.12	0.20	1.10

Determination of Low Density Lipid (LDL) (mg%)

Supplementation of 5 and 10 per cent *P.eous* diet with normal feed recorded appreciable decrease in the LDL during the various period of observation. The LDL level was found increase with an increase in the supplementation with cholesterol feed (Group E). The maximum reduction in the level of LDL was observed in the rats in Group C and Group D (23.35 and 22.16mg %) on the 90th day of observation, while Group H recorded a LDL level of 22.76 mg % when compared to its respective control Group E (24.16 mg %) .

Table.4.Effect of *P.eous* on the LDL (mg %) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	25.13	25.29.	25.96
Group B (2.5% *P. eous*)	28.06	26.30	24.96
Group C (5% *P. eous*)	24.96	24.60	23.35
Group D (10% *P. florida*)	23.86	23.46	22.16
Group E (Cholesterol feed)	21.59	22.13	24.16
Group F (Cholesterol feed + 2.5% *P. eous)*	29.35	27.10	26.16
Group G (Cholesterol feed + 5% *P. eous)*	26.18	25.86	24.73
Group H (Cholesterol feed + 10% *P. eous)*	25.26	23.31	22.76
SE	0.43	0.06	0.36
CD (P=0.05)	0.12	0.20	1.10

Determination of triacylglycerol (TGL) (mg%)

The data present indicates the hypocholesterolemic effect of *P.eous* on the treated animals. The control rats (Group A) and the rats fed with cholesterol feed (Group E) recorded an increased accumulation of serum triacylglycerol with an increase in the duration of the treatment. Rats fed with normal feed plus mushroom at various concentrations *i.e.*2.5 per cent, 5 per cent and 10 per cent recorded a significant increase in TGL with 136.69, 138.83 and 140.94 mg % respectively on the 90th day of observation when compared to control (117.83 mg %). Also, the rats in Group F, Group G and Group H recorded a gradual increase of TGL (143.03, 144.47 and 145.74mg %, respectively) when compared to control (117.83 mg %) and pathological control (Group E) (145.01mg %) on the 90th day of observation

Table.5.Effect of *P.eous*on the triacylglycerol (mg %)content in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	104.83	110.83	117.83
Group B (2.5% *P. eous*)	93.91	94.44	136.69
Group C (5% *P. eous*)	96.03	100.07	138.83
Group D (10% *P. eous*)	98.03	98.23	140.94
Group E (Cholesterol feed)	144.09	144.90	145.01
Group F (Cholesterol feed + 2.5% *P. eous)*	141.34	142.32	143.03
Group G (Cholesterol feed + 5% *P. eous)*	142.74	143.53	144.47
Group H (Cholesterol feed + 10% *P. eous)*	143.84	144.80	145.74
SE	0.26	0.27	0.26
CD (P=0.05)	0.80	0.82	0.79

DISCUSSION

Determination of liver total cholesterol and serum total cholesterol

Several authors have reported the hypercholesterolemic effect of edible mushroom. The male Wistar rats fed with the diet containing oyster mushroom strikingly had reduced cholesterol content in the serum and liver [2]. Similarly, *P. ostreatus* reduced the serum cholesterol conc., in rat [1]. Likewise, simultaneous ingestion of mushrooms with high fat diets significantly decrease the total cholesterol levels [9].

Thus, the hypocholesterolemic effects observed in the present study *P.eous* were effective in lowering the serum total and LDL cholesterol levels and the amount of liver total cholesterol. Darwin Christdas Henry, [5] reported that a reduction in the level of total cholesterol and total lipid in the albino Wistar rats fed with a supplemented diet of *V. volvacea* with an increase in the period of treatment. Sang Chul Jeong *et al*., [11] reported that a decrease in total cholesterol, LDL, and triglyceride concentration was accompanied by a significant increase in plasma high – density lipoprotine concentrations.

Determination of High Density Lipid and Low Density Lipid

After 28th day, feeding trial with mushroom diet, plasma total cholesterol and low density lipoprotein cholesterol concentration were found significantly lower than control while the HDL level were significantly higher [9]. The present findings are in line with these earlier reports. In the present study, a significant decrease in total cholesterol, LDL-cholesterol levels and increased levels of HDL in *P.eous* treated group of animals were observed as compared to high- fat diet exclusive group.

Clinical studies have demonstrated a positive correlation between LDL cholesterol conc. in serum and the incidence of coronary heart disease in human being [15] the increase in the HDL to LDL ratio is of great importance in prevention of atherosclerosis [7, 8]. The high levels of cholesterol particularly LDL cholesterol is mainly responsible for the onset of coronary heart disease [16].

In the present study, an increase in HDL levels was observed in the group of rat that were co-administered with various levels of *P.eous* diet. Conversely, LDL levels were significantly lower in rats fed with mushroom diets than the control. Thus the LDL/ HDL cholesterol ratio which is thought to be the athereogenic index [6] of lipoproteins were lower in the rats fed with mushroom diet than the control rats. Sang Chul Jeong *et al*., [11] reported that a decrease in total cholesterol, LDL, and triglyceride concentration was accompanied by a significant increase in plasma high – density lipoprotein concentrations.

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