Hypocholesterolemic activity of *Lentinus edodes* on the serum profile in the male albino rats

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Abstract

Shiitake (*Lentinus edodes)* is an edible mushroom from East Asia which is cultivated and eaten in many Asian countries as well as in Europe. It is a species with medicinal properties, which is used primarily in traditional medicine and also in conventional oncology treatments. Cardiovascular diseases are among the main causes of death in our society and there is a strong correlation between enhanced blood cholesterol levels and the development of such diseases. The hypocholesterolemic action of this mushroom was extensively examined in rats. It was demonstrated that when rats were fed a diet supplemented with 10 % (dry weight) of *L. edodes* fruiting bodies for 90 days the plasma cholesterol, triglyceride (TG), low density lipoprotein cholesterol (LDL-c) levels were found significantly lower than control. The rats fed with 10 percent *L. edodes* (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 (1.05 mg%) on the 90th day of observation when compared to control (0.91 mg %) In the present study, a significant decrease in total cholesterol, LDL-cholesterol levels and increased levels of HDL in *L. edodes* treated group of animals were observed as compared to high- fat diet exclusive group thus making *L. edodes* as an ideal food supplement for the human society considering the increase in the coronary diseases.

Key words: *Lentinus edodes*, serum profile and Male albino wistar rats.

Introduction

Shiitake (*Lentinus edodes)* is an edible mushroom from East Asia which is cultivated and eaten in many Asian countries as well as in Europe. It is a species with medicinal properties, which is used primarily in traditional medicine and also in conventional oncology treatments (Akramiene *et al*, 2007). Cardiovascular diseases are among the main causes of death in our society and there is a strong correlation between enhanced blood cholesterol levels and the development of such diseases. *L. edodes* is revered in Asian medicine for its health-promoting effects, including anticancer, antidiabetic, hypotensive, hypocholesterolemic and antimicrobial activities (Khan *et al*., 2009). Also, it is important nutritionally because of its higher protein, dietary fibers and important mineral contents (Zhanhai yu *et al*., 2010).

Pharmacological functions of polysaccharides isolated from *L. edodes* have been reported in china (Shen *et al*., 2007). Administration of lentinan and schizophyllan the polysaccharides isolated from *L.edodes* was known to inhibit the growth of various transplantable tumors in experimental animals and increased the survival rate (Liu *et al*., 2007). Hyperlipidaemia is considered a risk factor involved in the development of cardiovascular disease (Wasser, 2005; Zhong *et al.*, 2007) and lentinan prevented heart disease by lowering blood pressure and cholesterol levels and acts as an anticoagulant (Cheung, 2008).

With such unmatched potentials the commercial interest in the shiitake mushroom has increased in recent years, mainly because of its high value on the international market and an increase in dried mushrooms imports by some countries. This has occurred, not only because of its excellent aroma, flavor and nutritional profile, but also because of its medicinal properties **(**Rigoberto *et al.,* 2011).

Materials and methods:

Medicinal properties of *Lentinus edodes*

The medicinal properties of *L. edodes* were evaluated by testing the serum chemistry of *L. edodes* supplemented diet on male albino wistar rats. Accordingly, serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein (HDL-c) were among the parameters investigated.

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 *L.edodes* was powdered and mixed thoroughly with the lab stock diet with the help of a little amount of hot water, and made into pellet form and air dried. Then it was stored in an air tight container at room temp.

Cholesterol feed: Feed rich in cholesterol, viz, groundnut oil and egg yolk were mixed with normal feed and used.

Cholesterol plus mushroom feed : Hundred g of cholesterol feed was powdered, then 2.5, 5 and 10 g of *L.edodes* was powdered and mixed thoroughly with the help of little amount of hot water, and made in to pellet form and then air dried. Then it was stored in an air tight container at room temp.

Animals and diets

Male wistar rats weighing 100 g and five weeks old were used for the study. The rats were individually housed in wire mesh cages and kept in an isolated room at a controlled temp. of 28±2°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 five per centage powdered *L. edodes* 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 with standard management practices and clinical as well as other parameters were recorded at 30 days, 60 days and 90 days duration.

Experimental Design:

The experimental rats were grouped as

Group A Rats fed with Normal Feed

Group B Rats fed with Normal Feed + 2.5% *L.edodes*

Group C Rats fed with Normal Feed + 5% *L.edodes*

Group D Rats fed with Normal Feed + 10 % *L.edodes*

Group E Rats fed with Cholesterol feed

Group F Rats fed with Cholesterol feed + 2.5% *L.edodes*

Group G Rats fed with Cholesterol feed + 5% *L.edodes*

Group H Rats fed with Cholesterol feed + 10 % *L.edodes*

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 profile was done by using ERBA CHEM semi auto analyzer. The values were taken on 30, 60 and 90th day of experiment.

RESULT AND DISCUSSION

Effect of *L. edodes* on the serum total cholesterol (mmol/l) in the male albino rats

Serum total cholesterol level in the test animal recorded (Table 1) a significant decline with an increase in the level of supplementation of *L. edodes* and with an increase in the duration of the treatment when compared to their respective controls. Among the three levels of *L. edodes* 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 serum.

The serum total cholesterol level of rats fed with 10 per cent of *L. edodes* diet (Group D) over a period of 30, 60 and 90 days recorded 4.82,4.90 and 4.95 mmol/l of cholesterol level respectively when compared to control (Group A) (5.28,5.52and 5.75 mmol/l). Similarly, the animal fed with cholesterol feed plus 10 per cent *L. edodes* diet (Group H) showed significantly decreased cholesterol level on the 30, 60 and 90th day of observation (5.28,5.84 and 4.91 mmol/l respectively) when compared with their respective controls.

Administration of polysaccharides from *L. edodes* significantly reduced serum total cholesterol and enhanced serum antioxidant enzyme activity (Chen Xu *et al.*, 2008).The various properties of the soluble β-glucan type polysaccharides could have probably lowered the cholesterol absorption by inhibiting the formation of micelles in the small intestine and altering the physical characteristic of the intestinal mucosa of rats as observed by Zhanhai yu *et al*., (2010).

Determination of High Density Lipid (HDL) and Low Density Lipid (LDL)

The data in table 2 clearly indicated that the rats fed with 10 percent *L. edodes* (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 (1.05 mg%) on the 90th day of observation when compared to control (0.91 mg %) whereas, when 5 and 10 per cent *L. edodes* 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 (1.02 mg %) on the 90th day of observation when compared to control (0.95 mg %)

Supplementation of 5 and 10 per cent *L. edodes* diet with normal feed recorded (Table 3) appreciable decrease in the LDL during the various period of observation. The LDL level was found increased 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 D (3.91 mg %) on the 90th day of observation, while Group H recorded a LDL level of 4.00 mg % when compared to its respective control Group E (4.82 mg %)

Several studies have reported the ability of edible mushroom in reducing plasma total cholesterol level. The oyster mushroom *P. florida* has been shown to possess hypolipidemic and hypocholesterolemic activity (Bobek, 1996). A dose of high fat diet supplemented with oyster mushroom diet daily for different period of time showed a significant reduction in all lipid components including LDL- cholesterol (Bobek, 1998). Similarly in study of feeding trial with mushroom *(L. edodes)* diet, plasma total cholesterol and low density lipoprotein cholesterol concentration were found significantly lower than control while the HDL level were significantly higher (Yasuko *et al*., 2010). The present findings are in line with these earlier reports.

Clinical studies have demonstrated a positive correlation between LDL cholesterol conc. in serum and the incidence of coronary heart disease in human being (Vazques-Freire *et al.*, 1996). The increase in the HDL to LDL ratio is of great importance in prevention of atherosclerosis (Farias *et al.*, 1996; Ghasi *et al*., 2000). The high levels of cholesterol particularly LDL cholesterol is mainly responsible for the onset of coronary heart disease (Farias *et al.*, 1996; Yokozawa *et al.*, 2003).

In the present study, a significant decrease in total cholesterol, LDL-cholesterol levels and increased levels of HDL in *L. edodes* treated group of animals were observed as compared to high- fat diet exclusive group thus making *L. edodes* as an ideal food supplement for the human society considering the increase in the coronary diseases.

Determination of triacylglycerol (TGL) (mg%)and liver total lipids(mg/kg^{-1})

The data presented in table 4 clearly indicated the hypocholesterolemic effect of *L. edodes* 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 mushroom at various concentrations *i.e.*2.5 per cent, 5 per cent and 10 per cent recorded a significant decrease in TGL with 1.61,1.58 and 1.50 mg % respectively on the 90th day of observation when compared to control (1.65 mg %). Also, the rats in Group F, Group G and Group H recorded a gradual decrease of TGL (1.69, 1.65 and 1.60 mg %, respectively) when compared to control Group E (1.72 mg %) on the 90th day of observation

The present findings are in line with the earlier report of Shimada (2009) who reported that, low density lipoprotein cholesterol and triglycerides concentrations were found to be significantly lesser than control, while HDL level were significantly higher in rats fed with *L. edodes* diet.

The reduction in triglyceride level was observed in the group of rats that were co-administered with various levels of *P. florida* diet and the elevated triglyceride levels are frequently associated with low HDL levels (Kelly, 1992). HDL concentrations vary reciprocally with plasma triglyceride conc. and directly with the activity of lipoprotein lipase (Murray *et al.*, 2003).

Hyperlipidemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart diseases (CHD). Cardiovascular diseases, including atherosclerosis are the most common cause of mortality and morbidity worldwide (Yokozawa *et al.*, 2003). Lowering of lipids and cholesterol levels by a drug or dietary intervention with the use of edible mushrooms especially *L. edodes* could reduce the risk of CHD.

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Table 1. Effect of *L.edodes* on the total cholesterol (mmol/L) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	5.28_{d}	5.52_{d}	5.75_{d}
Group B (2.5% *L.edodes*)	5.10_{c}	5.43_{c}	5.62_{c}
Group C (5% *L.edodes*)	4.95_{b}	5.21_{b}	5.42_{b}
Group D (10% *L.edodes*)	4.82_{a}	4.90_{a}	4.95_{a}
Group E (Cholesterol feed)	6.25_{g}	6.74_{h}	6.95_{h}
Group F (Cholesterol feed + 2.5% *L.edodes)*	6.12_{f}	6.51_{g}	6.62_{g}
Group G (Cholesterol feed + 5% *L.edodes)*	5.91_{e}	6.02_{f}	6.12_{f}
Group H (Cholesterol feed + 10% *L.edodes)*	5.28_{d}	5.84_{e}	5.91_{e}

Table 2. Effect of *L.edodes* on the HDL (mg %) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	0.75_g	0.82_f	0.91_h
Group B (2.5% *L.edodes*)	0.79_f	0.84_e	0.94_g
Group C (5% *L.edodes*)	0.82_e	0.89_d	0.99_c
Group D (10% *L.edodes*)	0.85_b	0.92_c	1.05_a
Group E (Cholesterol feed)	0.90_c	0.92_c	0.95_f
Group F (Cholesterol feed + 2.5% *L.edodes)*	0.91_c	0.93_c	0.97_e
Group G (Cholesterol feed + 5% *L.edodes)*	0.93_b	0.95_b	0.98_d
Group H (Cholesterol feed + 10% *L.edodes)*	0.95_a	0.97_a	1.02_b

Table 3: Effect of *L.edodes* on the LDL (mg %) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	4.25_d	4.31_f	4.62_f
Group B (2.5% *L.edodes*)	4.10_c	4.20_e	4.45_e

Group C (5% *L.edodes*)	3.82_b	4.05_c	4.12_c
Group D (10% *L.edodes*)	3.61_a	3.84_a	3.91_a
Group E (Cholesterol feed)	4.35_e	4.58_g	4.82_h
Group F (Cholesterol feed + 2.5% *L.edodes)*	4.21_d	4.31_f	4.51_f
Group G (Cholesterol feed + 5% *L.edodes)*	4.18_c	4.10_d	4.28_d
Group H (Cholesterol feed + 10% *L.edodes)*	3.81_b	3.92_b	4.00_b

Table 4: Effect of *L.edodes* on the triacylglycerol (mg %)content in the serum of male albino wistar rats

Groups	**30 days**	**60 days**	**90 days**
Group A (Normal Feed)	1.51_c	1.58_e	1.65_d
Group B (2.5% *L.edodes*)	1.49_b	1.51_c	1.61_c
Group C (5% *L.edodes*)	1.42_a	1.47_b	1.58_b
Group D (10% *L.edodes*)	1.40_a	1.42_a	1.50_a
Group E (Cholesterol feed)	1.62_f	1.68_h	1.72_f
Group F (Cholesterol feed + 2.5% *L.edodes)*	1.58_e	1.62_g	1.69_e
	1.55_d	1.59_f	1.65_d

Group G (Cholesterol feed + 5% *L.edodes)*			
Group H (Cholesterol feed + 10% *L.edodes)*	1.50_c	1.56_d	1.60_c