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Effect of Feeding *Agaricus Bisporus* (White Button) Mushroom on Serum and Liver Cholesterol and Excretion of Cholesterol and Bile Acids in Rats

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Abstract

This study investigated the hypocholesterolic effects of (*Agaricus bisporus*) an Indian edible mushroom, in male Albino rats. The study revealed a significant reduction in serum total cholesterol content in experimental groups I and II, fed with 5 and 10% mushroom diet, respectively. The decrease in cholesterol was attributed to a reduction in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). No significant changes were observed in the concentration of serum high-density lipoproteins (HDLs) between the control and experimental groups. However, a significant increase was noted when HDL cholesterol was expressed as a percentage of total cholesterol. Liver cholesterol levels (total, LDL, and VLDL) were significantly reduced in the experimental groups compared to the control group. The liver HDL cholesterol content in the experimental groups I and II increased by 13 % and 28 %, respectively. Additionally, there was a significant increase in the bulk of faces produced by the experimental groups compared to the control group. Fecal excretion of cholesterol and bile acids also increased in rats fed a mushroom diet. Present findings suggest a potential hypocholesterolemic effect of *Agaricus bisporus*, highlighting its possible role in promoting cardiovascular health.

Keywords: *Agaricus bisporus*; Bile acids; Cholesterol; LDL Cholesterol; Rats.

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1. Introduction

Mushrooms, unique in texture and flavor, belong to the vegetable Kingdom. The term “edible mushroom” refers to the fruiting bodies of macroscopic fungi cultivated commercially or grown under controlled conditions. In India, commercial cultivation primarily focuses on 5 mushroom species: White button mushroom (*Agaricus bisporus*), Oyster mushroom (*Pleurotus* spp), Paddy straw mushroom (*Volvariella volvacea*), Milky mushroom (*Calocybe indica*), and Shitake mushroom (*Lentinula edodes*). But still, White button mushroom commands the largest market share, comprising 85 %, while oyster mushrooms and paddy mushrooms hold 7 and 6 % market share respectively [1-3].

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Agaricus bisporus, particularly popular in North India, is a low calorie food rich in protein, vitamins(including C, D, and B12), minerals, dietary fiber, and antioxidants [4,5]. The fatty acid composition of *Agaricus bisporus* reveals a high percentage of unsaturated fatty acids (80, 41 %), contributing to overall health and reducing the risk of atherosclerosis [6,7]. Mushrooms, being low in fat and high in dietary fiber, are considered an ideal dietary choice for preventing various diseases, including cardiovascular diseases [8].

Cardiovascular diseases stand as the primary cause of global mortality [9]. The increasing interest in recent years revolves around understanding the link between dietary components, particularly those rich in protein, fiber, and unsaturated fatty acids, and their impact on cardiovascular diseases, which are leading causes of global mortality. Cardiovascular diseases, often rooted in atherosclerosis, are exacerbated by elevated blood cholesterol levels [10,11]. Atherosclerosis, a major contributor to cardiovascular diseases, is influenced by various factors such as a sedentary lifestyle, genetic predisposition, and lipid abnormalities. Elevated levels of low-density lipoprotein (LDL), commonly known as" bad cholesterol," contribute to the progression of atherosclerosis by providing more circulating lipids for incorporation into arterial walls. Conversely, high-density lipoprotein(HDL) cholesterol, often referred to as" good cholesterol", plays a protective role by transporting cholesterol away from blood vessels to the liver for breakdown, destruction, and excretion [12].

In light of these considerations, there is a consistent effort to include foods in our diet that not only lower "bad cholesterol" levels but also raise "good cholesterol" levels. This dietary approach aims to promote cardiovascular health and mitigate the risk of diseases associated with the heart and blood vessels.

In the context of exploring dietary interventions for cardiovascular health, various mushroom species, including Oyster mushrooms [13], *Pleurotus florida* [14], *Pleurotus ostreatus* [15], and *Agaricus bisporus* [16], have demonstrated the potential to lower plasma cholesterol levels in rats. This study specifically aims to investigate the impact of incorporating white button mushrooms on the concentration of total cholesterol, LDL cholesterol, and HDL cholesterol in both the serum and liver of the rats.

2. Materials and Methods

2.1. *Samples*

The white button mushroom (*Agaricus bisporus*) used in this study was obtained from the Department of Plant Pathology, CCSHAU, Hisar (India), and washed with water to remove the dust and other foreign material. The cleaned mushroom was dried in an oven at a temperature of 60 °C+2 °C. The dried samples were further pulverized using a cyclo-tech mill (Tecator, Hoganas, Sweden) to achieve a particle size small enough to pass through a 0.5 mm sieve. The milled mushroom sample was then stored in air-tight plastic bags in desiccators at room temperature (27 °C).

2.2. *Composition of diet*

Diets were prepared according to AIN-76 purified diets for rats containing 10 percent protein (Table 1). The protein content of the diet was adjusted after taking into account the protein content of the test material. Cholesterol was added to all the diets at 1 % level (except in group I) in order to induce alimentary hypercholesterolemia in rats. For the preparation of diets, the ingredients were mixed thoroughly and passed through 70 mesh sieve to ensure uniform distribution of vitamins and minerals.

Table 1. Nutrient content in control and experimental diets (g/Kg).

Ingredients	Type of Diet			
	Control Diet I (Cellulose (g) without cholesterol)	Control Diet II (Cellulose (g) with cholesterol)	Experimental Diet I (*Agaricus bisporus* (5 %)	Experimental Diet II (*Agaricus bisporus* (10 %)
Albumin powder	126.98	126.98	111.51	96.03
Fat (hydrogenated veg. oil)	50	50	50	50
Mineral mixture	40	40	40	40
Vitamin mixture	10	10	10	10
Sucrose	100	100	100	100
Choline chloride	2	2	2	2
Methionine	3	3	3	3
Cholesterol	-	10	10	10
Agaricus bisporus (5%)	-	-	50	-
Agaricus bisporus (10%)	-	-	-	100
Cellulose	50	50	-	-
Starch	608.02	608.02	623.49	588.97

Mineral and vitamin mixtures recommended by the BARR Committee 1972 were used in the diet. Hydrogenated vegetable oil and egg albumin were the sources of fat and protein, respectively. The diet for one week's consumption was prepared at one time and stored in the refrigerator.

2.3. *Experimental design*

Twenty-eight weanling albino male rats weighing 30-40 g were obtained from the Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar. Animals transported from the animal house to the test laboratory were weighed when received and fed standardized laboratory rat chow for acclimatization for a period of 2 days. Then, the rats were randomly divided into four groups, each consisting of seven rats, and they were fed the following diets for 42 days:

Group I: Synthetic diet
Group II: Synthetic diet + cholesterol (1 %)
Group III: Synthetic diet + cholesterol (1 %) + Mushroom (*A. bisporus* 5 %)
Group IV: Synthetic diet + cholesterol (1 %) + Mushroom (*A. bisporus* 10 %)

The rats were individually housed in polypropylene cages, maintained under controlled conditions with a temperature range of 18-26 °C and a 12 h light/dark cycle. Food and water were given *ad libitum* to rats for 42 days. At the end of the experiment, the rats were deprived of food for 16 h and anesthetized using diethyl ether. After anesthesia, blood samples were collected via heart puncture and transferred to test tubes without anticoagulant. These blood samples were then centrifuged at 3000 rpm for 30 min to obtain the serum samples. Livers extracted from animals were washed with saline, promptly frozen in liquid nitrogen, and subsequently stored in a deep freeze for further analysis.

2.4. *Analytical methods*

The serum was analyzed for total cholesterol [17] and HDL cholesterol [18] using a fully automatic blood chemistry analyzer. LDL and VLDL cholesterol were estimated by using the following formulae [19]

LDL cholesterol = Total cholesterol -HDL cholesterol-1/5th Triglycerides

VLDL cholesterol= Total cholesterol – (HDL cholesterol + LDL cholesterol)

The liver total lipids were extracted from about 1-2 g of liver with chloroform: methanol (2:1 v/v) [20,21]. After extraction, the volume of the lipid solution was adjusted to 10 mL with chloroform, and it was used for estimation of liver total, HDL, LDL, and VLDL cholesterol by the methods described earlier for serum.

2.5. *Faecal analysis*

The fecal sample was analyzed for bile acid [18]. Cholesterol in feces was determined with a fully automatic blood chemistry analyzer.

2.*6. Ethical approval*

The present study and all experimental procedures were approved and performed according to the care and use of animals as prescribed by the Animal Welfare and Experimentation Ehics Committee, India, CCS Haryana Agricultural University, Hisar-125004, India.

2.7. *Statistical analysis*

The statistical analysis of the data was conducted using a completely randomized design (CRD) for calculating mean, standard deviation, and percent according to the standard method [22]. On the basis of CRD, critical difference (CD) has been calculated. Significance at the 5 % level ($P<0.05$) was established when the differences between the two treatments exceeded the calculated CD value.

3. Results and Discussion

Results in Table 2 summarize the effect of feeding 5 and 10 % *Agaricus bisporus* mushroom on serum cholesterol levels of rats.

Table 2. Impact of white button mushroom (*Agaricus bisporus*) consumption on serum cholesterol levels in rats (mg/100 mL).

Dietary group	Total cholesterol	HDL cholesterol		LDL cholesterol	VLDL cholesterol
		Total	% of Total cholesterol		
Control group I (without cholesterol)	83.4 ± 0.37 (63.50)	34.3 ± 0.49 (62.39)	41.13 ±0.65	22.16 ± 0.67 (74.52)	26.94 ± 0.06 (46.45)
Control group II (with cholesterol)	228.5 ± 0.61	91.2 ± 0.61	39.91 ± 0.31	86.99 ± 0.98	50.31 ± 0.06
Experimental group I *Agaricus bisporus* (5%)	215.3 ±0.64 (5.78)	90.4 ± 0.57 (0.88)	41.94 ± 0.21	77.6 ± 0.66 (10.79)	47.3 ± 0.05 (5.98)
Experimental group II *Agaricus bisporus* (10%)	197.3 ± 0.40 (13.65)	89.8 ± 0.65 (1.53)	45.51 ± 0.34	64.34 ± 0.74 (26.04)	43.16 ± 0.11 (14.21)
CD (P<0.05)	1.55	1.74	1.22	2.30	0.22

Values are mean ± SD of six replicates,
Values in parenthesis are per cent decrease over control group II

Experimental rats exhibited a significant reduction in serum levels of total, LDL, and VLDL cholesterol compared to the control group II. The group of rats fed with a higher percentage (10 %) of mushrooms showed significantly lower serum levels of total, LDL, and VLDL cholesterol as compared to the group of rats fed with 5 % of mushrooms.

When comparing the two control groups, I and II, it was observed that control group I, which was fed a diet devoid of cholesterol, had significantly lower serum lipid profiles. However, no significant differences were detected in the concentration of HDL cholesterol between the two experimental groups and control group II. Nevertheless, when serum HDL cholesterol levels were expressed as a percentage of total cholesterol, they were found to be significantly higher in control group I and in both experimental groups as compared to control group II.

The findings presented in Table 3 indicate that hepatic levels of total, LDL, and VLDL cholesterol were significantly lower in control group I and experimental group I and II (fed 5 and 10 % mushroom) compared to control group II fed with basal diet plus cholesterol. The deposition of cholesterol in the liver was found to be significantly lower in the group fed 10 % mushroom. However, HDL cholesterol levels were found to be significantly higher in groups fed mushroom and control group I.

Table 3. Influence of white button mushroom (*Agaricus bisporus*) diet on liver cholesterol level in rats (g/100 g tissue)

Dietary group	Total cholesterol	*HDL cholesterol*		LDL cholesterol	VLDL cholesterol
		Total	% of total		
Control group I (without cholesterol)	0.23± 0.03 - (89.54)	0.013± .00 (88.89)	6.67±1.05	0.15 ± 0.04 - (90.62)	0.07 ± 0.01 - (85.42)

Control group II (with cholesterol)	2.2 ± 0.05	0.117±0.02	5.29±0.77	1.60 ± 0.05	0.48 ± 0.01
Experimental group I (*Agaricus bisporus*5%)	1.87± 0.07 - (15.0)	0.133±0.02 + (13.67)	7.27+1.30	1.27 ± 0.07 - (20.62)	0.47 ± 0.01 - (2.08)
Experimental group II (*Agaricus bisporus*10%)	1.47± 0.04 - (33.18)	0.15 ± 0.02 + (28.20)	10.26±1.56	0.89 ± 0.05 - (44.37)	0.43 ± 0.01 - (10.42)
CD (P<0.05)	0.15	0.05	NS	0.16	0.04

Values are mean ± SD of six replicates.
Values in parenthesis are per cent decrease (-) or increase (+) or over the control group (II)
NS= Non-significant

The results presented in Table 4 show a significant increase in the production of feces upon the addition of 5 and 10 % mushrooms to the basal diet. The excretion of cholesterol in feces witnessed a rise of 7.57 and 19.7 % in experimental groups I and II as compared to control group II, which was fed a basal diet with no mushroom supplement. A similar pattern was observed in excretion of bile acids in the feces of rats in experimental groups.

Table 4. Impact of white button mushroom (*Agaricus bisporus*) diet on fecal weight, cholesterol, and bile acid levels in rats.

Dietary group	Fecal weight (g)	Cholesterol (g/100 g)	Bile acids (mg/100 g)
Control group I (without cholesterol)	1.28 ± 0.01 + (5.78)	0.15 ± 0.02 - (88.64)	190.83 ± 3.52 - (66.62)
Control group II (with cholesterol)	1.21 ± 0.01	1.32 ± 0.05	571.77 ± 8.43
Experimental group I *Agaricus bisporus* (5%)	1.31 ± 0.01 ± (8.26)	1.42 ± 0.05 + (7.57)	641.77 + 3.57 + (12.23)
Experimental group II *Agaricus bisporus* (10%)	1.43 ± 0.01 + (18.18)	1.58 ± 0.05 ± (19.70)	730.83 ± 6.11 + (27.82)
CD (P<0.05)	0.02	0.13	17.17

Values are mean ± SD of six replicates.
Values in parenthesis are per cent increase (+) or decrease (-) over the control group (II)

The observed reduction in serum cholesterol levels of rats in the present experiment aligns with findings reported in previous studies [23-25]. The potential mechanism behind the lower plasma cholesterol level in rats could be attributed to the inhibition of cholesterol synthesis and/or the acceleration of cholesterol metabolism induced by the diet containing mushrooms.

The process driving the initiation of atherogenesis has been demonstrated to stem from the building up of lipids within the arterial wall. Elevated levels of low-density lipoproteins (LDL) cholesterol in the bloodstream constitute the primary risk factor for atherosclerosis, contributing to the development of cardiovascular diseases [26,27]. In the present investigation, the efficacy of a diet supplemented with 1 % cholesterol administered to rats over a 42-day period has been affirmed. This dietary intervention effectively resulted in increased serum total cholesterol, LDL, and VLDL levels in the control group of rats.

In the present study, the addition of 5 and 10 % mushroom diets to rats demonstrated a significant inhibition of the rise in plasma cholesterol levels. Mushrooms are known to

contain the agent mevinolin (monacolin K, lovastatin), a hypocholesterolemic agent. This compound may play a role in reducing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase enzyme, the rate-limiting factor in the biosynthesis of cholesterol [23,26]. Another study suggested that the major degradation products of cholesterol, namely bile acids, were excreted in feces when the diet included oyster mushrooms, indicating an impact on cholesterol catabolism [28]. The elimination of cholesterol from the body initiates with the hydrolysis of esters in LDL cholesterol, converting them into free cholesterol within the liver. Subsequently, this free cholesterol is either secreted or transformed into bile acids within the bile ducts [25]. The present study (Table 4) and others have reported an increased fecal output of bile acids with various types of mushrooms [29].

The presence of fiber in mushrooms may interfere with the undigested residue of mushroom proteins, leading to an elevated fecal excretion of bile acids. This reduction of bile acid re-absorption could potentially stimulate the activity of liver cholesterol 7α-hydroxylase, a rate-limiting enzyme in the degradation of cholesterol to bile acids [30]. Kim *et al*. [31] developed noodles that contained *L. edodes* paste, resulting in a higher quality, fiber-rich functional food with antioxidant and hypocholesterolemic properties.

Agaricus bisporus mushroom is low in fat, comprising about 80 % unsaturated fatty acid, especially linoleic acid, which is particularly beneficial for the prevention of atherosclerosis [5,6]. The results of the study align with earlier research findings on the impact of linoleic acid [5,32]. Linoleic acid, an essential fatty acid for human health, plays a significant role in various physiological functions, and its presence in diet is linked to a decreased risk of cardiovascular diseases, triglyceride levels, and blood pressure. The incorporation of a diet rich in polyunsaturated fatty acid contributes to increased fecal excretion of cholesterol, leading to a reduction in cholesterol absorption in the gut. This dietary shift causes a reduction in its synthesis by the body or directs its redistribution from plasma to other body components [33,34].

5. Conclusion

The rising incidence of atherosclerosis and coronary heart disease has sparked interest in exploring foods with potential health benefits and a role in reducing cholesterol levels. The findings from the present study indicate that *Agaricus bisporus* mushroom led to a significant reduction in both serum (5.78 % and 13.65 %) and liver (15 and 33.18 %) cholesterol levels in rats fed 5 and 10 % mushroom diet. Besides, liver HDL cholesterol levels increased significantly (13.67 and 28.2 %), and the excretion of both cholesterol and bile acids increased significantly, establishing their potential as a hypocholesterolemic agent. The findings emphasize the versatility of white button mushrooms as a potential dietary supplement for individuals looking to manage their lipid profiles and support cardiovascular health benefits.

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