Annals. Food Science and Technology
2014

A STUDY ON CHOLESTEROL LOWERING EFFECT OF *PLEUROTUS SAJOR-CAJU* MUSHROOM IN HYPERCHOLESTEROLEMIC RATS

Rajni Goyal[*1], R.B. Grewal[2]

[1] Department of Home Science,University College, Kurukshetra University, Kurukshetra
[2]Department of Foods & Nutrition, CCS Haryana Agricultural University Hisar-125004.INDIA
*E-mail: shubhi__rgoyal@yahoo.com

Abstract
The present study was conducted to evaluate the hypocholesterolemic effect of Pleurotus sajor-caju mushroom in hypercholesterolemic rats. Rats were fed hypercholesterolemic semi-synthetic diet containing 1 % cholesterol and supplemented with 5 and 10 % dried powder of the mushroom. The feeding experiment was carried out for a period of 7 weeks. The results showed that serum total cholesterol content in experimental group of rats (I & II) fed 5 and 10 % mushroom diet, respectively was reduced significantly by 7.13 and 16.59%, respectively. The reduction of cholesterol was mainly due to decreased cholesterol content in low density lipoproteins (LDL) and very low density lipoprotein (VLDL). No significant change was observed in concentration of serum high-density lipoproteins (HDLs) between control & experimental groups. However, when HDL cholesterol was expressed as per cent of total cholesterol, it increased significantly. The total, LDL and VLDL cholesterol levels in liver were reduced significantly in experimental groups as compared to control group. The liver HDL cholesterol content of experimental groups I & II was increased by 28.2 and 42.73 %, respectively. A significant increase in bulk of feces produced as well as excretion of cholesterol and bile acids in feces was found in rats fed mushroom diet as compared to control group.

Keywords: Pleurotus sajor-caju, rats, total cholesterol, LDL cholesterol, bile acids, India

Submitted: 08.03.2014 Reviewed: 29.04.2014 Accepted: 14.05.2014

1. INTRODUCTION

Mushrooms are fleshy spore-bearing organ of the fungi (Miles & Chang, 1997). They are considered as efficient means for conversion of waste agro materials into valuable protein rich food. At present mushroom cultivation is the major fermentation industry which involves the bioconversion of cellulosic wastes into edible biomass. The word ‘mushroom’ is thought to be derived from the French word ‘mousseron’, ‘mouse’ or ‘moss’, while in India, it is known as Ksumpa in Sanskrit and Khumbi or Kukurmutta in Hindi. Artificial cultivation of mushroom was initiated in France around 1650 AD and spread rapidly to the entire Europe. Early people believed mushrooms to be wild food but now it has become very popular and valuable food item in the modern dietary regimes because of its nutritional value. Mushrooms need organic matter to grow. Mushrooms secrete enzymes to digest surrounding foodstuff and to get nutrients from organic matter, which is generally called as compost. Nutritional value of mushroom as a result largely depend on chemical composition of the compost which is a mixture of straw or hay, corn cobs, water, cotton seed meal and nitrogen supplement (Tshinyangu, 1996). Thus there are variations in the composition data of same species of mushrooms as reported by different authors. In Northern Indian markets, species of Pleurotus are popularly sold by the name 'Dhingri'. Nutritionally, *Pleurotus sajor-caju* lies between vegetables and meat. The food experts have realized the importance of mushrooms and now they had started appreciating the food value of mushrooms because of their low calorific value, very high content of proteins, B-complex vitamins, minerals and unsaturated fatty acids.

A number of feeding trials have been conducted in the past to study the effect of dietary fiber from various sources of food on

level of lipids in serum and liver. Various foods such as oat, barley, beans, guar gum and soybean have been reported to lower plasma cholesterol in rats or human beings. Besides, replacement of saturated fats in the diet by polyunsaturated fats from vegetable sources changes the lipid profile that is associated with a decreased risk of cardiovascular disease (Goodnight et al., 1982). Mushrooms being low in fat, energy, rich in fiber and unsaturated fatty acids are the choice of the people suffering from hypertension and atherosclerosis. Higher fungi are almost ideal food with respect to the dietetic prevention, development and treatment of hypercholesterolemia. The present study was conducted to explore the effect of *Pleurotus sajor-caju* (dhingri) mushroom on serum and liver lipids and on fecal excretion of bile acids and cholesterol in rats.

2. MATERIAL AND METHODS

2.1. Procurement of material

Fresh *Pleurotus sajor-caju* mushroom was procured 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 oven at $60+2^0$C. The dried samples were pulverized by using a cyclotech mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm sieve. The milled mushroom sample was then stored in air tight plastic bags in desicators at room temperature (27^0C).

2.2. Nutritional evaluation of mushroom

2.2.1. Proximate nutrients

Moisture, crude protein, fat, ash and crude fiber contents of the mushroom sample was estimated using the standard method of analysis (AOAC, 1990). The total carbohydrate content was determined using following equation:

Total carbohydrate = 100 – (Moisture + protein + fat + ash + crude fiber)

2.2.2.Fatty acid composition

Fatty acid composition of mushrooms was analyzed by GLC (Gas Liquid Chromatography) using the method of Luddy *et. al*. (1968).

2.2.3.Dietary fibre constituents

The mushroom was analyzed for dietary fiber constituents by the method of Van Soest and Wine (1967) modified by Arora (1981).

2.3. Biological experiment

2.3.1.Experimental Design

Before starting this experiment approval has been taken from the Institutional Review Committee for animals**.** 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 following diets for 42 days:

Group I: Synthetic diet
Group II: Synthetic diet + cholesterol (1%)
Group III: Synthetic diet + cholesterol (1%) + Mushroom (P.sajor-caju 5%)
Group IV: Synthetic diet + cholesterol (1%) + Mushroom (P.sajor-caju 10%)

The rats were housed individually in polypropylene cages kept under the conditions of 18-260C with 12h 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 16h and anesthetized using diethyl ether. After anesthesia, blood samples taken from the heart puncture were collected in the test tubes without anticoagulant and were centrifuged at 3000rpm for 30 minutes to give the serum samples. Liver removed from animals was washed with saline and frozen in liquid nitrogen immediately and kept in a deep freeze.

2.3.2. Composition of Diet

Diets were prepared according to AIN-76 purified diets for rats containing 10 percent protein (Table 1). The protein content of diet was adjusted after taking into account the

protein content of test material. Cholesterol was added to all the diets at 1% level (except in group I) in order to induce an 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. Mineral and vitamin mixtures recommended by BARR committee, 1972 were used in the diet. Hydrogenated vegetable oil and egg albumin were the source of fat and protein, respectively. The diet for one week's consumption was prepared at one time and stored in refrigerator.

2.3.3. Body fluid and organ

At the end of the experiment, the rats were deprived of food for 16h and anesthetized using diethyl ether. After anesthesia, blood samples taken from the heart puncture were collected in the test tubes without anticoagulant and were centrifuged at 3000rpm for 30 minutes to give the serum samples. Liver removed from animals was washed with saline solution, cleaned of adhering matter and blotted in filter paper. It was frozen in liquid nitrogen immediately and kept in a deep freeze till further analysis.

2.4. Analytical methods

2.4.1. Serum analysis

The serum was analyzed for total cholesterol (Roeschlau *et al.*, 1974) and HDL cholesterol (Rocos & Farenbach, 1963) using fully automatic blood chemistry analyzer. LDL and VLDL cholesterol were estimated by using following formulae (Friedwald *et al.*, 1972).

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 1-2g of liver with chloroform: methanol (2:1 v/v) according to the method of Folch *et al.* (1957) as modified by Chauhan (1974). After extraction the volume of the lipid solution was adjusted to 10ml with chloroform and it was used for estimation of liver total, HDL, LDL and VLDL cholesterol by the methods described earlier for serum.

2.4.2. Fecal Analysis

Fecal sample was analyzed for bile acid by the method of Rocos & Farenbach (1963). Cholesterol in feces was determined with fully automatic blood chemistry analyzer.

2.4.3. Statistical Analysis

All data were statistically analyzed in completely randomized design (CRD) for mean, standard deviation and per cent using standard method of Panse and Sukhatme(1961). On the basis of CRD, critical difference (CD) has been calculated. Whenever, the differences between two treatments were more than the CD value, the differences were significant at the 5% level ($P<0.05$).

Table1. Biochemical Composition of Experimental Diets /Kg.

	Cellulose (without cholesterol)	Cellulose	*Pleurotus sajor caju* (5 %)	*Pleurotus sajor caju* (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
Pleurotus sajor caju (5%)	-	-	50	-
Pleurotus sajor caju (10%)	-	-	-	100
Cellulose	50	50	-	-
Starch	608.02	608.02	623.49	588.97

Table 2. Proximate composition, dietary fibre constituents and fatty acid composition of *Pleurotus sajor caju* mushroom

Nutrients	g/100g
Protein	25.65 ± 0.05
Fat	1.96 ± 0.12
Ash	7.46 ± 0.30
Crude fiber	12.13 ± 0.03
Dietary fibre constituents	
Hemi cellulose	26.32 ± 0.77
Cellulose	10.17± .08
Lignin	7.22 ± 0.02
Fatty acids	
Saturated Fatty acids	
a) Total	22.92
b) Myristic acid	1.02
c) Palmitic acid	13.50
d) Stearic acid	2.92
e) Behanic acid	5.483
Unsaturated Fatty acids	
a)Total	77.08
b) Oleic acid	9.46
c) Linoleic acid	65.67
d) Linolenic acid	1.95

3. RESULTS AND DISCUSSION

3.1. Nutritional evaluation

The proximate composition of *Pleurotus sajor-caju* mushroom presented in Table 2. reveals that *Pleurotus sajor-caju* mushroom was low in fat content; rich in protein and had good amount of ash and crude fiber. The fatty acid composition of mushrooms given in Table 2 reveals that saturated fatty acid content was 22.92 % and palmitic acid was the predominant saturated fatty acid. The total unsaturated fatty acid (USFA) content in *Pleurotus sajor-caju* mushroom was quite high (77.08%) and among various unsaturated fatty acids present, linolenic acid was present in highest amount (65.67 %).

The higher amount of linoleic acid present in mushroom is comparable to that present in Safflower oil which is most suitable for prevention of atherosclerosis (Hughes, 1972).

Pleurotus sajor caju mushroom is good source of fiber(Table 2).Hemi cellulose content determined by subtracting ADF(acid detergent fiber) from NDF(neutral detergent fiber) was 26.32 g/100g, where as the amount of cellulose and lignin were 10.17 and 7.22 percent in dhingri mushroom. Total dietary fiber, as determined from sum of hemicelluloses, cellulose and lignin was 43.73 percent in *Pleurotus sajor caju* mushroom.

3.2. Serum and liver analysis

Both mushroom diets (5&10%) significantly reduced the levels of serum total cholesterol, LDL cholesterol and VLDL cholesterol (Table 3). Inclusion of dhingri mushroom (Pleurotus sajor caju) at 5 and 10 percent level in the diets of experimental rats resulted in 7.13 and 16.59 percent lower serum cholesterol levels as compared to group of rats fed on control diet (II) containing cholesterol. With increase in percent of dhingri (Pleurotus sajor caju) mushroom in the diets of experimental group, the cholesterol lowering effect was further increase. No significant difference was found in concentration of HDL cholesterol among the three diets group. However, when HDL cholesterol was expressed as per cent of total cholesterol, it increased significantly ($P<0.05$) in experimental groups I & II.Table 4 shows the effect of feeding P.sajor-caju mushroom on levels of liver total, LDL, HDL and VLDL cholesterol. The results reveal that addition of Pleurotus sajor-caju to control plus cholesterol diet significantly decreased the level of total cholesterol, LDL cholesterol and VLDL cholesterol in liver. However, a significant increase in liver HDL cholesterol was observed in experimental rats.

Table 3. Effect of feeding dhingri mushroom (*Pleurotus sajor caju*) on serum cholesterol (mg/100ml) levels in rats

Dietary group	Total cholesterol	HDL cholesterol Total	% of Total cholesterol	LDL cholesterol	VLDL 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 *Pleurotus sajor caju* (5%)	212.2 ± 0.23 (7.13)	90.1 ± 0.57 (1.21)	42.46 ± 0.25	75.25 ± 0.52 (13.49)	46.85 ± 0.17 (6.88)
Experimental group II *Pleurotus sajor caju* (10%)	190.6 ± 0.39 (16.59)	89.3 ± 0.69 (2.08)	46.85 ± 0.32	59.82 ± 0.63 (31.23)	41.48 ± 0.10 (17.55)
CD (P<0.05)	1.25	1.77	1.23	2.15	0.32

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

Table 4. Effect of feeding mushroom dhingri mushroom (*Pleurotus sajor caju*) on liver cholesterol (g/100g tissue) levels in rats

Dietary group	Total cholesterol	*A. HDL cholesterol* Total	% of total	LDL cholesterol	VLDL cholesterol
Control group I (without cholesterol)	0.23 ± 0.03 - (89.54)	0.013 ± 0.00 + (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 *Pleurotus sajor caju* (5%)	1.78 ± 0.05 - (19.09)	0.15 ± 0.02 + (28.20)	8.32 ± 1.1	1.18 ± 0.03 - (26.25)	0.45 ± 0.02 - (6.25)
Experimental group II *Pleurotus sajor caju* (10%)	1.27 ± 0.03 - (42.27)	0.167 ± 0.02 + (42.73)	13.06 ± 1.53	0.69 ± 0.03 - (56.87)	0.41 ± 0.01 - (14.58)
CD (P<0.05)	0.13	0.05	3.01	0.11	0.04

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

Table 5. Effect of feeding dhingri mushroom (*Pleurotus sajor caju*) on fecal weight, cholesterol and bile acid in rats

Dietary group	Fecal weight (g)	Cholesterol (g/100g)	Bile acids (mg/100g)
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 *Pleurotus sajor caju* (5%)	1.34 ± 0.01 + (10.74)	1.45 ± 0.04 + (9.85)	658.33 ± 7.15 + (15.14)
Experimental group II *Pleurotus sajor caju* (10%)	1.51 ± 0.00 + (24.79)	1.60 ± 0.04 + (21.21)	760.00 ± 7.64 - (32.96)
CD (P<0.05)	0.02	0.13	20.63

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

3.3. Fecal Analysis

The excretion of feces increased significantly in experimental group of rats fed on mushroom diets (Table 5). The per cent increase in excretion of cholesterol was 9.85 and 15.14 per cent in the feces of experimental groups I &II respectively. The excretion of bile acids in stools increased by 21.21 and 15.14 per cent in experimental groups I and II, respectively.

Results obtained in the present study revealed that Pleurotus sajor-caju was effective in

reducing the level of serum total cholesterol, VLDL cholesterol and LDL cholesterol. A low plasma cholesterol level in rats may be due to the inhibition in cholesterol synthesis and or acceleration of cholesterol metabolism by the diet containing mushroom.

Besides dhingri (Pleurotus sajor caju) mushroom is good source of fiber, vegetable protein, low in fat containing more than 70 percent unsaturated fatty acid (Table 2)and devoid of cholesterol (Rai, 1995). Polyunsaturated rich diet had stronger influence on serum cholesterol levels Presence of polyunsaturated fatty acid in diet is associated with decreased risk of cardiovascular disease. According to Grundy (1979) inclusion of diet rich in polyunsaturated fatty acid leads to increased fecal excretion of cholesterol, causes decrease in cholesterol absorption in the gut, causes reduction in its synthesis by body or causes shift in its contents from plasma to other body components. Thus presence of these components along with dietary fiber in the mushroom might interact with the bile acids and reduce their reabsorption.

4. CONCLUSIONS

Thus it can be concluded that inclusion of Pleurotus sajor caju mushroom in our diet is quite beneficial for good health as it is a good source of fiber, protein and polyunsaturated fatty acids. The Pleurotus sajor caju mushroom is boon for obese persons as this food is almost devoid of fats and contains very good amount of polyunsaturated fatty acids and dietary fiber which helps to lower the level of cholesterol in serum and liver.

5. REFERENCES

[1] Miles, P.G. and Chang, S.T. 1997. Introduction to principles of production. II. Worldwide trends over the past decade. In: Mushroom Biology-Concise Basics and Current Development. World Scientific Publishers, Singapore.pp. 86-96 and 124-126

[2] Tshinyangu, K.K. 1996. Effect of grass hay substrate on nutritional value of *Pleurotus ostreatus* var. Columbinus. Die Nahrung, **40**: 79-83.

[3] Goodnight,S.H., Harris,W.S., Connor, W.E. and llingworth, D.R.(1982): Polyunsaturated fatty acids, hyperlipidemia and thrombosis. *Artheriosclerosis* **2**(2):87-113.

[4] AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. Arlington, Virginia, USA, pp.2-15.

[5] Luddy, F.E, Bardford,R.A.,and Heind, P.M.1968. A rapid quantitative for the preparation of methyl esters fat and other fat. Journal of American Oil Chemists, **42**: 549-552.

[6] Van Soest, P.J.and Wine, R.H. 1967. Use of detergents in the analysis of fibrous foods. Determination of plant cell wall constituents. Journalof Association Official Analytical Chemistry, **50**: 50-55.

[7] Arora, S.K. 1981. Analytical techniques for quality evaluation. Forage Research, **7A**:197-213.

[8] Roeschlau, D., Bernt and Gruber, W.A. 1974. Clinical Chemistry Clinical Biochemistry, **12**: 226.

[9] Rocos, H.G. and Farenbach, M.J. 1963. Analytical Biochemistry, **61**: 520-529.

[10] Friedewald, W.T., Levy, R.I. and Frederickson, D.P.S. 1972. Estimation of plasma low density lipoprotein cholesterol concentration without use of preparative ultracentrifuge. Clinical Chemistry, **18**: 499

[11] Folch, J., Lees, M. and Sloane-Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. **226**: 407.

[12] Chauhan, B.M.1974. Effect of plant fiber in decreasing plasma total cholesterol and increasing high density lipoprotein cholesterol. Proceedings Society Experimental Biology, **162**: 310-313.

[13] Panse, V.G. and Sukhatme, P.V.1961. Statistical methods of agricultural coworkers. 2nd ed:. Indian Council of Agricultural Research, New Delhi,pp. 12- 87.

[14] Hughes,D.H.1972. Preliminary characterization of the lipid constituents of the cultivated mushroom *Agaricus campestris.* Mushroom Science, **5**: 540-545.

[15] Rai, R.D. 1995. Nutritional and medicinal values of mushrooms. Advances in Horticulture Vol 13. Mushroom. (eds). K.L. Chadha and S.R. Sharma.pp537-551.

[16] Grundy, S.M. Dietary fats and sterols. In: Nutrition, Lipids and Coronary Heart Disease. R.I. Levy, B.M. Rifkind, B.H. Dennis, N. Ernst (eds) New York, Raven Press, 1979.pp. 89-118