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Comparative Effects of Oyster Mushrooms on Plasma Lipid Profile of Hypercholesterolaemic Rats

Nuhu Alam, Md. Shahdat Hossain[1], Abul Khair, S.M. Ruhul Amin[2] and Asaduzzaman Khan[1]
Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

The feeding of hypercholesterolaemic rats with 5% powder of fruiting bodies of oyster mushrooms i.e., *Pleurotus ostreatus, P. sajor-caju*, and *P. florida* reduced the plasma total cholesterol level by 37%, 21% and 16%, respectively and triglyceride level by 45%, 24% and 14%, respectively. LDL/HDL ratio decreased by 64%, 45% and 41% for *P. sajor-caju, P. ostreatus*, and *P. florida* fed rats, respectively. Mushroom feeding also reduced body weight slightly in hypercholesterolaemic rats. However, it had no adverse effect on plasma bilirubin, creatinin and urea nitrogen level. The present study reveals that 5% oyster mushroom supplementation provides health benefits, at least partially, by acting on the atherogenic lipid profile in the hypercholesterolaemic condition.

Key words: Comparative effect, oyster mushrooms, lipid profile and hypercholesterolaemic rats.

INTRODUCTION

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease. But mushroom consumption in many developing countries, particularly in Bangladesh, is extremely limited. There would be many but one reason probably being that the nutritional or health benefits derived from various edible mushrooms are largely unknown.

Mushrooms provide a wide variety of physiologically ameliorative active components: *Pleurotus sajor-caju* inhibits hypertensive effects through its active ingredients, which affect the renin-angiotensin system(Chang, 1996), *Trichloma mongolcium* produces vasorelaxation because of its lectin content (Wang *et al.*, 1996), *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic effects in experimentally induced diabetics (Chorvathova *et al.*, 1993), *Lentinus edodes* and *Grifola frondosa* have antihypertensive effects in spontaneously hypertensive rats (Kabir *et al.*, 1987) and *Agaricus bisporus* decreases serum low-density lipoprotein-cholesterol (LDL-C) by increasing the expression of LDL receptor at mRNA level and LDL receptor activity (Fukushima *et al.*, 2000).

[1] Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[2] National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh.

Considerable experimental evidence suggests that one of the most important food components that help to reduce serum cholesterol is its polyunsaturated fatty acid (PUFA) content (Hashimoto *et al.*, 1999, 2001, Gamoh *et al.*, 1999, 2001, Hossain MS *et al.*, 1999). Arachidonic acid exacerbates platelet functions (Hossain *et al.*, 1999a), whereas LNA acts as a precursor of the physiologically important PUFA, such as eicosapentaenoic acid (EPA; $C_{20:5}$, ω-3) and docosahexaenoic acid (DHA; $C_{22:6}$, ω3) (Schmidt *et al.*, 2001). There is considerable data supporting the belief that the health benefit obtained through the lowering of blood cholesterol may be derived from the effects of EPA and DHA (Hashimoto *et al*, 1998, 1999a). In addition to their roles in the development and function of the central nervous system (Lepage and Roy, 1986), these two fatty acids play an important role in the physiological functions of the cardiovascular system.

Thus, one of the objectives of the present study was to generate awareness of the beneficial effects of edible mushrooms, particularly of oyster mushrooms, on hypercholesterolaemia, which poses serious health problems in both developed and developing countries.

MATERIALS AND METHODS

Animals: Twenty five young Long Evans rats *(Rattus rattus*) of 114 ± 12g (mean±SD) were used in the present study. Rats were housed in animal room at 23 ± 2^0 C, under 12 h dark-light cycles and then divided randomly into five groups. Rats were fed a basal diet supplemented with, no cholesterol or mushroom (normocholesterolaemic control rats; NC), 1% cholesterol (hypercholesterolaemic rats; HC), 1% cholesterol and 5% powder of *Pleurotus ostreatus* (HC+PO group rats), 1% cholesterol and 5% powder of *Pleurotus sajor-caju* (HC+PS group rats), 1% cholesterol and 5% powder of *Pleurotus florida* (HC+PF group rats).

The composition of the basal diet was as follows (g/100g): Wheat flower- 50; rice powder- 11; wheat bran-19; casein (non fat)-8; egg white-10; soybean oil-1; table salt-0.5, vitamin mixture-0.25 and mineral mixture-0.25. The composition of vitamin mixture in the diet was as follows (gram/100g vitamin mixture):retinyl acetate-9.5×10^{-4}; cholecalciferol-1.2×10^{-3}; α-tochoferol acetate-0.05; thiamin hydrochloride-2.4; nicotinic acid-12; riboflavin-2.4; D-calcium pantothenate-9.6; pyridoxine hydrochloride-1.2; folic acid-9.5×10^{-2}; vitamin K-0.25; cyanocobalamine-9.5×10^{-3}; inositol-47.95 and ascorbic acid-24.0. The composition of mineral mixture added to diet was as follows (g/100g of mineral): calcium gluconate-28.5; K_2HPO_4-17.3; $CaCO_3$-26; $MgSO_4$-12.6; KCl-12.6; $CuSO_4$-0.06; $FeSO_4$-0.3; $MnSO_4$-0.55; NaF-2.5×10^{-4}; KI-9×10^{-4}; sodium molybdate-3×10^{-4}; SeO_2-3×10^{-4}; $CrSO_2$-1.5×10^{-3}.
Rats were fed for 40 days.

Collection of oyster mushrooms: Mature fruiting bodies of *Pleurotus ostreatus, P. sajor-caju,* and *P. florida* were collected from the National Mushroom Development and

Extension Centre, Savar, Dhaka, Bangladesh. The fruiting bodies were dried in sunlight and crushed into powder. The powder was mixed with the basal diet.

Chemical analysis of plasma: Plasma total cholesterol (TC) was measured enzymatically using the cholesterol oxidase assay, whereas high-density lipoprotein cholesterol (HDL-C) was measured by the same procedure after precipiting low-density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) using magnesium sulfate and phosphotungstic acid, using test kits (Cholesterol and HDL-Cholesterol Liquicolor; Human Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany. human@human.de). Tgriglycerides, bilirubin, creatinine, and blood urea nitrogen were also measured with commercially available reagent kits (Triglycerides liquicolor^{mono}, Bilirubin liquicolor, Creatinine liquicolor and Urea liquicolor; Human Gesellschaft für Biochemica und Diagnostica, Wiesbaden,Germany. human @human.de).

Low-density lipoprotein cholesterol was calculated as follows:

$$LDL\text{-}C = \{TC – (HDL\text{-}C + TG/5)\}$$

And very low density lipoprotein cholesterol was calculated as:

$$VLDL\text{-}C = \{TC – (HDL\text{-}C + LDL\text{-}C)\}$$

Statistical analysis: Results were expressed as mean ± SEM (for body weight, mean±SD). All parameters for inter group differences were analyzed by one-way ANOVA. The correlations were evaluated by simple regression analysis. The statistical program used was SPSS 11.5. ($P<0.05$ was considered statistically significant).

RESULTS AND DISCUSSION

Effects of mushroom feeding on body weight: Mushroom feeding reduced body weight in hypercholesterolaemic rats slightly but not significantly. This effect is depicted in Table 1.

Table 1. Effect of *P. ostreatus, P. sajor-caju,* and *P. florida* feeding on the body weight of hypercholesterolaemic rats

Parameter (gm)	NC	HC	HC+PS	HC+PO	HC+PF
Body weight	244±12.8	244±18.5	225±22	216±20.7	215±16.8

The results are the mean±SD. *NC, normocholesterolaemic rats; HC, hypercholesterolaemic rats; HC+ PS, P. sajor-caju fed hypercholesterolaemic rats; HC+ PO, P. ostreatus fed hypercholesterolaemic rats; HC+ PF, P. florida fed hypercholesterolaemic rats.*

Effects of mushroom feeding on plasma lipid profile and other parameters: Plasma TC, TG, HDL-C, LDL-C, VLDL-C, bilirubin, creatinin and blood urea nitrogen (BUN) levels in NC, HC, HC+PS, HC+PO, HC+PF rats after mushroom feeding for 40 days have been presented in Table 2.

In HC rats, plasma TC increased by 21% compared with levels in NC rats. Plasma TC concentrations decreased by 21% in HC+PS rats, by 37% in HC+PO rats and by 16% in HC+PF rats compared with HC rats. In HC rats, plasma TG increased by 55% compared with levels in NC rats. Plasma TG concentrations decreased by 24% in HC+PS rats, by 45% in HC+PO and by 14% in HC+PF rats compared with HC rats. In HC rats, plasma HDL-C level decreased by 35% and plasma LDL-C level increased by 144% compared with levels in NC rats. Plasma HDL-C level increased slightly, but not significantly in HC+PS and HC+PF rats compared with HC rats. But HC + PS, HC+PO and HC+PF rats showed significant decrease in plasma LDL-C levels by 59%, 47% and 41%, respectively compared with HC rats.

The ratio of plasma LDL-C to HDL-C is shown in Fig. 1. In HC rats, this ratio increased by 266%, compared with NC rats. But mushroom feeding reduced the ratio significantly in HC + PS, HC+PO and HC+PF rats by 64%, 45% and 41% respectively compared with HC rats. There was no significant difference in plasma bilirubin, creatinin and BUN levels in the normo-, hypercholesterolaemic and mushroom-fed hypercholesterolaemic rats.

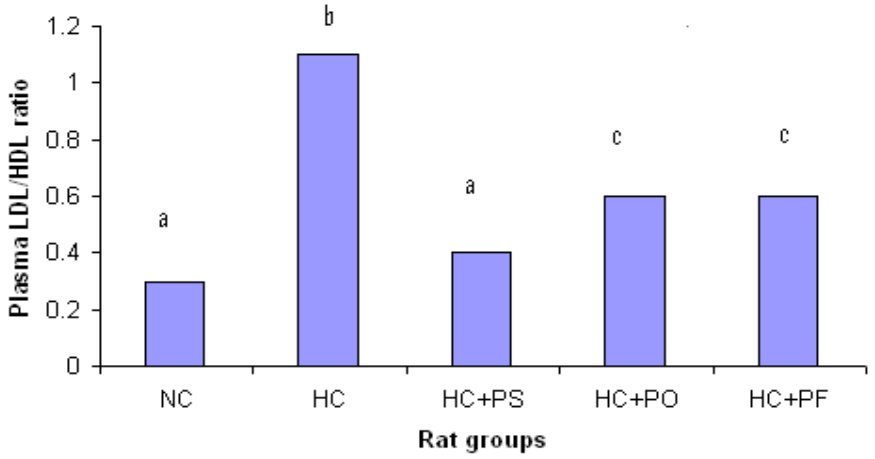
Table 2. Effects of *Pleurotus sajor-caju, P. ostreatus and P. florida* mushrooms on plasma lipid profiles of hypercholesterolaemic rats

Parameters (mg/dl)	NC	HC	HC+PS	HC+PO	HC+PF
TC	59.4±6.3^{a}	72± 5^{b}	57 ± 5^{a}	46.3±5.9^{a,c}	60.4 ±7.5^{a,b}
TG	92 ± 7.8^{a}	143±15.8^{b}	108 ±5.4^{a}	78.8 ±2^{c}	122.9 12.8^{a,b}
HDL-C	31.9±4.4^{a}	20.6± 3.4^{b}	25.6 ± 4^{a,b}	18.9 ± 2.9^{b}	22.8 ±5.3^{b}
LDL-C	9.1 ± 2.8^{a}	22.2± 5.5^{b}	9.6 ± 3.7^{a}	11.7 ± 3.1^{a}	13.1 ±7.3^{c}
VLDL-C	18.4±1.6^{a}	28.6± 3.2^{b}	21.7 ± 1.1^{a}	15.8 ± 0.4^{a}	24.5 ±2.6^{a,b}
Bilirubin	0.28± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.25 ± 0.1	0.28± 0.1
Creatinin	0.5±0.1	0.6± 0.1	0.5±0.2	0.5±0.1	0.4±0.1
BUN	18.8±0.3	21.1±1.0	20.4±1.4	19.3±1.1	19.6±0.8

The results are the mean±SEM. Values in the same row that do not share a common superscript are significantly different at P<0.05 (one way ANOVA then LSD post hoc comparison) *NC, normocholesterolemic rats; HC, hypercholesterolemic rats; HC+PS, P. sajor caju fed hypercholesterolemic rats; HC+ PO, P. ostreatus fed hypercholesterolemic rats; HC+PF, P. florida fed hypercholesterolemic rats. TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; BUN, blood urea nitrogen.*

The present study provides evidence that the feeding of 5% oyster mushrooms to rats significantly ameliorates the plasma atherogenic lipid profiles in experimentally induced hypercholesterolaemic rats. Rats are particularly resistant to the development of hypercholesterolaemia and atherosclerosis (Wissier *et al.*, 1954, Fillius *et al.*, 1956 and Malinow *et al.*, 1954) and have a strong capability to maintain their plasma cholesterol (Fujioka *et al.*, 1995, Spady and Cuthbert, 1992 and Roach *et al.*, 1993). Therefore, in order to induce hypercholesterolaemia or atherosclerosis in rats, cholesterol feeding is associated with other additives, including bile acids and propylthiouracil (an anti-thyroid drug), which increase the intestinal absorption of cholesterol (Dolphin and Forsyth, 1983

and Pathe and Chevallier, 1976). However, in the present study, the addition of 1% cholesterol to the basal diet without bile acids and/or anti-thyroid drugs produced hypercholesterolaemia in rats, because cholesterol feeding itself increases bile acid secretion by approximately three to four fold in rats (Uchida *et al.*, 1996). The 21% increase in plasma cholesterol in the hypercholesterolaemic rats in the present study was comparable with that reported by Bobek *et al.* (1995), who fed rats cholesterol (0.3%) diet with added bile acids (0.5%) and showed a 1.7-fold higher cholesterolaemia in their cholesterol fed rats compared with normal rats.



Columns with different symbol notations indicate significant differences at P<0.05 (one way ANOVA then LSD post hoc comparison)

Fig 1. Effects of *Pleurotus sajor-caju, P. ostreatus and P. florida* on plasma LDL-C/HDL-C ratio of hypercholesterolemic rats.

In this experimental paradigm, 5% mushroom feeding significantly repressed the increment of plasma cholesterol. The mechanism by which mushrooms reduce plasma TC levels in hypercholesterolaemic rats is not clearly understood. Mushrooms contain the hypocholesterolaemic agent mevnolin (monacolin K, lovastatin) (Gunde-Cimermann *et al.*, 1993), which may be involved in decreasing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme (Bobek *et al.*, 1995), the rate-limiting enzyme for cholesterol biosynthesis. Thus, mushroom feeding may involve the suppression of endogenous cholesterol biosynthesis by inhibiting the activity of HMG-CoA reductase activity. In addition, mushroom contains water soluble gel forming substances including β-1,3-D-glucan and pectin, which bind to bile acids, thereby inhibiting cholesterol-bile micelle formation and cholesterol resorption.

The LDL-C level in control rats was low suggesting that the principal cholesterol carrying lipoprotein in normocholesterolaemic rodents, including rats are not the LDL particles, rather they are HDL and VLDL. However, when rats become hypercholesterolaemic, the

LDL-C level increased 2-3 folds of that of normocholesterolaemic rats, again demonstrating that the principal cholesterol carrying lipoprotein in hypercholesterolaemic rats was LDL and then the carriers were probably the HDL and VLDL. Our results of reduced LDL-C after mushroom feeding are consistent with a similar report suggesting increased clearance of radioiodinated LDL from rat blood (Chorvathova *et al*, 1993). Usually, a high level of LDL-C and a low level of HDL-C indicate an imbalance between cholesterol transport from the liver to extrahepatic tissues and back to the liver. Mushroom feeding significantly decreased LDL/HDL ratio in hypercholesterolaemic rats. Thus, mushrooms may provide an important health benefit by increasing plasma HDL-C and decreasing plasma LDL-C. The process of excretion of cholesterol from the body begins with the hydrolysis, in the liver, of LDL-C and HDL-C ester into free cholesterol, which is secreted as such and/or after its conversion into bile acids in the bile ducts. The fruiting bodies of mushrooms increase faecal cholesterol (Kubo and Nanba, 1997) .Thus, the decreased plasma cholesterol may also be attributed to such a mechanism. The higher level of plasma HDL-C indicates that more cholesterol from peripheral tissues was returning to the liver for catabolism and subsequent excretion. Plasma VLDL-C and TG content in mushroom fed hypercholesterolaemic rats were lower compared to the hypercholesterolaemic control rats. VLDL-C is the major transport vehicle for the TG from the liver to extrahepatic tissues, whereas LDL-C is not secreted as such the liver; rather, it seems to be formed from VLDL-C after partial removal of TG by lipoprotein lipase (Mayes, 1997). After feeding cholesterol to rats, LDL-C became the prime carrier for cholesterol, then consequently leading to a decreased cholesterol content of VLDL-C and HDL-C in mushroom fed hypercholesterolaemic rats. The present results suggest that oyster mushroom ingestion has significant health benefits through the modulation of physiological functions that include various atherogenic lipid profiles in hypercholesterolaemia. Therefore, oyster mushroom may be a good source of nutrition that may also act as a prophylactic against hypercholesterolaemia, hyperlipidaemia and related complications, which are the risk factors of atherosclerosis.

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